SciTechnol

VEGETOS

International Journal of Plant Research

Online ISSN: 2229-4473

Print ISSN: 0970-4078

Das and Dhakar, Vegetos 2016, 29:3
10.5958/2229-4473.2016.00059.8

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Effect of Interstock on Growth of Vigorous Mango Cultivars under Eastern Plateau and Hill Region of India

Bikash Das* and Mahesh Kumar Dhakar

Abstract

Investigations were undertaken at Ranchi of Jharkhand state of India to test the efficacy of mango genotypes Amrapali and Latra be used as interstocks for reducing the plant size of vigorous but popular mango varieties viz. Langra, Himsagar and Bombay Green under eastern plateau and hill conditions of India. Data on plant growth parameters during 2012-13, 2013-14 and 2014-15 indicated decrease in rate of growth of plant height, canopy spread, girth of rootstock, girth of interstock, girth of scion, average length of shoot extension growth in cultivars Himsagar and Bombay Green with interstock of Amrapali. Hence, it was concluded that mango cultivar Amrapali can be effectively used as interstock for reducing plant vigour of mango cultivars Himsagar and Bombay Green.

Keywords

Interstock; Amrapali; Latra; Girth of interstock

Introduction

The eastern plateau and hill agroclimatic zone of India offers suitable climatic conditions for successful cultivation of a number of fruit crops. During the last decade, the region has received attention of the policy makers for largescale area expansion under fruit crops due to the availability of huge tracts of fallow uplands. Among the different fruit crops, mango occupies the most important position with respect to the preference of the growers of the region which is evident from the fact that the crop occupies more than 67% of the total area under fruit crops even if the productivity in the region is significantly lower than other fruit crops. The low productivity of mango in the region is attributed to edaphic constraints like low organic carbon in the soil, soil acidity, deficiency of phosphorus, boron etc. High density orcharding can be the most important practice for improving the productivity of mango in the region. Nath have already reported the feasibility of high density orcharding of mango cv Amrapali under this agroclimatic zone of India.

Among the mango cultivars, the major share under the area expansion programme in the region during the last decade has come from cv Amrapali. This is mainly due to its prolific bearing and high yielding ability. Due to the the dwarf canopy size, the plants of mango cv Amrapali can be planted in high density system which also contribute towards higher productivity of this cultivar. However, late fruit maturity coinciding with heavy rainfall results in poor fruit quality of mango cv Amrapali which fetches lower market price in the region (Banerjee et al. 2015). Fruits of traditional mango cultivars like Langra, Himsagar and Bombay Green fetch higher market price due to the superior fruit quality and time of availability of the fruits under the eastern plateau and hill conditions. However, lower yield of these cultivars, results in unprofitability of traditional mango orcharding under the eastern plateau and hill conditions. Development of high density orcharding can ensure profitability of mango cultivation of these traditional vigorous mango cultivars. However, strategies for high density orcharding have not yet been developed for vigorous cultivars like Langra, Bombay Green and Himsagar.

Control of tree vigour is the first strategy for high density orcharding in any fruit crops. Use of dwarfing rootstock and growth retardants are some of the commonly recommended practices for control of tree vigour in mango Among the growth retardants, use of Paclobutrazol have been found most effective in mango. However, apprehensions on residue in the fruit and harmful effects of paclobutrazol on soil microflora is a limiting factor. Although rootstocks have several applications such as improving fruit quality, imparting adaptability to climatic and edaphic conditions and inducing dwarfing, the priorities of rootstocks selection in the tropics and subtropics have been focused mainly on vigour management and securing regular high fruit yields [1]. Although a number of polyembryonic dwarfing rootstocks have been identified in mango [2] their unavailability on a largescale is a major constraint for their use in high density orcharding. Use of interstocks have also been found to be effective in reducing vigour of mango plants [3-8] although no effect of interstock on plant vigour has also been reported by many workers [9-11]. The interstock is a segment of a tree trunk that it is grafted between the rootstock and the tree. It has been used with different fruit trees to modulate the tree size, fruit production and quality, and the aging of the tree.

The mango hybrid Amrapali and and genotype Latra are known for their dwarf tree size. Although fruits of Amrapali and Latra are abundantly available, due their monoembryonic nature limits their use as dwarfing rootstocks. No information is available on behavior of mango cv Amrapali and Latra as interstock. Keeping this fact in view we undertook an investigation to test the efficacy of these genotypes to be used as interstocks for reducing the plant size of vigorous but popular mango varieties viz. Langra, Himsagar and Bombay Green under eastern plateau and hill conditions.

Materials and Methods

The investigations were undertaken during 2011-2015 at ICAR Research Complex for Eastern Region, Research Centre, Ranchi, Jharkhand, India. The area is situated at 23^{0}25' N and 85^{0}20' E and 620 m above msl. The treatment comprised of combinations of three interstocks viz., no interstock, interstock of Latra and interstock of Amrapali and three vigorous mango cultivars viz. Langra, Himsagar and Bombay Green used as scions. The plants were planted in the field in July, 2010 at a spacing of 5 m x 5 m in a factorial randomized block design with four replications and 10 plants per replication. In case of double grafted plants with interstocks, the uniform length of

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Received: February 24, 2016 **Accepted:** July 04, 2016 **Published:** July 11, 2016

interstock was maintained (10 cm). However, due to the drying of scions of double grafted plants with Latra as interstock during the first year, in-situ grafting of scion on plants with Latra interstock was done during August, 2011. Yearly data were recorded during 2012-13, 2013-14 and 2014-15 on plant growth parameters like height, canopy spread, girth of rootstock, girth of interstock, girth of scion and number of vegetative flushes per year and average shoot length of vegetative flushes. Girth of the rootstock was measured at 5 cm below the first graft union while girth of interstock and scion were measured at 5 cm above the first and second graft union, respectively. The data on plant growth parameters were recorded during the month of September in each year. Values on percent increase over previous season in different growth parameters were also estimated for better interpretation of results. The year wise data were subjected to analysis of variance.

Results

Effect on plant height

During all the three years, significant differences were recorded with respect to effect of interstock and interaction between Interstock x Genotypes (Table 1). In case of two year old plants (2012-13), the minimum plant height was recorded in case of plants with Latra as interstock whereas plants with Amrapali interstock and no interstock did not differ significantly. The lower values of Latra were attributed to the mortality at the time of plant establishment. With respect to interaction effect, the difference in plant height between No interstock and Interstock of Amrapali in all the three genotypes were non-significant. In case of three year old plants (2013-14), the plant height with Interstock of Latra was significantly lower than other treatments although the % increase in plant height over previous season (29.44%) was markedly higher. This can be attributed to their delayed field establishment and persisting juvenility. Although the plant height with No interstock and Interstock of Amrapali did not differ significantly, the rate of increase with Interstock of Amrapali (5.76%) was significantly lower than that with No interstock (18.48%). With respect to the interaction effect, plants of cv Langra with Amrapali interstock had significantly lower plant height than that with no interstock and no such difference could be recorded in case of cvs Himsagar and Bombay Green. However, significantly lower values of % increase in plant height were recorded with both the interstocks in case of the cultivars Himsagar and Bombay Green. This hinted at reduced plant height of mango cultivars Himsagar and Bombay Green with interstock of Latra and Amrapali after three years of planting. During the fourth year of planting (2014-15), both the interstocks resulted in significantly lower values of plant height than that with no interstock. With respect to the interaction effect, the plant height of cultivar Langra and Bombay Green was significantly low with use of interstocks. However, significantly lower values of % increase in plant height was recorded in case of Himsagar on interstock of Latra (2.29% than 18.53% in case of no interstock) and Bombay Green on interstock of Amrapali (10.36% than 34.86% in case of no interstock). Hence, after four years of planting, reduction in plant height of mango cultivars Himsagar and Bombay Green was recorded with use of interstocks of Latra and Amrapali, respectively.

Effect on canopy spread

With respect to average canopy spread, significant effects of interstock, genotypes and interaction between interstock x genotype was recorded during 2012-13 and 2013-14 while during 2014-15, the effect of interstock was significant only (Table 2). However, significantly lower canopy spread of double grafted plants was recorded only in cultivar Langra during 2012-13. With respect to % increase over previous year, significantly lower values than the single grafted plants were recorded in case of cultivar Bombay Green on Amrapali interstock during both the years. Hence, as recorded in case of plant height, use of interstock of Amrapali resulted in reduction in the canopy spread of mango cultivar Bombay Green after four years of planting.

Effect on girth of rootstock

With respect to girth of rootstock, significant effect of interstock and interaction between Interstock x Genotype was recorded throughout the experimental period (Table 3). During 2012-13, the girth of rootstock with interstock of Latra was significantly lower which was attributed to delayed plant establishment. In case of cv Langra with interstock of Amrapali, the value was significantly lower than that in No interstock while no such difference was recorded in other genotypes. During 2013-14, similar trend was recorded. However, marked reduction in the values of % increase over the previous season was recorded during this year with use of interstock particularly in the cultivars Himsagar and Bobmay Green. During 2013-14, the girth of rootstock with interstock of Latra was significantly lower than that with No interstock in all the three cultivars. In case of interstock of Amrapali, the girth was significantly lower than thant of No interstock in mango cultivars Langra and Himsagar. However, the value of % increase over the previous season was markedly lower than that of No interstock only in case of Himsagar on Amrapali interstock. Hence, after four years of planting reduction in the growth rate of rootstock was recorded in of Mango cv Himsagar on interstock of Amrapali. Reduction in circumference of rootstock has also been reported by Yonemoto [8] by use of dwarf variety Khom as interstock indicating.

Effect on girth of interstock

During all the three years of experimentation, the girth of interstock differed significantly and it was significantly higher in case of Amrapali (Table 4). With respect to % increase in the girth

Table 1: Effect of interstock on plant height (m) of mango plants.

	2012-13				2013-14				2014-15			
	No interstock	Interstock of Latra	Interstock of Amrapali	Genotype	No interstock	Interstock of Latra	Interstock of Amrapali	Genotype	No interstock	Interstock of Latra	Interstock of Amrapali	Genotype
Langra	2.35	0.54	1.79	1.56	2.54	0.86	1.92	1.77	2.75	1.74	2.23	2.24
Himsagar	1.59	1.13	1.74	1.48	1.78	1.31	1.81	1.63	2.11	1.34	2.24	1.90
Bombay Green	1.61	1.51	1.82	1.65	2.18	1.72	1.93	1.94	2.94	1.68	2.13	2.25
Average	1.85	1.06	1.78		2.17	1.30	1.89		2.60	1.59	2.20	
SEm±	Interstock (I): 0.17; Genotypes (G): 0.11; I x G: 0.29				Interstock: 0.08, Genotypes: 0.12, I x G:0.15				Interstock: 0.13, Genotypes: 0.11, I x G:0.18			
C.D. at 5%	Interstock (I): 0.48; Genotypes (G): ns; I X G: 0.83				Interstock: 0.24, Genotypes: ns, I x G: 0.46				Interstock: 0.36, Genotypes: ns, I x G: 0.51			

over previous year, during 2013-14, the values in cultivar Langra was markedly lower in case of interstock of Amrapali whereas during 2014-15, values in cultivars Langra and Bombay Green were markedly lower in case of interstock of Amrapali. Hence, the results clearly indicated influence of scion on the growth of interstock also and interstock of Amrapali with cultivar Langra had slower growth rate than that of Latra.

Effect on girth of scion

Significant effects of interstocks and its interaction with Genotypes were recorded on girth of scion during all the three years (Table 5). However, significantly lower girth was recorded in case of interstock combinations of cultivar Langra only. With respect to % increase over previous season, during both 2013-14 and 2014-15, the values in case of cultivars Himsagar and Bombay Green with interstock of Amrapali were markedly lower than that with No interstock. Hence, after four years of planting, interstock of Amrapali resulted in slower growth rate of scion of mango cultivar Himsagar and Bombay Green.

Effect on vegetative flushing and shoot growth

During all the three years, number of vegetative flushes was significantly influenced by interstock and during 2014-15, the effect of interstock x genotype was significant (Table 6). During 2012-13 and 2013-14, the number flushes with interstock Latra was significantly higher which was attributed the juvenility due to delayed establishment. During 2014-15, the number of flushes in cultivar Bombay Green was significantly lower with interstock of Amrapali than that with no interstock. The average length of extension growth was significantly influenced by interstock and interaction between interstock x genotype during all the three years (Figure 1). During 2012-13, in all the cultivars, the length was significantly lower with interstock of Amrapali than with no interstock. However, during 2013-14 and 2014-15, the length with interstock of Amrapali was significantly lower than that with no interstock only in case of Himsagar and Bombay Green.

Discussion

Use of dwarfing rootstock for control of tree vigour is already a common practice in many temparate fruit crops. Plant vigour is an interaction between different plant growth parameters and dwarfing effect of interstock is the resultant of its effect on diffferent plant growth parameters. The present study clearly indicated effects of different interstock of plant growth parameters where interaction between interstock and genotype played important role. Use of interstock of Amrapali successfully resulted in decrease in the plant height, canopy spread of mango cultivars Bombay Green whereas no such consistent

Table 2: Effect of interstock on average spread of mango plants (m).

	2012-13				2013-14				2014-15			
	No interstock	Interstock of Latra	Interstock of Amrapali	Genotype	No interstock	Interstock of Latra	Interstock of Amrapali	Genotype	No interstock	Interstock of Latra	Interstock of Amrapali	Genotype
Langra	2.11	0.78	0.95	1.28	2.15	0.86	1.15	1.38	2.34	1.17	1.54	1.68
Himsagar	1.40	1.02	1.37	1.27	1.42	1.15	1.43	1.33	1.58	1.35	1.57	1.50
Bombay Green	0.88	0.74	0.97	0.87	1.08	0.86	1.13	1.02	1.63	1.56	1.50	1.56
Interstock	1.46	0.85	1.10		1.55	0.95	1.24		1.85	1.36	1.53	
SEm±	Interstock (I): 0.11; Genotypes (G): 0.12; I x G: 0.34				Interstock: 0.10, Genotypes: 0.12, I x G: 0.33				Interstock: 0.11, Genotypes: 0.12, I x G: 0.35			
C.D. at 5%	Interstock: 0.31; Genotypes : 0.34; I x G: 0.98				Interstock: 0.31, Genotypes: 0.33 , I x G: 1.04				Interstock: 0.31, Genotypes: ns, I x G: ns			

Table 3: Effect of interstock on girth of rootstock of mango plants (cm).

	2012-13				2013-14				2014-15			
	No interstock	Interstock of Latra	Interstock of Amrapali	Genotype	No interstock	Interstock of Latra	Interstock of Amrapali	Genotype	No interstock	Interstock of Latra	Interstock of Amrapali	Genotype
Langra	88.11	15.11	55.24	52.82	114.97	28.32	75.32	79.54	121.08	74.64	87.25	94.32
Himsagar	59.18	46.52	58.95	54.88	87.71	51.41	72.34	70.49	105.10	62.00	82.83	83.31
Bombay Green	62.41	48.95	61.29	57.55	98.32	50.32	74.31	74.32	108.36	81.18	82.99	90.84
Average	69.90	36.86	58.49		100.33	43.35	73.99		111.51	72.61	84.35	
SEm±	Interstock (I): 4.54; Genotypes (G): 3.15; I x G: 6.79				Interstock: 6.78, Genotypes: 1.48, I x G: 9.04				Interstock: 8.46, Genotypes: 4.48, I x G: 7.18			
C.D. at 5%	Interstock (I): 13.86; Genotypes (G): ns; I x G: 19.14				Interstock: 18.3, Genotypes: 5.34, I x G: 28.63				Interstock: 24.64, Genotypes: ns, I x G: 22.46			

Table 4: Effect of interstock combinations on girth of interstock of mango plants (cm).

	2012-13			2013-14			2014-15		
	Interstock of Latra	Interstock of Amrapali	Average	Interstock of Latra	Interstock of Amrapali	Genotype	Interstock of Latra	Interstock of Amrapali	Genotype
Langra	8.45	28.74	18.59	28.16	56.80	42.48	53.39	62.78	58.08
Himsagar	30.69	33.44	32.06	50.45	65.40	57.92	51.84	72.74	62.29
Bombay Green	29.26	36.45	32.85	37.66	53.08	45.37	57.56	65.39	61.47
Average	22.80	32.88		38.76	58.43		54.26	66.97	
SEm±	Interstock (I): 3.66; Genotypes (G): 3.98; I x G: 4.94			Interstock: 5.64, Genotypes: 6.73, I x G: 9.42			Interstock: 3.98, Genotypes: 2.66, I x G: 7.59		
C.D. at 5%	Interstock (I): 9.46; Genotypes (G): 10.42; I x G: ns			Interstock: 16.61, Genotypes: ns, I x G: ns			Interstock: 11.18, Genotypes: ns, I x G: ns		

Table 5: Effect of interstock on girth of scion of mango plants (cm).

	2012-13				2013-14				2014-15			
	No interstock	Interstock of Latra	Interstock of Amrapali	Average	No interstock	Interstock of Latra	Interstock of Amrapali	Average	No interstock	Interstock of Latra	Interstock of Amrapali	Average
Langra	76.20	5.96	30.74	37.64	81.42	10.48	32.31	41.40	102.23	46.40	49.03	65.89
Himsagar	47.81	27.49	32.09	35.80	55.36	36.61	35.28	42.42	85.53	39.75	49.15	58.15
Bombay Green	49.53	28.73	35.36	37.87	59.61	33.51	37.44	43.52	88.68	57.31	48.09	64.69
Average	57.85	20.73	32.73		65.46	26.87	35.01		92.15	47.82	48.76	
SEm±	Interstock (I): 4.19; Genotypes (G): 2.44; I x G: 8.38				Interstock: 7.83, Genotypes: 3.65, I x G: 13.46				Interstock: 7.46, Genotypes: 2.44, I x G: 15.11			
C.D. at 5%	Interstock: 12.68; Genotypes : ns; I x G: 28.63				Interstock): 22.11, Genotypes: ns, I x G: 41.16				Interstock: 21.49, Genotypes: ns, I x G: 44.61			

Table 6: Effect of interstock on total number of vegetative flushes per year.

	2012-13				2013-14				2014-15			
	No interstock	Interstock of Latra	Interstock of Amrapali	Genotype	No interstock	Interstock of Latra	Interstock of Amrapali	Genotype	No interstock	Interstock of Latra	Interstock of Amrapali	Genotype
Langra	3.16	4.68	3.19	3.67	3.64	4.31	3.71	3.89	3.19	3.84	2.84	3.29
Himsagar	3.61	4.52	3.24	3.79	3.28	4.16	3.86	3.77	2.98	3.79	2.11	2.96
Bombay Green	3.61	4.66	3.14	3.80	3.11	4.24	4.01	3.79	3.11	3.98	2.18	3.09
Interstock	3.46	4.62	3.19		3.34	4.24	3.86		3.09	3.87	2.37	
SEm±	Interstock (I): 0.24, Genotypes (G): 0.18, I x G: 0.71				Interstock: 0.19, Genotypes: 0.24, I x G: 0.51				Interstock: 0.23, Genotypes: 0.11, I x G: 0.39			
C.D. at 5%	Interstock (I): 0.74, Genotypes (G): ns, I x G: ns				Interstock: 0.62, Genotypes: ns, I x G: ns				Interstock: 0.68, Genotypes: ns, I x G: 0.91			

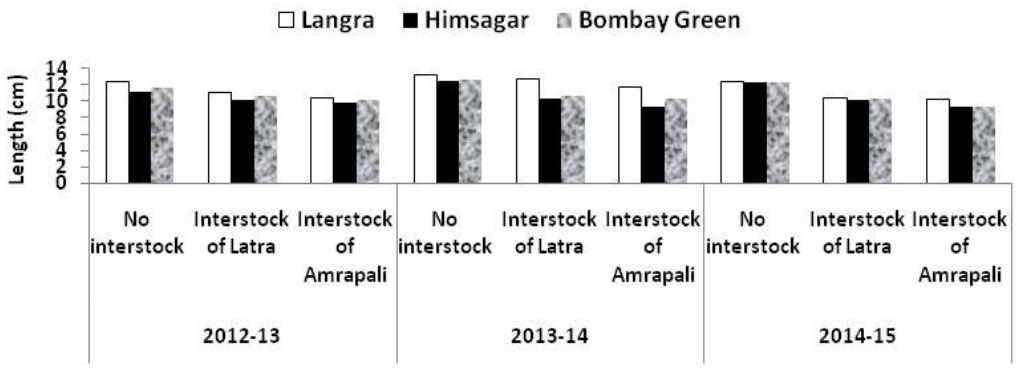

Figure 1: Effect of interstock on average length of shoot extension growth in mango.

trend was recorded in other interstock-scion combinations. Efficacy of interstocks in mango have been found to vary according to the rootstock-interstock-scion combinations [12,13] . Willis and Marler [14] have shown that rate of vegetative growth of mango appears to be more dependent on scion genotype than on the rootstock. Hence non-dwarfing effect of interstock as reported by different workers [9-11] can be attributed to incompatible combination of scion-interstock in their respective studies. Avila [7] have also reported reduction in tree height and canopy spread of 9 year old mango cv Manila on interstock of Thomas on rootstock of Esmeralda.

Tree crown weight is closely correlated with trunk girth or its crosssectional area and trunk measurements are the most commonly used method of estimating tree size. After four years of planting reduction in the growth rate of rootstock was recorded in of Mango cv Himsagar on interstock of Amrapali. Although the mechanism of stionic effects on rootstock has not been studied in mango, reduction in circumference of rootstock has also been reported by Yonemoto [8] by use of dwarf variety Khom as interstock. Use of interstock of Amrapali also resulted in decrease in the girth of scion of mango cvs Himsagar and Bombay Green. Reduction in the scion girth of mango with interstock has also been reported by Yonemoto [8]. Although the mechanism of action of interstock on scion has not been studied, Zhu [13] recorded variation in leaf nutrient composition in apple with different interstock. Jones [14] had reported depleted nutrient concentration in the xylem sap of apple plants with interstock which contributed towards the dwarfing effect of interstock. The same study also indicated production of growth inhibitors on the extending shoots from the interstock which contributed towards dwarfing in cherry.

Decrease in canopy size may result from reduction in shoot growth, changes in branching pattern and crop load. In mango, shoot growth is cyclic with distinct periods of extension followed by no extension [15] and the periods of extension is preceded by emergences of vegetative flushes. Yonemoto [8] have reported decrease in the shoot length of mango cultivars Irwin and Keitt by use of dwarf genotype Khom as interstock. Hence, reduction in number of flushing and average length of extension growth contributed towards reduction in the overall plant growth of mango cultivars Himsagar and Bombay

Green with interstock of Amrapali. Although, rootstocks which result in reduced annual shoot growth may not necessarily be dwarfing, if trees simply grow slowly but at maturity are of large size, the slow growth rate of shoots ensures merit by the ease in maintaining canopy size through pruning [16].

Conclusion

The study indicated decrease in the plant vigour of mango cultivars Himsagar and Bombay Green with the use of mango hybrid Amrapali as interstock whereas no such trend was observed in case of mango cultivar Langra. The reduction in the vigour due to interstock was apparent through decrease in the rate of increase in plant height, spread, girth of rootstock, interstock, scion and decrease in length of extension growth of shoot. Hence, use of interstock of Amrapali can be an effective strategy for reducing the plant vigour of mango cultivars Himsagar and Bombay Green under the eastern plateau and hill conditions of India.

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Singh et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00060.4

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Effect of Elevated Temperature on *In Vitro* Microtuberization of Potato Genotypes with Different Thermotolerance Levels

Anupama Singh*, Nimisha Kaushal, Reena Sharma, Vinay Bhardwaj, Brajesh Singh and Rajinder Singh

Abstract

Extension of potato cultivation to tropical areas has been constrained by the thermosensitivity of most cultivars which require low temperature during induction of and their subsequent development. Development of culivars for heat tolerance requires robust phenotyping methods for evaluating germplasm which is generally maintained as *in vitro* plant cultures. Microtuber production behaviour of five potato genotypes with contrasting themotolerance levels was studied to develop an *in vitro* assay system for screening potato genotypes for tolerance to high temperatures. Significant differences were observed with respect to tuber number, tuber weight and plant weight in all the genotypes at ambient (18°C) and elevated temperature (25°C). Kufri Himalini and CP4054 produced highest tuber number at 18°C and 24°C respectively. Although plant weight increased with an increase in temperature in all genotypes, total biomass increased only in tolerant genotypes. Heat tolerant genotypes exhibited comparable tuberization at both the temperatures whereas tuber formation was reduced in heat sensitive genotypes at elevated temperature. All genotypes formed micro tubers *in vitro* but only thermotolerant genotypes formed tubers in soil at elevated temperature, suggesting that mixotropic culture media overcome the effect of high temperature in the inhibition of tuberization indicating the need to use culture media devoid of hormones for developing a reliable *in vitro* assay system.

Keywords

Potato; Microtuberization; Thermotolerance

Introduction

Tuberization in potato is affected by several factors including light intensity, photoperiod, temperature and genotype [1]. However one of the major constraints in the tuberization process is its thermosensitivity in most potato cultivars limiting the extension of potato cultivation to tropical areas.

Under field conditions tuberization is reduced when night temperatures are above 20°C and there may not be any tuberization at 25°C or above [2]. To extend potato cultivation to relatively warmer areas where night temperatures are usually considerably higher than 20°C and in view of global climate warming, development of heat tolerant potato varieties needs urgent attention. Since there is considerable evidence for strong and consistent correlation between the formation of micro tubers under *in vitro* conditions and tuber formation under field conditions, for their structure as well as starch and protein composition [3], development of an *in vitro* assay system to evaluate the effect of high temperature on micro tuber formation will be highly desirable to facilitate early clone selection and considerably lessen the need for field evaluation of large number of genotypes under high night temperature conditions, thus accelerating breeding programs.

Under *in vitro* conditions, a temperature range of 20-25°C is optimum for plantlet growth whereas for micro tuber induction, the temperatures required are generally lower (15-18°C) [4,5]. Although much work has been done on the effect of day and night temperatures on the developmental physiology and growth of the potato crop, relatively little is known about the effects of temperature on micro tuber production in different potato cultivars.

In the present study we evaluated the effect of two temperature regimes on micro tuber formation in five heat tolerant and heat sensitive genotypes of potato to standardise an *in vitro* system for early selection of heat tolerant clones.

Material and Methods

Plant material

Tuber samples of three Indian cultivars; Kufri Surya (KS), Kufri Himalini (KH) and Kufri Chandramukhi (KCM) and two advanced heat tolerant clones from CIP (Lima, Peru); CP 4398 and CP 4054, were drawn from the National Potato Breeding Program at the Central Potato Research Institute, for the present study (Table 1).

Well sprouted tubers of similar size were planted in potting mixture in 15 cm pots with five replications per genotype. Plants were grown for a month under non tuberizing conditions at 24°C under continuous light (600 $\mu Es^{-1}m^{-2}$) in a controlled environment chamber (Conviron, Model E-15, Canada). After 30 days, the plants were subjected to day/ night temperature treatments of 18/18°C and 24 /24°C with 12 h photoperiod.

Establishment of axenic cultures

Single nodal cuttings, 0.5 to 1.0 cm long, were obtained from etiolated sprouts of each cultivar/clone. These were disinfected with a mixture of 0.1% $HgCl_2$ and 0.1% sodium lauryl sulphate for 5 min, washed thrice with sterile water and cultured aseptically in test tubes with one segment per tube [1]. Each tube contained approximately 15 ml of semisolid (7 g agar L^{-1}) MS basal medium [6] with 3% sucrose. The cultures were incubated under 16 h/ day photoperiod of 38 µmol m^{-2} s^{-1} and at day and night temperatures of 22 ± 2°C.

Microtuberization

Eight week old axenic plantlets were sub cultured aseptically in 250 ml Erlenmeyer flasks containing 25 mL liquid MS medium with 3% sucrose, each flask containing 10 nodal cuttings. The cultures were incubated under 16 h/ day photoperiod of 38 µmol m^{-2} $s^{-1}$and at day and night temperatures of 22 ± 2°C for 30 days. For tuber induction, the culture medium was replaced with MS medium containing 8% sucrose and 10 mg L^{-1} BAP. Flasks were incubated in the dark at

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Received: July 05, 2016 **Accepted:** September 08, 2016 **Published:** September 11, 2016

two temperatures: 18°C and 25°C The experiment was conducted in a randomized complete block design (5 genotypes × 2 temperatures) with 4 replications. Each replication consisted of one flask with 10 nodal sections. Thus there were 40 plantlets per genotype per culture condition.

Data recording and statistical analysis

After sixty days of incubation in tuber induction media plantlets were taken out of the culture vessels, the medium was rinsed off and data were recorded for number, weight and total biomass of micro tubers. As the aim of the study was to compare the effect of temperature on microtuberization, the fixed effect model was used (for what?)

Results and Discussion

Microtuber formation occurred in all the genotypes at both the temperatures. The time required for micro tuber induction was almost the same for all genotypes but differed at two temperature regimes (Figure 1). Tuber formation was induced after 5 days at 25°C and after 8 days at 18°C. However, only three genotypes formed tubers when potted plants were given heat stress at 24°C with 12 h photoperiod (Figure 2).

Analysis of variance showed that mean squares due to genotypes for all parameters were significant at $p \leq 0.05$, indicating genetic variability among the genotypes for micro tuber number, weight, plantlet weight and total biomass. However, mean squares due to treatment were significant only for tuber number and average tuber weight, suggesting that the temperature influenced only these two attributes. These results are similar to those observed under field conditions, where moderately elevated temperature affects tuberization only and not the haulm growth [7]. The significant effect of genotype x treatment interactions for all the characters indicated differential response of genotypes to low and elevated temperatures (Table 2).

KH produced maximum number of tubers at lower temperature followed by KCM (Figure 3). At elevated temperature, tuber number was highest for CP 4054. Number of tubers was reduced significantly at elevated temperature in KH, KCM and KS. At 18°C KH had the highest average tuber weight and total tuber weight per flask, which was reduced significantly at elevated temperature. The tolerant genotypes showed an increase in tuber weight per flask at 25°C as compared to 18°C, with CP 4054 recording the highest tuber weight per flask as well as average tuber weight. These findings suggest

Table 1: List of potato clones used for in vitro heat tolerance assay.

Clone selection code	Variety/ CPRI Accession No.	Pedigree/Parentage	Heat tolerance
HT/92-621	Kufri Surya	Kufri Laukar x LT-1	Tolerant
SM/91-1515	Kufri Himalini	I-1062(E) X Bulk pollen	Sensitive
A-2708	Kufri Chandramukhi	Seedling 4485 X Kufri Kuber	Sensitive
CIP-397068.28	CP 4054	C90.266 X C93.154	Tolerant
CIP 304394.56	CP 4398	Shepody X LR 93.050	Tolerant

Figure 1: Tuber formation in potted plants 21 d after heat stress at 24°C in controlled environment chambers. (a) CP 4054 (b) CP 4398 (c) Kufri Surya (d) Kufri Chandramukhi (e) Kufri Himalini. Well sprouted tubers of similar size were planted in potting mixture in 15 cm pots with five replications per cultivar for each experiment. Plants were grown under non tuberizing conditions at 24°C under continuous light (600 $\mu Es^{-1}m^{-2}$) in a controlled environment chamber (Conviron, Model E-15, Canada). After 30 days, the plants were subjected to heat stress treatment of (24 /24 °C) with 12 h photoperiod. Tuber formation was observed 21 days after stress.

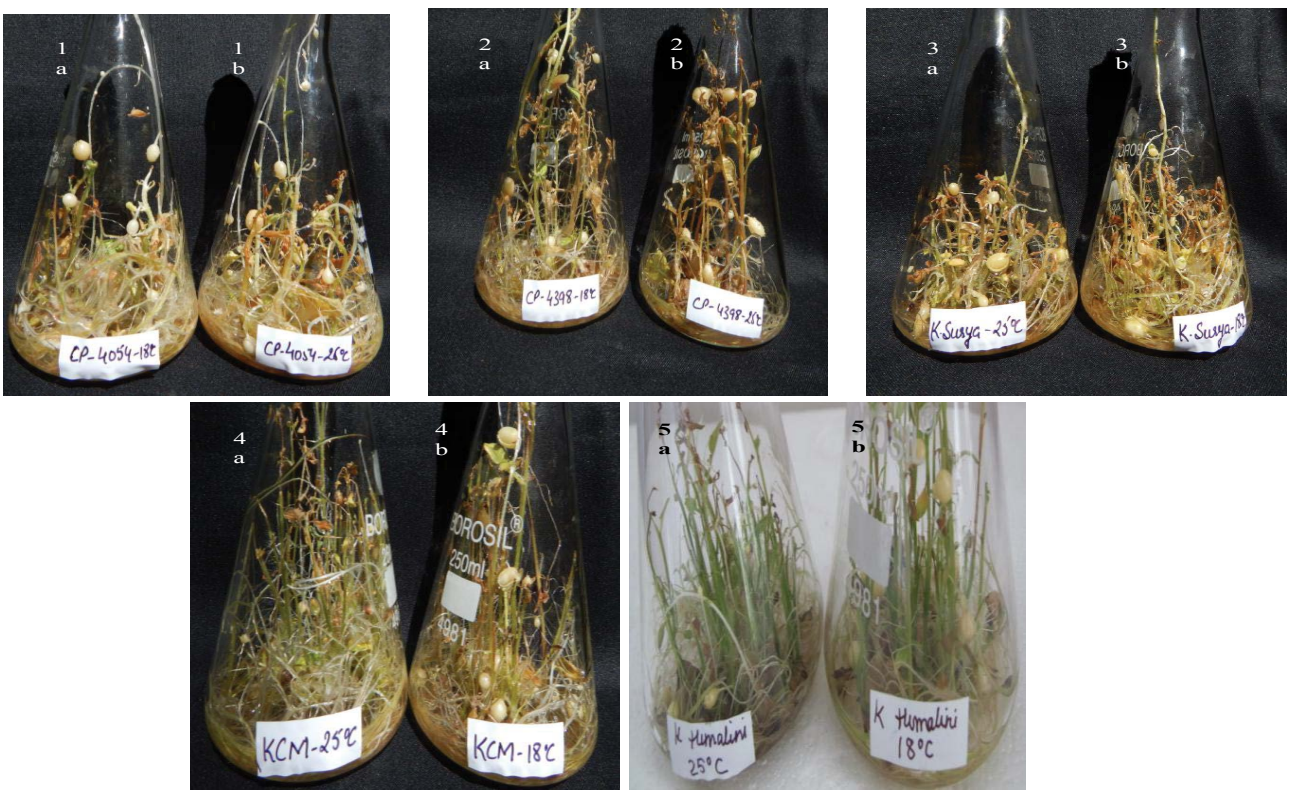

Figure 2: Microtuberization in heat tolerant and susceptible potato genotypes. 1. CP 4054 at (a) 18°C (b) 25 °C. 2. CP 4398 at (a) 18°C (b) 25°C .3. Kufri Surya at (a) 25°C and (b) 18 °C. 4. Kufri Chandramukhi at (a) 25°C and (b) 18 °C. 5. Kufri Himalini at (a) 25°C and (b) 18 °C.

Table 2: Analysis of variance of *in vitro* performance of 5 genotypes for various characters.

		Tuber number (per flask)	Tuber yield (mg per flask)	Plant weight (mg)	Total biomass (mg)	Average tuber weight (mg)
Source of Variation	df	MS				
Genotypes	4	23.65*	*0.86	10.289*	15.95*	*0.0039
Treatments	1	6.40*	0.009	0.139	0.223353	*0.0019
Interaction	4	24.27*	*1.44	1.635*	4.77*	*0.0041
Error	30	1.3	0.009	0.171	0.15629	0.0002
Total	39					
CD(G)		1.162	0.096	0.422	0.403242	0.013
CD(t)		0.735	0.061	0.267	0.255033	0.008
CD(GXT)		1.644	0.136	0.596	0.570271	0.018

* Significant at P<0.05

that the *in vitro* plantlets exhibited a similar level of tolerance to elevated temperature with respect to tuber induction as under natural conditions. Similar results were reported by Khan et al. [8] where *in vitro* assay of heat tolerant breeding population positively correlated to tuber family evaluation in field conditions in a semiarid tropical environment.

CP 4054 exhibited maximum fresh plant weight and total biomass both at 18°C and 25°C. Plant weight was higher at elevated temperature in all the genotypes except KCM. However, total biomass increased only in the tolerant genotypes KS, CP 4054 and CP4398 at 25°C. These results indicate that higher temperature has a greater influence on substrate partitioning in sensitive genotypes than in tolerant genotypes. Induction of tuberization even in heat sensitive genotypes at elevated temperature may be due to externally supplied BAP. Promotion of tuberization on cultured shoots by cytokinin as been demonstrated by many workers [1,5,9]. According to Garner and Blake [10] innate physiological responses may be evaluated best in growth regulator free culture media, when culture conditions mimic the major components of field environment. Therefore, standardizing *in vitro*

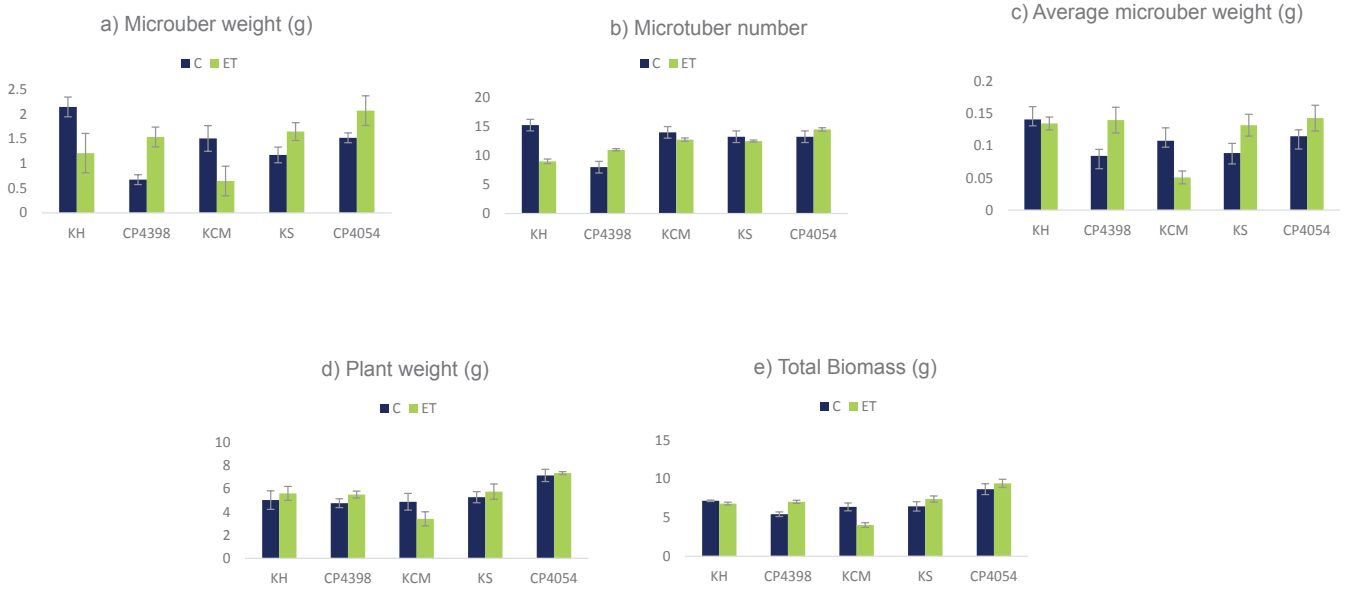

Figure 3: Effect of temperature on a) microtuber weight b) microtuber number c) average microtuber weight d) microplant weight e) total biomass in five potato genotypes.

assays for tuber induction in hormone free media may better reflect actual differences in thermotolerance in various genotypes [11].

Acknowledgements

The present study was supported by DST (WOS-A) grant no SR/WOS-A/LS-166/2012 and DST (SERB) grant no. YSS/2015/001995 from Department of Science and Technology, Government of India, to AS. The overall financial assistance and infrastructure provided by Indian Council of Agricultural Research is duly acknowledged.

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Shantharaja et al., Vegetos 2016, 29:2
10.5958/2229-4473.2016.00061.6

Vegetos- An International Journal of Plant Research

Research Article

A SCITECHNOL JOURNAL

Effect of Sowing Dates, Plant Density, Seed Treatment and Fertilizers on Performance and Quality Seed Production in Mungbean [*Vigna Radiata* (L.) Wilczek]

Shantharaja CS*, Mahajan SS, Rajora MP and Bhatt RK

Abstract

Variation in agro-ecological conditions due to climate change encourages modern research approaches to optimise the time for sowing, plant density and use of seed treatment and fertilizers to maximise the seed yield of Mungbean. It may vary from variety to variety and season to season. Therefore, an experiment was conducted in mungbean (var. SML 668) comprising three dates of sowing (July 15, August 1 and 16), two plant geometry (25 x 10 cm) and (30 x 10 cm) and six combinations of seed treatments and fertilizers, control (No fertilizer application), recommended dose of fertilizer (RDF) as basal dose (12.5 kg/ha N and 40 kg/ha P_2O_5), seed inoculation with *Rhizobium* and phosphate solubilizing bacteria (PSB), seed inoculation with *Rhizobium* and PSB + RDF as basal dose, seed inoculation with *Rhizobium* and PSB + 50 % more RDF as basal dose and seed inoculation with *Rhizobium* and PSB + RDF as basal dose + Borax spray (100 ppm) at flower initiation during *Kharif* 2013 and 2014 at CAZRI, Jodhpur. Studies revealed that delayed sowing in August reduced seed yield and affected yield attributes (plant height, pods/plant, seeds/pod, seed yield/plant and 1000-seed weight). Early sowing (15th July), wider spacing (30 x 10 cm) and recommended dose of fertilizer + seed treatment with *Rhizobium* and PSB + Borax spray (100 ppm) at flower initiation enhanced the seed yield and yield attributes in mungbean.

Keywords

Mungbean; Plant density; Seed production; Seed treatment; Seed quality

Introduction

Among the *kharif* pulses, mung bean is an important pulse crop grown in most of the tropical and sub-tropical parts of the world. It is also grown extensively in India under varying soil types and climatic conditions. It is a short duration crop that can be grown over a range of environments. In Rajasthan, it is mostly grown under rain fed condition in 7.59 lakh ha area with total production of 2.71 lakh tones. The average productivity of mungbean is 357 kg/ha which is very much low as compared to other mungbean growing states in the country (2006-07). Traditional method of cultivation practices adopted by most of the farmers is one of the reasons for low productivity in these areas. Moreover, yield barriers due to different genetic, physiological, biochemical constitutions and lack of required packages of practices required during the critical stages of crop growth posed a big challenge before suppliers to provide good quality seed of improved variety at reasonable price to the farmers. The development of high yielding varieties and better management practices recommended during recent years anticipated much scope for further increase in seed yield. Available technologies indicated that timely sowing at the proper plant densities along with application of recommended doses of fertilizer; microbial seed inoculants and foliar spray with micronutrients have been found to overcome such bottlenecks to a considerable extent in pulses. However, changing climatic conditions and untimely rains varying from July to August or its further delay in the area encourages adoption of different technological approaches for harnessing the true potential of the crops. Such studies are meagre in mung bean and particularly in arid zone of Rajasthan. The present study was therefore undertaken to study the effect of different sowing time, plant geometry, recommended dose of fertilizer, seed treated with *Rhizobium* and Phosphate Solubilizing Bacteria (PSB) alone or in combination and boron spray prior to flowering for optimising improvement of seed yield and seed quality parameters in mung bean.

Material and Methods

A field experiment comprising three dates of sowing, 15th of July, 1st of August and 16th of August; two crop geometry, (25 × 10 cm) and (30 × 10 cm) and six combinations of seed treatments and fertilizers, Control (No Fertilizer Application) (F_1), Recommended dose of Fertilizer (RDF) as basal dose (12.5 kg/ha N and 40 kg/ha P_2O_5) (F_2), Seed inoculation with Rhizobium and Phosphate Solubilizing Bacteria (PSB) (F_3), Seed inoculation with Rhizobium and PSB + RDF as basal dose (F_4), Seed inoculation with Rhizobium and PSB + 50 % more RDF as basal dose (F_5) and Seed inoculation with Rhizobium and PSB + RDF as basal dose + Borax spray (100 ppm) at flower initiation (F_6) was conducted in Factorial Randomized Block Design with three replications during *Kharif* 2013 and 2014. The mungbean variety SML 668 was sown in flat bed of plot size 2.5 x 5 m on sandy soil of the institute farm at CAZRI, Jodhpur. Weeding management was done by spraying Stomp 30 EC @ 2.5 lit/ha as pre-emergence using 500 lit/ha water followed by two hand weeding. The experiment was conducted in rain-fed conditions but irrigation with sprinkler was provided whenever the shortage of rains observed and required for the crop growth.

The seeds were inoculated with Rhizobium and Phosphate Solubilizing Bacteria @ 4 g/kg seed, dried for 30 minutes in shade followed by sowing in the field. The required quantity of fertilizers in the form of Urea and DAP for the plot size was applied and mixed in the soil before sowing. The data was recorded for plant height at 30 and 60 days after sowing, flowering days, pods per plant, pod length, seeds per pod, seed yield per plant, seed yield per square meter, 1000-seed weight and harvest index. The seed quality attributes were recorded in the laboratory for seed germination as per standard procedure and vigour indices were calculated by multiplying normal

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Received: June 18, 2016 **Accepted:** July 05, 2016 **Published:** July 10, 2016

seedling percentages with seedling length and seedling dry weight. Data were evaluated by split-plot analysis of variance (ANOVA) by using the SPSS program.

Results and Discussion

The data revealed that dates of sowing, crop geometry and fertilizers and seed treatments had significant influence on plant growth characteristics, seed yield and seed quality (Table 1 and 2).

The early sowing (15th July) recorded maximum plant height at 30 DAS & 60 DAS during both the years. Days to flowering were earlier in 16th Aug sowing during 2013, but it was 15th July during 2014. No. of pods per plant was maximum for 16th Aug. sowing in 2013 whereas, during 2014 early sowing recorded maximum No. of pods per plant (Table 1). Khairnar et al. [1] evaluated 22 genotypes of mungbean under condition of the kharif season; they found wide variability in most of yield components and grain yield per ha. The characters viz., pod length; seeds per pod, seed yield per unit area and harvest index were significantly higher in early sowing during both the years (Table 1). Over all the early sowing, 15th July found to be better sowing date for better seed yield. Boe et al. [2] recorded that delayed mungbean planting date from May to July produced forage higher with 2.2 ton/ha Under Egyptian condition. Similarly, Ashour et al. [3] and El-Kramany [4], recorded that some genotypes of mungbean gave a suitable vegetative growth and yields when sown lately around mid of July.

Effect of crop geometry was found non-significant for most of the characters studied except, No. of pods per plant (Table 1) [5]. Over all wider spacing (30 x 10 cm) recorded higher values for the most of the seed yield attributing characters and yield compare to narrow spacing (25 x10 cm) during both the years.

Fertilizer treatments had significant effect on no. of pods and seed yield during 2013 and 2014. Seed treated with PSB + RDF as basal dose + Borax spray (100 ppm) at flower initiation (F_6) recorded maximum no. of pods (20.1), pod length (8.8 cm) during 2014. Similarly, seeds per pod (8.9 & 8.7) and seed yield (71.9 & 227.6 g/m^2) during both the years [6,7]. The characters, no. of pods per plant were shown significant during 2013 and 2014 with F_5 and F_6 treatments respectively. Plant height at 30 DAS & 60 DAS was recorded maximum for the treatment F5 during both the years (Table 1). Seed treatment with rhizobium + Phosphate Solubilizing Bacteria (PSB)

Table 1: Effect of dates of sowing, crop geometry and fertilizers on plant characters, seed yield and its components in mungbean.

Treatments	Plant height at 30 DAS (cm)		Plant height at 60 DAS (cm)		Days to flowering		No. of Pods/ plant		Pod Length (cm)		Seeds/pod (Nos.)		Seed Yield/Sq. m (g)		Harvest Index	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Date of Sowing (D)																
15th July	31.1	27.8	40.1	51.2	27.8	31.7	11.9	20.1	9.1	8.8	9.3	9.2	78.8	223.4	0.44	0.56
1st August	16.1	20.3	25.1	46.3	28.9	35.6	8.8	16.4	8.6	8.5	9.3	8.5	52.8	189.8	0.35	0.51
16th August	25.3	22.1	27.5	48.2	27.4	34.5	13.7	16.0	7.9	8.1	7.8	7.9	45.8	219.5	0.31	0.51
S.Em±	0.40	0.81	0.60	1.36	0.20	0.14	0.4	0.57	0.1	0.06	0.1	0.09	2.7	6.51	0.01	0.01
CD at 5%	1.30	2.27	1.70	3.83	0.50	0.40	1.1	1.60	0.3	0.16	0.4	0.27	7.7	18.37	0.03	0.02
Crop Geometry (G)																
25 × 10 cm	24.0	23.4	30.4	46.8	28.3	33.9	10.3	15.8	8.5	8.4	8.7	8.3	57.4	203.8	0.37	0.52
30 × 10 cm	24.3	23.3	31.5	50.4	27.8	34.0	12.7	19.1	8.6	8.6	8.8	8.7	60.8	218.1	0.36	0.54
S.Em ±	0.40	0.66	0.50	1.11	0.20	0.11	0.3	0.46	0.1	0.05	0.1	0.08	2.2	5.32	0.01	0.01
CD at 5%	NS	NS	NS	3.13	NS	NS	0.9	1.31	NS	0.13	NS	0.22	NS	NS	NS	0.02
Fertilizers (F)																
F1	22.4	21.8	28.0	46.2	28.8	34.2	10.9	14.9	8.4	8.2	8.7	8.3	49.0	188.7	0.38	0.50
F2	23.6	23.5	30.5	48.0	28.1	34.1	11.4	17.5	8.3	8.4	8.7	8.4	54.3	211.8	0.35	0.54
F3	23.2	23.1	29.8	47.1	28.3	34.1	12.0	15.7	8.4	8.5	8.8	8.6	50.9	200.5	0.34	0.52
F4	25.1	23.8	31.7	48.7	27.8	33.8	11.4	18.1	8.6	8.5	8.8	8.6	65.2	214.4	0.37	0.51
F5	25.6	24.4	33.2	51.1	27.3	33.9	12.8	18.7	8.7	8.5	8.7	8.7	63.2	222.6	0.38	0.56
F6	25.2	23.6	32.4	50.4	27.9	33.4	10.5	20.1	8.7	8.8	8.9	8.7	71.9	227.6	0.38	0.55
S.Em±	0.60	1.14	0.80	1.92	0.30	0.20	0.56	0.80	0.2	0.08	0.2	0.13	3.9	9.21	0.02	0.01
CD at 5%	NS	3.22	2.3	NS	0.80	NS	1.59	2.27	NS	0.23	NS	NS	10.9	28.38	NS	0.03
Interactions																
D x G	NS	3.22	NS	NS	NS	NS	NS	2.27	NS	NS	NS	NS	NS	NS	NS	NS
D x F	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
G X F	NS	NS	3.3	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.06	NS
D X G X F	NS	NS	NS	NS	NS	NS	3.89	NS	NS	NS	NS	NS	NS	NS	NS	NS

F1 - Control (No Fertilizer Application); F2 - Recommended dose of Fertilizer (RDF) as basal dose (12.5 kg/ha N and 40 kg/ha P2O5); F3 – Seed treated with Rhizobium and Phosphate Solubilizing Bacteria (PSB); F4 - Seed treated with PSB + RDF as basal dose; F5 - Seed treated with PSB + 50 % more RDF as basal dose; F6 - Seed treated with PSB + RDF as basal dose + Borax spray (100 ppm) at flower initiation

Table 2: Effect of dates of sowing, crop geometry and fertilizers on seed quality attributes in mungbean.

Treatments	Test weight (gm)		Seed Germination (%)		Vigour Index I		Vigour Index II	
	2013	2014	2013	2014	2013	2014	2013	2014
Date of Sowing (D)								
15th July	59.5	71.1	74.9	88.1	2798	2844	2435	2760
1st August	52.5	63.1	80.3	92.6	2920	2655	2787	2679
16th August	45.7	66.0	74.5	96.3	2582	2545	1916	2963
S.Em±	0.57	0.43	1.2	0.91	77	61	166	48
CD at 5%	1.61	1.23	3.4	2.56	218	173	467	134
Crop Geometry (G)								
25 × 10 cm	52.4	65.7	75.8	90.9	2737	2569	2212	2642
30 × 10 cm	52.7	67.8	77.3	93.7	2796	2793	2547	2959
S.Em±	0.47	0.35	1.0	0.74	63	50	135	39
CD at 5%	NS	1.0	NS	2.09	NS	142	NS	109
Fertilizers (F)								
F1	50.7	65.1	73.7	87.9	2600	2315	2028	2405
F2	53.2	66.1	78.2	93.6	2877	2707	2420	2624
F3	51.2	66.2	74.9	91	2643	2672	2364	2678
F4	52.6	67.2	74.5	93	2689	2742	2141	3034
F5	53.3	67.8	79.7	94.4	2954	2843	2810	3090
F6	54.5	67.9	78.4	94	2836	2808	2512	2971
S.Em±	0.81	0.61	1.7	1.29	109	87	234	67
CD at 5%	2.28	1.73	NS	3.63	NS	245	NS	190
Interactions								
D x G	2.3	NS	NS	NS	NS	NS	NS	190
D x F	NS	NS	NS	NS	NS	NS	NS	NS
G X F	NS	NS	NS	NS	NS	NS	NS	NS
D X G X F	NS	NS	NS	NS	NS	NS	NS	NS

F_1 - Control (No Fertilizer Application); **F_2** - Recommended dose of Fertilizer (RDF) as basal dose (12.5 kg/ha N and 40 kg/ha P_2O_5); **F_3** – Seed treated with Rhizobium and Phosphate Solubilizing Bacteria (PSB); **F_4** - Seed treated with PSB + RDF as basal dose; **F_5** - Seed treated with PSB + 50 % more RDF as basal dose; **F_6** - Seed treated with PSB + RDF as basal dose + Borax spray (100 ppm) at flower initiation.

+ RDF as basal dose+ borax spray (100 ppm) at flower initiation had maximum seed yield produced highest yield (71.9 and 227.6 g/m^2) followed by Rhizobium and PSB + RDF as basal dose and seed treated with Rhizobium and PSB + 50% more of RDF (Table 1).

The seed quality parameters like test weight, germination % and vigour Index I & II were found significant for date of sowing. Early sowing recorded highest test weight for both the year [8]. Whereas late sowing (1st Aug & 16th Aug) recorded maximum germination % during 2013 and 2014 respectively and similar results were shown for vigour indices also (Table 2). Effect of crop geometry on seed quality traits shown that, the wider spacing (30 x 10) recorded maximum values for all the seed quality traits during both the years (Table 2).

Seed treated with PSB + 50 % more RDF as basal dose (F_5) recorded maximum values for seed germination (79.7 & 94.4) % and vigour index I (2954 & 2843) and vigour index II (2810 &3090) followed by Seed treatment with rhizobium + Phosphate Solubilizing Bacteria (PSB) + RDF as basal dose + borax spray (100 ppm) at flower initiation (Table 2) [9].

From the results, it can be concluded that early sowing (15th July), wider spacing (30 x 10 cm) and recommended dose of fertilizer + seed treatment with Rhizobium and PSB + Borax spray (100 ppm) at flower initiation were the better treatments for increased seed yield in mung bean.

Acknowledgement

The authors would like to acknowledge Indian Council for Agricultural Research (ICAR) for financial funding through AICRP-NSP (Crops) Project.

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Roy et al., Vegetos 2016, 29:2
10.5958/2229-4473.2016.00062.8

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Differential response of Germplasm to Inter-Specific Hybridization of Brassica spp.

Chandan Roy*, Jha RN, Chandan Kishore, Singh PK and Tomar JB

Abstract

Understanding the fact behind the incompatible reaction in inter specific crosses is important for attempting successful crosses. Inter-specific hybridization between *Brassica juncea* and *Brassica oleracea var botrytis* were developed using reciprocal crosses. Four varieties of Indian mustard namely Varuna, Kranti, Pusa Bold and Rajendra Suflam were crossed reciprocally with one Cauliflower variety Sabour Agrim. Several barriers were observed right from the pre-fertilization to post fertilization during embryo developmental stages. 78.26%, 64.96% and 63.68% siliqua was retained after 6 days, 10 days and 15 days of pollination respectively when *B. juncea* was used as female parent whereas 99.35%, 45.51% and 43.22% of siliqua were retained after 6 days, 10 days and 15 days of pollination respectively when *Brassica oleracea* var *bortytis* was used as female parent. Both pre and post fertilization barriers were predominantly found when *B. juncea* was used as female parent whereas post fertilization barriers were found in the reciprocal crosses. Differential varietal response to siliqua drops was observed in the reciprocal crosses. Maximum response in the success of crosses was obtained for variety Kranti in B. juncea X B oleracea cross whereas variety Rajendra suflam was highly efficient in reciprocal crosses.

Keywords

Distant hybridization; Cauliflower; Indian mustard; *B. juncea*; *B. oleracea*

Introduction

There are six cultivated species in Brassica genus among which *Brassica oleracea* is vegetable crop species adapted to cooler region. Whereas, *Brassica juncea* is amphidiploids species, covered 80% of the total areas under oilseed crop in India. Several important traits have been introgressed from diploid progenitors of Brassica to amphdiploids like self incompitability [1,2] and cabbage aphid (*Brevicoryne brassicae*) resistance from *B. oleracea* to *B. napus*. Re-synthesis of tetraploid Brassica species using diploid proginators was done decade before for better insight of their genomic relationships [3]. Several pre and post fertilization barrier was reported in this process and few techniques like ovary or embryo culture has got a level of success. In a cross between Brssica napus and B rapa; Janetta et al., [4] identified pollen tubes penetrates into the ovule after 48 hrs of pollination [5]. It has been reported that embryo development starts immediately after fertilization in Brassica sp. Peng and Yu [6] isolated multicelluar embryos at 5 DAP in the wide crosses between Chinese cabbage and Kale. Limited reports are available about the crosses between *Brassica juncea* and *Brassica oleracea var botrytis*. Success up to 25 percent and 9% were reported using embryo culture technique when *Brassica juncea* and *Brassica oleracea* respectively used as female parent [7]. Inter-specific gene transfer in Brassica sp. was carried out for several genes but transfer of genes between *Brassica juncea* and *Brassica oleracea var botrytis* is very limited. Resistance to several diseases and pests are not available in C genome of Brassica that could be exploited through inter-specific hybridization. One of the limitations of such application is lack of understanding for crossability barriers between two species. That's why it is important to know the correct stage of barriers during fertilization process for increasing the efficiency of hybrid development between two species. Present investigation was carried out to find out the most commonly occurring hybridization barriers in two species.

Materials and Methods

The experiment was conducted in protected conditions under poly house conditions at Bihar Agricultural University, Sabour, Bhagalpur in Complete Randomized Design with three replications. Sowing was done at two different dates for coinciding the flowering period. Four varieties of Indian mustard namely Varuna, Kranti, Pusa Bold, Rajendra Suflam were crossed reciprocally with the recently released tropical Indian cauliflower variety Sabour Agrim. Flower buds which would be opening in the next two days were bagged using butter paper bag and emasculated. Rest of the flower buds was removed. Similarly, the inflorescence of male plants was also bagged before flowering. At time of pollination, freshly opened flowers were collected and used for pollination to the female flowers. After pollination the inflorescence was bagged using butter paper bag. Data recording started at different days after pollination (DAP) as percent of siliqua retained after pollination. Flower bud retention after pollination at 3 days, 6 days, 10 days, 15 days and 20 days after pollination (DAP) were recorded. In the present study retention of flower buds on or before 3 DAP was considered as pre fertilization barriers and after that as post-fertilization barriers. Multi-cellular embryos were detected after 5 days of pollination by Peng and Yu [6].

Results and Discussion

A total of 546 reciprocal crosses were attempted among which 391 crosses were made for *Brassica juncea X B oleracea var botrytis* and 155 were for reciprocal crosses. Progress of flower buds for the development of embryos after pollination was taken as effective measure for studying the fertilization barriers. Significant differences were observed between the reciprocal crosses for percent retention of siliqua at different dates after pollination. Maximum success was obtained where *B. juncea* was used as female parent. No siliqua were shattered three days after pollination. Maximum reduction of siliqua was observed at 6 days after pollination. However, no significant differences were found beyond 15 days of pollination. 99.74% siliqua retained three days after pollination. Siliqua drop drastically increased at six days and 10 days after pollination where only 78.26% and 64.96% siliqua were retained at respective days after pollination (Figure 1). Barriers at fertilization or immediately after fertilization

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Received: June 18, 2016 **Accepted:** July 05, 2016 **Published:** July 11, 2016

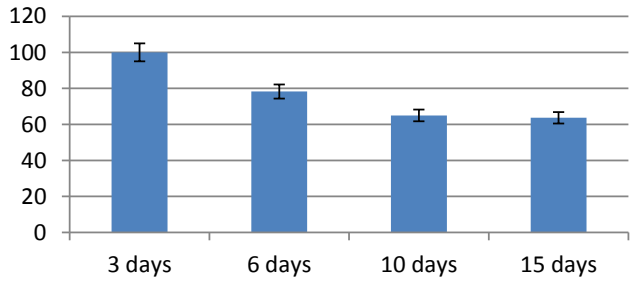

Figure 1: Percent retention of siliqua at different days after pollination (DAP) in *Brassica juncea X B. oleracea* cross.

at embryonic developmental stage were most prominent. *Sarmah and Sarla* [8] *reported pre-fertilization barriers in the crosses between Diplotaxis siettiana* and *Brassica juncea which could be overcome using irradiated pollen of Diplotaxis siettiana* by gamma rays where 5 day after pollination. Seventeen percent ovules showed entry of pollen tubes, ten days after pollination 27% ovules showed small globular embryos which did not grow further.

Peng and Yu [6] harvested multicellular embryos five days after pollination in the cross between Chinese cabbage and Kale, which showed maximum reduction due to lack of fertilization. Inter-specific crosses between *B. juncea* and *B. oleracea* were reported by Weerakoon et al. [7] observed success rate of pod formation by 25%. Success between tetraploid and diploid species is very less and to avoid crossibaility barriers it was suggested to go for ovule or embryo rescue techniques. Application of ovule culture technique was reported to increase the efficiency of BC_1 hybrids (*B napas* X *B. oleracea*//*B.oleracea*) 10 fold over the *in vivo* set [9].

In the reciprocal crosses, the drop of siliqua at three and six days after pollination was not observed. However, maximum drop was found at 10 DAP following which the rate of siliqua drop was markedly reduced. It clearly indicated the post fertilization barriers were prominent in such crosses. 99.36% siliqua were present at three as well as six days after pollination respectively but at 10 DAP and 15 DAP number of siliqua were retained 45.51% and 43.22% respectively (Figure 2). Peng and Yu [6] rescued embryo to develop inter-specific hybrids between *B. campestris* var *pekinensis* and *B. oleracea* var *acephale.*

Varietal interactions in reciprocal crosses were found significant. Maximum drop of siliqua was observed in the variety Pusa Bold at 6 DAP which was minimum in variety Kranti (Table 1). At 10 DAP the trend changed as maximum drop of siliqua was observed for variety Varuna (upto 30%) compared to minimum flower drop for Kranti and Pusa Bold (nearly 10%). It reflected that varietal response for fertilization barrier was different. Pre fertilization barrier was found higher in variety Pusa bold whereas post fertilization barrier is strong in variety Varuna. Among all the crosses maximum success was observed when variety Kranti used as female parent followed by using variety Rajendra Suflam. Hybrid seed was obtained in all the four crosses of *B. juncea* X *B. oleracea* var *botrytis*. However, the percentage of success was very low in all the four crosses. To increase the efficiency of hybrids produced in the crosses between *B. juncea* X *B. oleracea* var *botrytis*, embryo culture should be adopted. In the cross between *B. juncea* and *B. nigra,* Bhatt and Sarla [10] also reported both pre and post fertilization barrier in interspecific hybridization. Bud pollination and stump pollination along with ovary-ovule culture technique may be helpful in recovery of hybrids plants. The hybrid seed developed in the present study were sown and characterized morphologically along with their parents to confirm hybridity (Figure 3).

In the reciprocal crosses also varietal response towards the fertilization barrier was markedly differed. No significant difference was observed at three and six days after pollination (Table 2). However, drastic reduction of number of siliqua at 10 DAP was

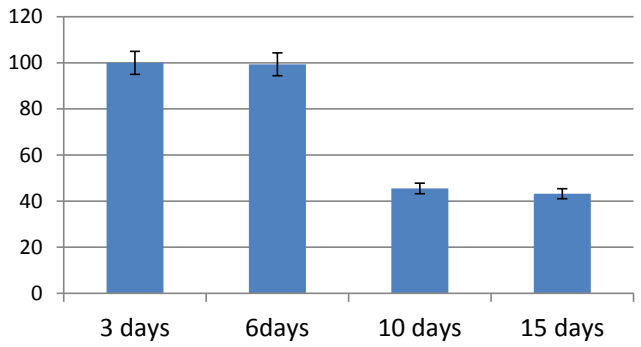

Figure 2: Percent retention of siliqua at different days after pollination (DAP) in *Brassica oleracea* var *botrytis X B. juncea* cross.

Table 1: Varietal responses for percent retention of siliqua in the cross of *B. juncea X B. oleracea.*

Varieties used as female parent	Total number of cross attempted	Number of siliqua retained/ percentage				Hybrid seed produced
		3DAP	6DAP	10DAP	15 DAP	
Varuna	98	98	84	54	54	2
Percentage (%)		**100**	**85.71**	**55.10**	**55.10**	**0.02**
Kranti	95	95	82	73	73	12
Percentage (%)		**100**	**86.31**	**76.84**	**76.84**	**0.12**
Pusa Bold	98	98	68	58	58	5
Percentage (%)		**100**	**69.38**	**59.19**	**59.19**	**0.05**
R. Suflam	100	100	78	65	64	14
Percentage (%)		**100**	**78**	**65**	**64**	**0.14**
CV (%)	**1.32**					
$CD_{0.05}$ (Variety X Days)	**1.59**					

Table 2: Varietal responses for percent retention of siliqua in the cross of *B. oleracea* X *B. juncea.*

Varieties used as male parent	Total number of cross attempted	Number of siliqua retained/ percentage			
		3DAP	6DAP	10DAP	15 DAP
Varuna	37	37	37	7	6
Percentage (%)		**100**	**100**	**18.91**	**16.21**
Kranti	38	38	38	18	18
Percentage (%)		**100**	**100**	**48.64**	**48.64**
Pusa Bold	40	40	40	22	22
Percentage (%)		**100**	**100**	**55**	**55**
R. Suflam	40	40	39	25	22
Percentage (%)		**100**	**97.5**	**62.5**	**55.55**
CV (%)	**1.79**				
$CD_{0.05}$ (Variety X Days)	**1.94**				

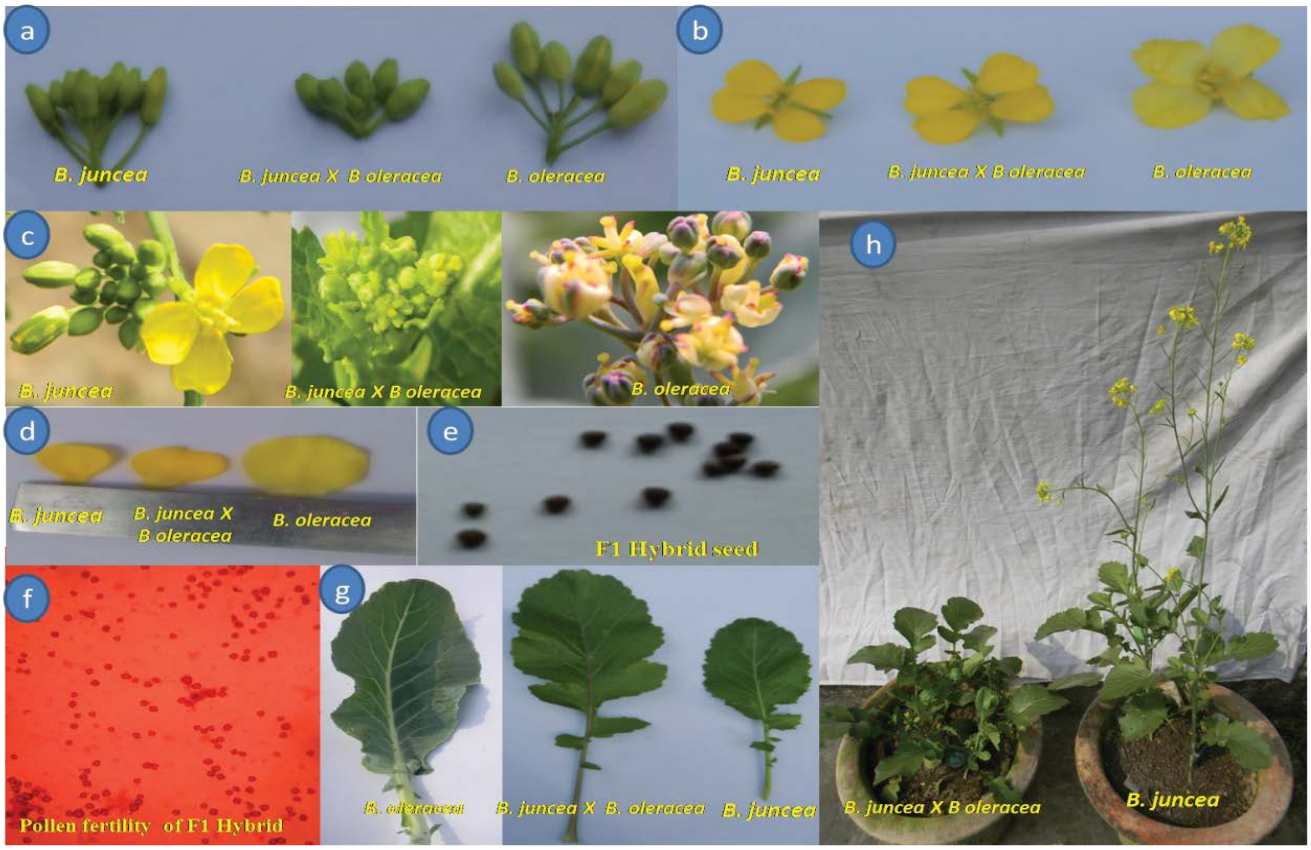

Figure 3: Comparison of F_1 hybrids (*B. juncea* cv. Rajendra Suflam L. X *B. oleracea* cv S. Agrim L.) with their parents for different characters. a. shape of flower bud; b. color and shape of petal; c. occurrence of deformities in flower buds; d. shape of flower petal; e. F_1 hybrid seed; f. F_1 pollen fertility status after colchicines treatment; g. leaf shape h. plant height during flowering.

observed in all the crosses followed by a mild reduction at 15 DAP pollination. At 10 DAP siliqua drops was recorded maximum in variety Varuna whereas in variety Rajendra suflam it was minimum. Much differences between the variety Pusa bold and Kranti was not observed. However, at 15 DAP variety Rajendra suflam followed by variety Varuna responded to flower drop to some extent. Although few crosses succeeded in almost all the crosses up to 20 DAP, none of them produced fully developed silqua no hybrid seed was obtained in any of the four crosses between *B. oleracea* var *botrytis* X *B. juncea*. It indicated only tetraploid species should be used as female parents to increase the efficiency of hybrids seed in interspecific hybridization.

Conclusion

In the present study, hybridization between *B. juncea* and *B. oleracea* var *botrytis,* both pre and post fertilization barriers commonly occurred using *B. juncea* as female parent. Whereas, post fertilization barriers were predominantly occurred using *B. oleracea* var *botrytis* as female parent. Varietal responses in reciprocal crosses was different as most efficient crosses were made using variety Kranti followed by Rajendra suflam as female parent. Though all the varieties were showing both pre and post fertilization barriers but Variety Pusa Bold was highly sensitive to pre-fertilization barriers while Varuna to Post fertilization barriers. In the reciprocal crosses Rajendra suflam was most efficient male parent for hybridization. To avoid pre and post fertilization barriers in brassica, ovule culture and embryo rescue technique may be utilized to increase efficiency. Variety Kranti as female parent could be useful for development of inter specific hybrids between *B. juncea* and *B. oleracea* var *botrytis*.

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Rashtian, Vegetos 2016, 29:3
10.5958/2229-4473.2016.00063.X

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Research Article

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Effects of Dirt roads on Vegetation and Diversity in Arid Rangelands (Case Study: Aliabad Pyshkoh of Yazd, Iran)

Anahita Rashtian*

Abstract

Since the road are considered as one of sustainable development indicators and many rural dusty roads are passed through rangelands, the effect of road networks on rangeland ecosystems is so important. In order to investigate the effects of roads, two regions were determined; one around the road, from the edge of road to 5 m and the other far from it, from 5 to 20 m. 10 transects of 50 m and 40 random plots of 1 m^2 were placed on each transect on each site and some vegetation characteristics were measured in Ali Abad Pishkuh rangelands in Yazd province. The results showed that road changes vegetation composition, production, and canopy cover percentage. So, grass production and canopy cover were decreased near the road. Important value of shrubs was declined near the road while it was increased in terms of grasses. Richness, evenness and diversity indices were reduced and distribution pattern moved to clumped. These factors indicate that plant communities moves less stable and they are crossed boundaries of ecological threshold around the road. Therefore, it is suggested that rural dusty roads construction should be with more study and proper reclamation.

Keywords

Rural road; Vegetation characteristics; Important value; Diversity indices

Introduction

In order to develope, human (directly or indirectly) has made many changes to natural ecosystems. Several studies indicate that the structure of plant communities is largely influenced by human factors [1]. Thus, human activities cause extensive changes in the environment. Roads and highways are like vital artery of a society; if they are not developed, social and economic sustainable development will be disrupted. On the other hand, improper road construction, as one of destroyer factors of natural resources, is proposed as one of watershed main problems [2]. Recognition and proper management of plant communities can make a balance between transportation quality and environment conservation.

During road construction, a large area of forests and rangelands are destroyed along the edge of road and a lot of stones and soils are moved. Environment, rangelands and forests degradation rates depend on different factors such as road type, topography, geologic formations and soil sensitivity [3].

The stages of road infrastructure can cause to remove some sensitive and less resistant species. The roads also cause changing microclimate, light regime, soil bulk density, pH and organic matters, microtopography, hydrologic regime, sedimentation and starting sensations [4,5,6]. Roads can increase plants density by establishing fast-growing and preferential species. Plants in the roadside grow more than other plants [7,8]. Many non-native or invasive species and even woody plants may accumulate at the edge of road [9,10]. Some species reproduction also decreases due to increasing traffic, soil compaction and reducing soil penetration [11]. People and vehicle movement can cause water, soil and air pollution and finally remove some species [12]; all these factors change species composition and diversity [13,14]; the effects of road decrease by getting away from it [15].

Quality of road construction effects on vegetation changes so that roads with more quality have more effects on vegetation dynamics [16]. This situation depends on railroad type, transport corridors or road and its superstructure characteristics such as paved or dusty roads [17,18], because the heat which absorbed by superstructure stuff during a day, emits to the atmosphere to the form of infrared waves and cause to create a heat island around roads corridors [17]. This situation changes according to the amount of road effect, vegetation density and its diversity [18,19,20]. The effects of road on plant communities can be seen up to 20 meters or even 60 meters far from the road in forests [21] but plant communities changes are significant to 5 meters in savannas or shrublands [22].

One of sustainable development indicators is to maintain natural resources for future generations [23]. According to this and the fact that the length of Iran road networks is 15137 km that the amount of 76749 km is paved and 37114 km is dusty [24], in this paper the effect of rural roads were investigated on vegetation characteristics and rangeland resources.

Materials and Methods

This study was conducted in Ali Abad Pishkuh steppe rangelands of Yazd province. These rangelands are located at 31° 39′ 01″ north latitude and 53° 47′ 09″ east longitude with annual precipitation of 212 mm. The average altitude is 2300 meters above sea level.

Firstly, the study area was determined using topographic maps and during field investigation, two regions were determined; one around the road, from the edge of road to 5 m and the other far from the road, from 5 to 20 m. some parameters such as canopy cover percentage, production, density, diversity and evenness were measured. Depending on vegetation type and condition, 10 transects of 50 m and 100 random plots of 1 m^2 were placed on each transect on each site and along 5 km of the road. Appropriate numbers of plots were calculated using statistical method. Species type, number of species, canopy cover and production were measured in each plot. The data was analyzed using T-test. Species important value was also determined using equation (1) [25].

Important value = relative frequency + relative dominance + relative density equation (1)

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Received: May 30, 2016 **Accepted:** July 09, 2016 **Published:** July 14, 2016

This index ranges from 0 to 300 and to show that as a coefficient, it was divided in 300. When it moves closer to 1, shows the species importance in the ecosystem and that species has a determinant role in species composition or ecological conditions prevailing in the region. It also has an important role in this species establishment. So species changes are important to interpret ecological conditions in the region. *EXCEL* and *MINITAB* software was used to analyze the data.

Some indices such as Jackknife (richness index), Simpson and Smith Wilson (evenness indices), Shannon Wiener and Brillion (diversity indices) were used to calculate diversity and richness. Differences between plant communities were determined using Tukey test.

Dispersion (variance/mean ratio), Morisita and Standardized Morisita indices were also used to determine species distribution [26].

Results and Discussion

Table 1 shows significant difference between the two regions in terms of production, density and canopy cover percentage ($p<0.05$), with production demonstrating a significant decrease in the rangelands near the road while density and canopy cover percentage have increased significantly.

In order to study the effect of roads on plant composition more precisely, plants were divided into three palatability classes and production, canopy cover and density of each palatability class were examined. The production of high palatable (Class I) species and the species with average palatability (Class II) has a significant reduction while canopy cover percentage of class I and production and canopy cover of class III species are higher in rangelands near the road (Table 2).

According to the results of the above table, shrubs production statistical analysis indicates a significant difference between two regions and it has decreased significantly, while grasses and forbs have had a significant increase in terms of canopy cover percentage and density in rangelands near the road. So, the road has caused shrubs reduction and grasses and forbs increase in plant composition around the road.

To determine important value of each life form, relative density, dominance and frequency were calculated. Important value of different life forms has shown in Table 4.

According to Table 5, important values of perennial and annual plants were investigated in rangelands near and far from the road.

Differences were detected between regions near the road and far from it, in terms of shrubs and forbs important value ($p<0.05$). In regions near the road, shrubs important value has reduced but this factor has increased in terms of forbs. It means that the road has caused favorable conditions for forbs growth. Important value of annuals has also increased significantly. This indicates that conditions have improved for annuals, but important value of perennial plants which play an important role in sustainability of rangeland ecosystems, has decreased.

Some indices such as Jackknife (richness index), Simpson and Smith Wilson (evenness indices) and Shannon Wiener and Brillion (diversity indices) were used to determine species diversity and richness and the effects of road on them. The results are shown in Figure 1.

Species richness index has a significant difference between the two communities. Richness in the plant community near the road has decreased significantly compared to the other. Plant communities far from the road have more evenness and diversity. Road construction has caused evenness and diversity reduction.

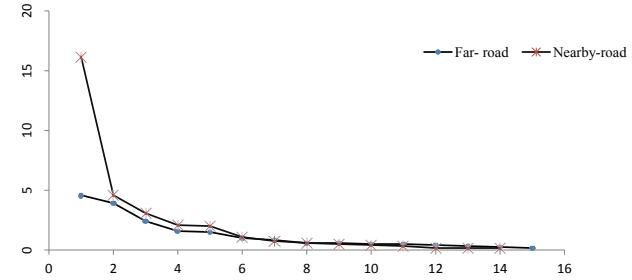

Figure 1: Whitaker rank-frequency (diversity-dominance) chart in rangelands near and far from the road.

Table 1: Investigated indices of rangelands near the road and far from it.

Paved region	Production (gr)	Canopy cover (%)	Density
Near rangelands	2.4 ± 28	4.2 ± 29.9	2.3 ± 32.1
Far rangelands	3.1 ± 38.3	3 ± 18.5	1.8 ± 19.17
P value	*0.017	* 0.039	*0.021

According to Table 2, richness, evenness and diversity indices have a significant reduction in rangelands near the road ($p<0.05$).

In order to recognize the complex structure of communities, Whitaker rank-frequency (diversity-dominance) chart was used. So, several species frequencies are so high in the community close to the road and it shows evenness reduction. The community far from the road has more evenness and there is also more species richness due to higher tension of the curve. Shorter length of curve indicates less species richness in the community near the road. Species distribution pattern is logarithmic in the two communities. This kind of distribution indicates that there are many species with low frequency. The number of species with high frequency is low. There are also immature communities with low species diversity.

Distribution pattern of different life forms was determined using quadrate distribution indices such as dispersion (variance/mean ratio), Morisita and Standardized Morisita indices (Table 6).

According to the results of Table 7, distribution pattern of shrubs, grasses and forbs are uniform on the basis of all indices in the rangelands far from the road. But based on Morisita and Standardized Morisita indices, there is clumped distribution pattern in rangelands near the road.

Discussion

The results showed that road causes to change plant composition, production and canopy cover percentage so that production decreased around the road. One of reasons can be reduction of perennial species density due to vehicles traffic, their pollution, waterways redirecting and finally more erosion. Decreasing canopy cover percentage can also cause more erosion. These results were also confirmed by Bolling [27], Coffin [12] and Liu et al. [15].

According to Tables 2 and 4, shrubs production and important value has decreased near the road while forbs and grasses important value, density and canopy cover percentage have increased. It seems that these species especially annuals are more opportunistic species [28]. They occupy open spaces (that is created in order to develop

Table2: Production, canopy cover percentage and density of different palatable classes in rangelands near and far from the road

Region	Production			Canopy cover			Density		
	Class I	Class II	ClassIII	Class I	Class II	ClassIII	Class I	Class II	ClassIII
Near of road	1.25+0.7	15.4 ± 19.7	1.7 ± 5.8	0.75 ± 0.2	14 ± 21.3	1.8 ± 5.8	0.91+ 0.5	13.7 ± 25	2.4 ± 6
Far of road	2.9 ± 4	11.2 ± 28.2	2.8 ± 3.3	0.9 ± 1.58	11.2 ± 15	1.1 ± 2	1.2 ± 4.9	10.4 ± 27.4	2.1 ± 3.5
P value	0.01 **	0.044*	*0.05	0.031*	0.232 ns	0.024*	0.001**	0.639 ns	0.182 ns

Table 3: Production and canopy cover percentage of different life forms in rangelands near and far from the road. Plant composition was also evaluated on the bases of life forms.

Region	Production (gr/m²)			Canopy cover (%)			Density (No/m²)		
	Shrub	Forb	Grass	Shrub	Forb	Grass	Shrub	Forb	Grass
Near of road	1.4 ± 5.9	2.3 ± 13.08	1.3 ± 8.75	0.66 ± 8	1.2 ± 13.83	1.5 ± 6.58	0.7 ± 3.5	1.6 ± 8.83	4.1 ± 19.8
Far of road	3.3 ± 18.8	2.5 ± 8.33	1.1 ± 8.58	3.4 ± 9.42	1.5 ± 5.92	0.8 ± 4.58	0.75 ± 5.75	0.83 ± 3.75	1.9 ± 10.1
P value	** 0.004	0.179 ns	0.062 ns	0.725	* 0.02	*0.03	0.41	*0.012	*0.04

Table 4: Important value of different life forms in rangelands near and far from the road.

Region	Important value		
	Shrub	Forb	Grass
Near of road	11 ± 57.2	10 ± 139.1	9.3 ± 103.7
Far of road	14 ± 128.4	8 ± 76.2	8.3 ± 99.6
P value	** 0.001	**0.002	0.748 ns

Table 5: Important values of perennial and annual plants in rangelands near and far from the road

Region	Important value	
	Perennial	Annual
Near of road	10 ± 179.8	10 ± 120.2
Far of road	7.1 ± 242.7	7.7 ± 57.3
P value	** 0.000	** 0.000

Table 6: Richness, evenness and diversity indices in rangelands near and far from the road.

Region	Richness	Evenness		Diversity		
	Jackknife	Simpson	Wilson	Simpson	Shannon Wiener	Brillion
Near of road	0.91 ± 14.9	0.3 ± 0.695	0.05 ± 0.4	0.315 ± 0.09	0.22 ± 2.27	0.15 ± 2.067
Far of road	0.78 ± 17.8	0.2 ± 0.820	0.16 ± 0.5	0.08 ± 0.521	0.32 ± 2.77	0.22 ± 2.418
P value	* 0.046	*0.017	ns 0.143	* 0.022	*0.047	*0.04

Table 7: Distribution index of different life forms in rangelands near and far from the road.

Distribution index		Near rangelands		Far rangelands	
		Calculated number	Distribution pattern	Calculated number	Distribution pattern
Shrub	Ratio	0.61	Uniform	0.62	Uniform
	Morisita	0.71	Uniform	1.013	Clumped
	Standardized Morisita	0.62	Clumped	0.636-	Uniform
Grass	Ratio	0.74	Uniform	0.93	Uniform
	Morisita	1.42	Clumped	0.388	Uniform
	Standardized Morisita	0.616-	Uniform	0.264-	Uniform
Forb	Ratio	0.63	Uniform	0.69	Uniform
	Morisita	1.013	Clumped	0.363	Uniform
	Standardized Morisita	0.664-	Uniform	-0.264	Uniform
Total	Ratio	0.54	Uniform	0.39	Uniform
	Morisita	1.19	Clumped	0.98	Uniform
	Standardized Morisita	0.508	Clumped	0.845-	Uniform

roads) around the roads quickly while shrubs couldn't occupy this space or niche because of their growth speed and lower establishment. Studies by Hayasakaa et al. [29] support this connection. They investigated plant communities' changes near rural and urban roads and found that there are invasive plants (Astraceae family) and grasses near the road more than other plants.

Plant community richness near the road is less than the rangeland far from the road and road construction has caused to remove some plant species; it is obvious in Whitaker rank-frequency chart. Plant communities' evenness has reduced near the road and Simpson index and the curve slope shows this fact. Species diversity is higher far from the road that corresponds with the results of studies by Bowering et al. [27,30], Khodadad and Sepehry [2] and Marcantonio et al. [31] but the study by Najafi et al. [32], who have studied forest cover diversity, doesn't confirm this. Distribution patterns of different life forms have become clumped that its reason can be the effect of microtopography due to road construction and changing soil water regime near the

road. These results were also confirmed by Tormo et al. [33], who found that soil water regime and plant species types effect rangelands restoration near the road, and Neher et al. [5], who declared that microtopography factor and water flows influence plant composition.

Therefore, due to higher sensitivity of arid and semi-arid vegetation, it is recommended to do any road construction and development according to its environmental consequences. It is also better to restore vegetation properly and at the same time to cause the least damage to natural resources adjacent to it.

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Sunil et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00064.1

Vegetos- An International Journal of Plant Research

Research Article

A SCITECHNOL JOURNAL

Analysis of Diversity and Distribution of *Pongamia [Pongamia pinnata* (L.) Pierre] Germplasm Collections from Two Distinct Eco-Geographical Regions in India

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Abstract

Collection and study of extent of diversity available in *Pongamia*-native oil bearing tree species is the fundamental and significant requirement for its effective utilization. Two survey missions undertaken in distinct eco-geographical regions of India *viz*. Chhattisgarh and Rajasthan states of India formed the basis of the present study. The data on *in-situ* traits *viz*. pod length, pod width, pod thickness, seed length, seed width, seed thickness and the nursery traits *viz*. seedling girth, seedling height and number of leaves was recorded. There was good variation for these traits among the accessions collected from both the states. The extent of variation was in conformity with the earlier studies from several other regions of the country, which implied that pod and seed traits have a limited range. It was also inferred that cross pollination (pollination by bees) and open pollination did not significantly impact the extent of variation among the germplasm collections from Chhattisgarh and Rajasthan respectively; and using the DIVA-GIS software we were able to identify the diversity rich pockets in these two states. The D^2 statistic grouped the accessions from Chhattisgarh into seven diverse clusters and those from Rajasthan into four. It was concluded that use of phenotypic data along with the molecular markers data would effectively bring out the extent of diversity for utilization in *Pongamia* crop improvement.

Keywords: DIVA-GIS; Diversity index; Heritability; Pollination; *Pongamia*; Variability

Introduction

The demand for different sources of energy *viz*. coal, electricity, natural gas and oil has increased tremendously as a result of population growth along with social and economic development drivers. Oil, being portable and efficient, is the most convenient source of energy. Although oil supplies match the demand, in the current scenario, the high greenhouse effect/impact of the fossil fuel, has made us to look for alternative sources [1]. Bio-fuels from tree borne oil seed is considered to be viable option [2]. Among the tree borne oils *Jatropha*, *Pongamia*, *Simarouba* etc are listed as the potential sources. Of these, *Pongamia*, native to India is nitrogen fixing tree species with a potential of bio-amelioration of degraded lands [3]. It scores over the non-native species in important factors *viz*. acceptability by farmers, adaptability to the local environment and in lacking possible invasive effects of the introduced tree species.

Pongamia pinnata (L.) Pierre known as *Karanj* belongs to family Fabaceae; sub-family-Papilionoideae and tribe Millettieae. It is hardy, non-edible seed oil producing tropical tree species. The economic part, the seed, is borne in a hard pod which varies in shape and size. The pods generally bear up to 1-3 seeds/pod, although four seeds/pod have also been reported [1]. The pods are borne either singly or in clusters. The natural distribution of *Karanj (Pongamia)* is along coasts and river banks in India and Myanmar [2]. In India, its occurrence and distribution of diversity is reported throughout the country in general and particularly in the Western Ghats. The distribution of variability along the altitudinal gradient in specific areas/ regions of diversity in Andhra Pradesh and Orissa states in India, using the DIVA-GIS was reported [4]. Good phenotypic diversity and variation is observed in traits, *viz*. flower colour, branching pattern, palmate leaf number, leaf colour, pod size and shape etc. in view of it being a cross pollinated species. Its attribute as a cool shade giving tree has made it an important species of urban forestry and also traditionally used to package and conserve the freshness of hot season fruits like Mango and tender nuts of Palmyra palm.

Harrington [5] demonstrated the suitability of fatty acid methyl esters (FAMEs) from *Pongamia* seed oil as a good fuel for diesel engines and several other research findings also confirmed the potential of *Pongamia* oil to be used as a biodiesel [6,7,8,9,10]. FAMEs as biodiesel are environmentally safe, non-toxic and biodegradable [11]. The composition of the *Pongamia* seed oil and the properties of the FAMEs meet the North American and European industry standards and its values for the pour and cloud points are satisfactory for tropical and some temperate regions [3]. *Pongamia* being a native, non-food species with good oil yielding potential has become a popular bio fuel crop. However, the major bottleneck is identification of diverse and promising lines for exploitation, which is a major challenge. Keeping this in view well planned surveys were undertaken by NBPGR throughout India.

This article documents the variation in diversity observed, identification of diversity rich regions and diverse accessions for crop improvement

Materials and Methods

Two surveys were undertaken by National Bureau of Plant Genetic Resources, Regional Station, Hyderabad, India in the states of Chhattisgarh and Rajasthan, which are characterized by diverse agro-ecological traits for collection of *Pongamia pinnata* germplasm during April-May 2009. In the state of Chhattisgarh, the mission covered Jagdalpur, Kanker, Dantewada, Narayanpur, Dhamtari and Durg districts in the Bastar plateau. The surveyed area lies between 18.97.71-81.60.15 N to 21.10.87 - 81.26.73 E with an altitude ranging from 178 m to 889 m In Rajasthan, the mission was undertaken in the districts of Ajmer, Bilwara, Chhittorgah, Banswara, Udaipur,

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Received: June 02, 2016 **Accepted:** July 08, 2016 **Published:** July 14, 2016

Dungarpur and Rajasmund. The surveyed area lies between 23.21.23-74.264 N to 25.58.14 - 75.0913 E. The general strategy adopted for germplasm collection was based on the theories suggested by Bennett, FAO and Huaman et al. [12,13,14]. Random sampling of matured pods from all sides of the trees was done. Latitude, longitude and altitude of the collection sites were recorded using Garmin 12, Global Positioning System (GPS). The Passport information consisting of the details with regards to the village, mandal and district of the site of collection were recorded. The collected germplasm was raised in nursery bags containing sand and clay in the ratio of 1:4 in polyhouse at ambient temperature with relative humidity ranging from 70 to 80 %. Data on seedling traits was recorded on two-month-old saplings. Average data for 10 nursery raised plants was recorded to study the diversity. The pod and seed data (recorded *in-situ*) along with the seedling (nursery) traits was subjected to statistical analysis using R 3.2.1 and GENRES softwares. DIVA-GIS software (developed by International Potato Centre, Lima, Peru) was also done to develop Grid maps on the diversity using point-to-grid analysis seeking square neighborhood method. Differentially colored grids were thus generated as Shanon diversity index to signify the extent of variation in the diversity. The diversity maps using GIS-DIVA software maps were generated for seed, pod and seedling traits.

Results and Discussion

Distribution of *Pongamia* is reported from India in diverse eco-geographical regions. There were reports of collection of germplasm from erstwhile Andhra Pradesh [1,15], wherein effort was made to collect phenotypically diverse germplasm lines to develop descriptor states and document the associated ITK and study the variability, heritability and group the accessions based on clustering analysis respectively. Mukta et al. [11] studied the variability in *Pongamia* for its potential for biodiesel traits. Scott et al. [3] published a timely review of the status of research on *Pongamia* and the way forward.

The two surveys undertaken in Chhattisgarh and Rajasthan resulted in the collection of 50 accessions from Chhattisgarh and 21 accessions from Rajasthan. The distribution of the *Pongamia* tree population in Chhattisgarh is denser and hence critical accession-wise evaluation based on plant traits was possible to sample the diverse accessions based on age of the tree, branching pattern, pod size, shape, bearing and also factors like assessment of local trees. Whereas, the distribution of the *Pongamia* in districts of Rajasthan was far and wide and the accessions were collected from single isolated trees. Solomon Raju and Purnachander Rao [16] reported reproductive behaviour of the crop leading to predominant cross fertilization, which is brought about by important pollinating insects such as *Apis dorsata, A. cerana indica*, *Amegilla* sp., *Megachile* sp., *Xylocopa latipes,* X. *Pubescens,* wasps viz., *Sphex* sp., *Vespa* sp., *Ropalidia spatulata, Delta pyriformes* and thrips (*Thrips hawaiiensis* and *Haplothrips tardus).* The distribution of *Pongamia* in Chhattisgarh models on cross pollination, as the dense population can easily be cross fertilized by bees and thrips, whereas the distribution of plant population in Rajasthan was not amenable for pollination by insects. Dhillon et al. [17] have reported cross-pollination up to 60-90% and open pollination 10-40% in *Pongamia.*

Estimation of variability using statistical measures

The descriptive statistic for pod, seed and seedling traits for both the states of Chhattisgarh and Rajasthan are provided in Table 1. In Chhattisgarh, maximum CV was recorded in the number of leaves per plant (33%) followed by seedling height (23%) and the lowest was recorded in pod length (5.4%). The maximum pod length recorded was 6.6 cm (VNKR-09-07) and there were five accessions which recorded a pod length of 6.0 cm and above; VNKR-09-49 recorded maximum pod width (3.0 cm). Pod thickness of 1.5 cm was recorded in VNKR-09-27, which was the highest. The seed length ranged from 1.8 (VNKR-09-38) to 2.7 (VNKR-09-18) and the maximum seed width was recorded in VNKR-09-44 (1.9 cm). Maximum seed thickness of 0.9 cm was recorded in VNKR-09-52. The seedling girth recorded was in the range of 2.8 cm (VNKR-09-03) - 5.4 cm (VNKR-09-42). The maximum seedling height of 34.1 cm was recorded in VNKR-09-47.

In Rajasthan, among the 21 accessions, maximum CV was recorded in number of leaves per plant (23%) followed by seed thickness (18.4%) and seedling height (18%). The maximum pod length and width was recorded in SNP-09-20 with 7.5 cm and 3.0 cm respectively and the same accession also recorded maximum seed length (1.9 cm), seed width (1.9 cm) and also number of leaves (20.0). Maximum seed thickness was observed in SNP-09-09 (0.8 cm) and also maximum seedling girth (6.1). The maximum seedling height was recorded in accession SNP-09-12 (41 cm). The variability assessment of the germplasm collected from Haryana [18], Maharashtra [19], Jharkhand [20], Andhra Pradesh [21] and Uttar Pradesh [22] was elucidated by different authors. The maximum pod length reported by Divakara et al. [20] was 65.5 mm and similar pod length was also recorded in the present study in Chhattisgarh however, in Rajasthan a pod length of 7.45 cm was recorded. Sunil et al. [1] reported maximum seed length, width and thickness of 2.6 cm, 1.8 cm and 1.0 cm respectively and similar results were reported from the observations in the present study. The studies depict the range of variation for pod and seed traits in the germplasm collected across the country and showed that what was observed in the present study is within the variation reported earlier for these traits. Analysis of variance revealed there was significant difference among the

Table 1: Summary statistics of the germplasm collected from Chhattisgarh and Rajasthan.

Trait	Pod length (cm)		Pod width (cm)		Pod thickness (cm)		Seed length (cm)		Seed width (cm)		Seed thickness (cm)		Seedling girth (mm)		Seedling height (cm)		No. of leaves		100-seed weight (g)	
	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R
Mean	4.9	5.4	2.1	2.3	1.1	1.0	2.3	1.9	1.5	1.5	0.8	0.7	4.2	4.7	22.6	28.0	12.8	14.5	150.7	164.5
Range	2.8	3.1	1.4	1.0	0.7	0.4	0.9	0.7	1.1	0.6	0.4	0.3	2.5	2.5	20.5	23.0	14.4	10.0	187.0	146.0
Minimum	3.8	4.3	1.6	2.0	0.8	0.9	1.8	1.2	0.9	1.3	0.5	0.5	2.8	3.6	13.6	18.0	5.3	10.0	75.0	124.6
Maximum	6.6	7.5	3.0	3.0	1.5	1.2	2.7	1.9	1.9	1.9	0.9	0.8	5.4	6.1	34.1	41.0	19.7	20.0	262.0	270.6
Standard Deviation	0.7		0.3	0.3	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.7	0.6	4.4	5.9	3.4	3.0	6.5	5.4
CV%	5.4	7.3	8.1	6.9	8.0	15.5	6.3	9.5	6.2	8.5	10.0	18.4	20.6	14.0	23.0	18.0	33.0	23.0	4.3	3.3

Note: C=Chhattisgarh; R= Rajasthan

accessions for all the traits recorded except for seed thickness among the accessions collected from both states (Table 2a and 2b).

We wanted to compare the extent of variability between the populations collected from Chhattisgarh and Rajasthan considering the typical distribution of diversity/sampling observed, as outlined above. The assessment of variability using simple measures of variability *viz.* Phenotypic and Genotypic coefficients of variation, heritability and genetic advance are provided in Table 3a and 3b. Phenotypic Coefficient of variation (PCV) was higher than Genotypic Coefficient of Variation (GCV) for all the traits signifying the influence of environment. Among the *in-situ* recorded plant traits highest PCV was recorded in 100-seed weight. 100-seed weight also recorded higher heritability (97% in both the regions). Similarly, high PCV and heritability values were recorded for pod length, seed length and pod breadth. Hence, these traits can be used for phenotypic selection in *Pongamia*. However, 100-seed weight is the only trait that has also recorded high Genetic Advance (GA) in addition to PCV and heritability. This indicates that heritability is due to additive gene effect and selection is effective in such cases. Similar finding was reported by Sunil et al. [1]. The comparison of variability of the accessions between two states revealed that there was not much difference in the cross pollinated (Chhattisgarh) and open pollinated (Rajasthan) conditions, clearly showing the non influence in sampling strategy under cross and open pollinated conditions. The study by Sahoo et al. [23] on the inter and intra- population variability in *Pongamia* also pointed out that, within population variability was up to 32.34% and the molecular polymorphism up to 67.18% with ten ISSR hinting at modest levels of genetic variation. Jiang et al. [24] also reported that the seed oil content and composition varied between the trees and within the progeny of the single parent tree. Use of efficient molecular markers like AFLP for diversity analysis in *Pongamia* [25] along with important phenotypic traits would more distinctly bring out the variation. These findings were in line with the finding of Thudi et al. [26], who showed that diverse germplasm lines could be collected from Karnataka state only, as the accessions collected from these two states showed similar variability among the traits studied.

Diversity analysis using DIVA-GIS

We also aimed to identify the pockets of diversity within these two eco-geographic regions. DIVA-GIS geo-referenced software was utilized for this, using the input data of traits recorded and the geo-reference (latitude and longitude) values recorded at the time

Table 2 A and B: Analysis of variance for seed and seedling traits of the germplasm collected from Chhattisgarh and Rajasthan.

Chhattisgarh	Mean sum of squares										
Source	Df	Pod length (cm)	Pod width (cm)	Pod thickness (cm)	Seed length (cm)	Seed width (cm)	Seed thickness (cm)	Seedling girth (mm)	Seedling height (cm)	No. of leaves	100-seed weight
Genotypes	49	1.2**	0.2**	0.3**	1.4**	1.0**	0.3**	1.0*	58.3**	35.0**	4330.3**
Replicates	2	0.9	0.6	0.2	1.2*	0.2	0.1	1.0	62.7	30.3	151.0*
Residuals	98	0.7	0.3	0.1	0.2	0.0	0.0	0.7	2590.7	16.4	42.0
Rajasthan	Mean sum of squares										
Genotypes	20	1.4**	0.2**	0.1**	0.3**	0.1**	0.0	1.0*	104.8**	26.8**	3188.3**
Replicates	2	0.1	0.1	0.0	0.1	0.0	0.0	0.2	111.6*	5.4	241.7**
Residuals	40	0.2	0.0	0.0	0.0	0.0	0.0	0.4	25.1	10.7	29.0

Note: **Significant at 1%; *Significant at 5%

Table 3 A and B: Estimation of genetic variable for pod, seed and seedling traits in Chhattisgarh and Rajasthan.

	GCV (%)	PCV (%)	Heritability (%)	Genetic Advance (%)
Chhattisgarh				
Pod length	12.5	13.6	84.3	11.6
Pod breadth	12.1	14.6	69	4.4
Pod width	7.7	11.2	48	1.2
Seed length	9	11	67	3.4
Seed breath	11.4	13	77.5	3.1
Seed width	10.8	14.8	53.7	1.2
Seedling girth	8.7	22.3	15.3	0.3
Seedling height	14.6	27.3	28.7	3.6
Number of leaves	20	38	27.3	2.7
100-seed weight	25	25.4	97	77
Rajasthan				
Pod length	12	14	73	1.1
Pod breadth	9.68	11.8	66.2	0.4
Pod width	10.6	18.7	32.1	0.1
Seed length	15.4	18.1	72.8	0.5
Seed breath	9	12.3	53	0.2
Seed width	6.4	19.4	11	0
Seedling girth	9.4	17	31	0.5
Seedling height	18.3	25.6	51.3	7.6
Number of leaves	16	27.7	33.2	2.7
100-seed weight	19.7	20	97.3	65.9

of germplasm collection. The Figure 1 shows the diversity index values of the germplasm lines recorded for pod traits *viz.*pod length, breadth and width in both the states. The red grid standing for the more diverse lines followed by the rest as detailed in the legend. The Shannon diversity index values for the germplasm collected from Chhattisgarh ranged from 0-4.0 and those from Rajasthan recorded up to 3.0 only. The South Central region of Chhattisgarh emerged as the region with highest diversity within Chhattisgarh state and whereas South Eastern region of Rajasthan recorded highest diversity index values between 1.66 and 3.0. The seed traits *viz.* seed length, seed width and thickness recorded similar diversity index values as the pod traits. The maximum Shannon diversity index for other nursery traits including the number of leaves, seedling height and girth ranged from 2.7 to 4.0 in Chhattisgarh and the maximum value recorded in Rajasthan was in the range of 1.5 to 2.0 and the same is depicted in the Figure 2. The broad findings *viz.* high diversity regions in Chhattisgarh are on the expected lines as the general density of the population was more in Chhattisgarh and the reproductive biology of *Pongamia*, as an outcrosser, giving rise to more diversity. Sunil et al. [4] had identified Rayagad district of Odissa state as the region for the collection of diverse germplasm lines for seed traits and oil content. Bastar region of Chhattisgarh, which forms a contiguous region of Odissa also can be tapped for diverse lines use of DIVA-GIS tool in identification of diversity rich regions was reported earlier in *Jatropha* [27], brinjal [28]. However, interestingly high mean values for all the traits were recorded in germplasm collected from Rajasthan except in case of pod thickness and seed length. The arid ecology and stressed and open pollination conditions prevailing, the isolated trees could not have been pollinated by bees as outlines by Solomon Raju and Purnachander Rao, [16]. Thus environmental conditions found in Rajasthan may be more suitable for the development of reproductive traits i.e. conditions suitable for quality over quantity of seed produced

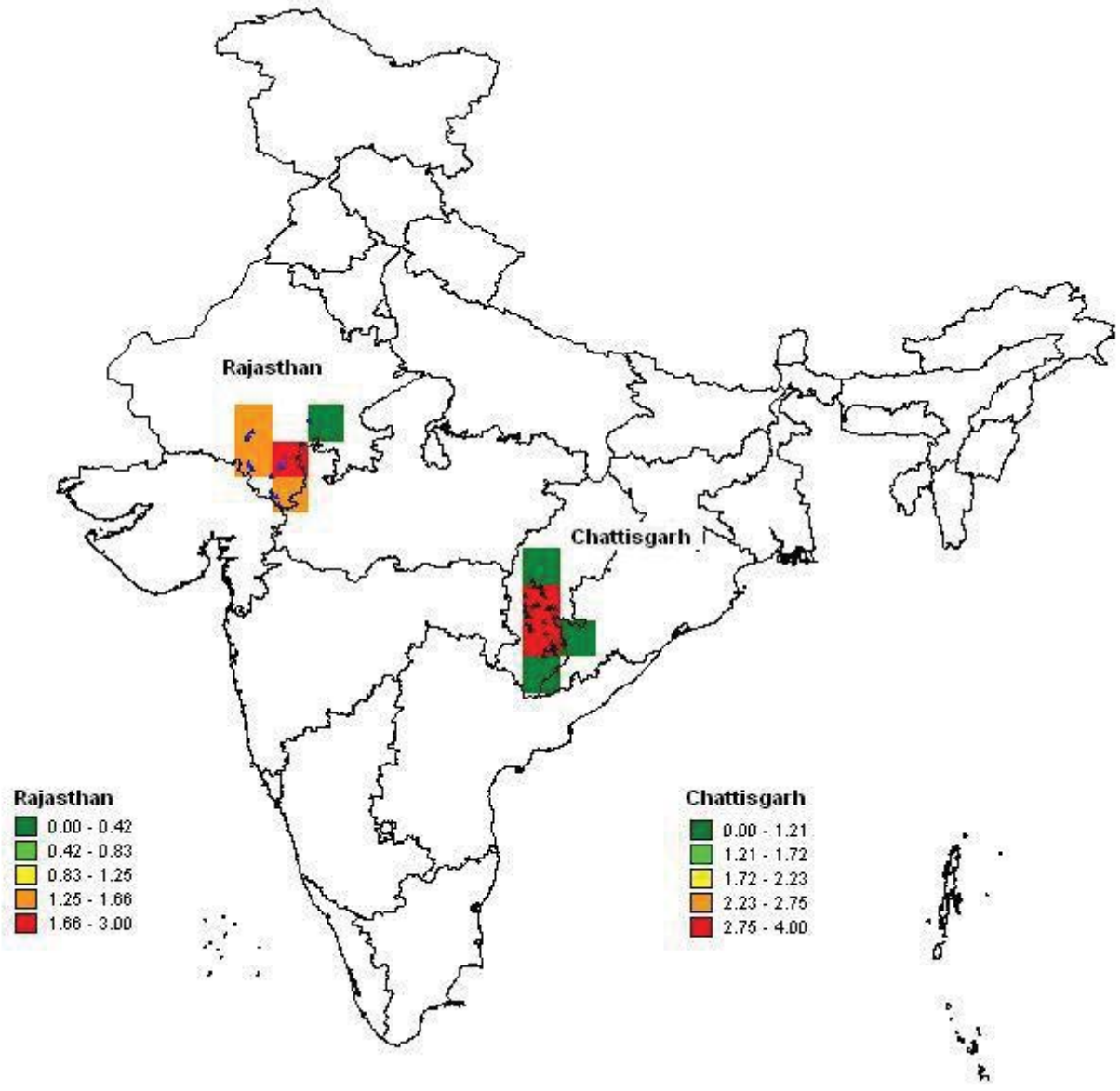

Figure 1: Shannon diversity index values of the pod traits recorded on the germplasm collected from Chhattisgarh and Rajasthan.

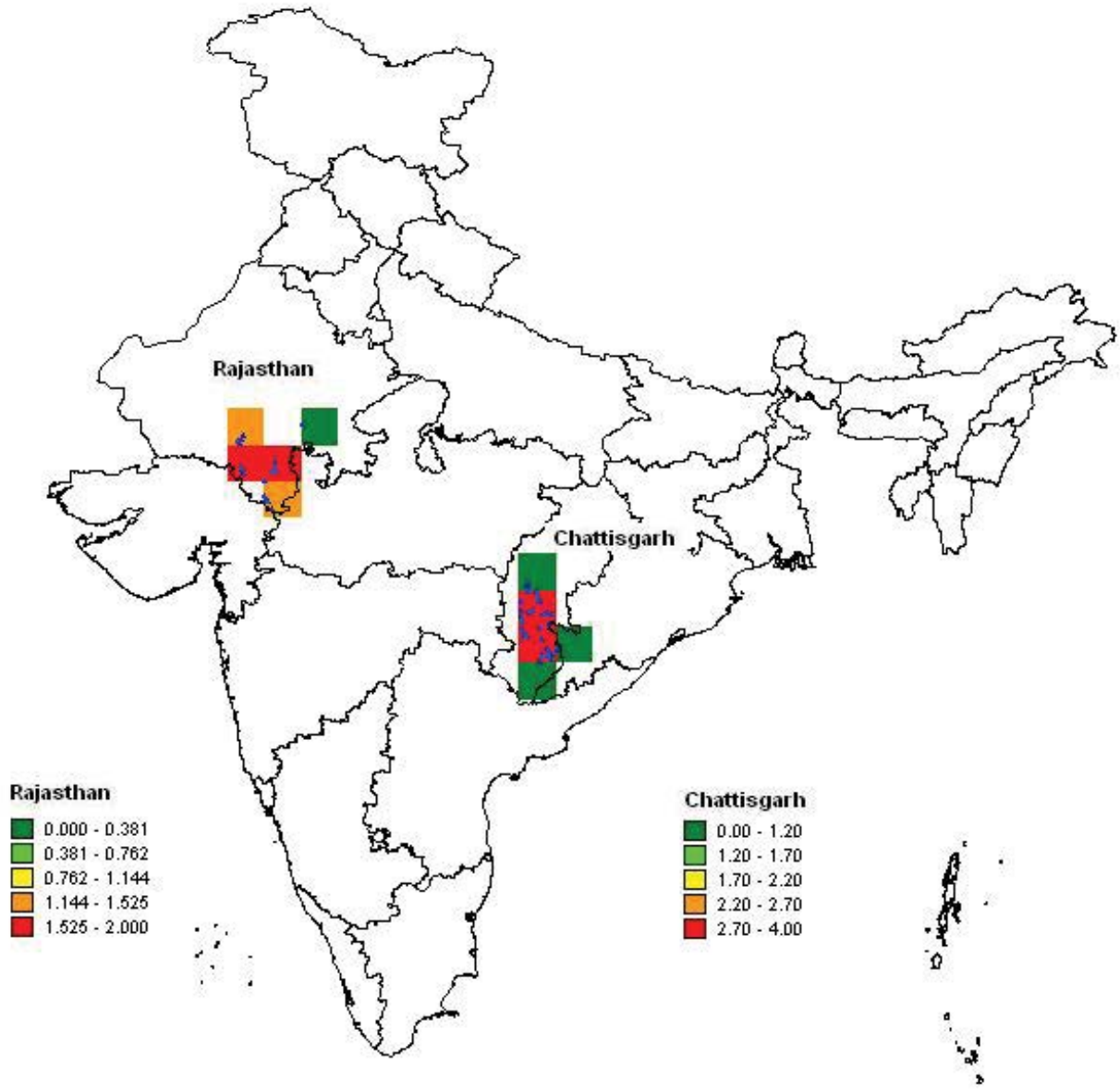

Figure 2: Shannon diversity index values for the nursery traits recorded in the germplasm collected from Chhattisgarh and Rajasthan.

and also the seedlings grown under optimal conditions expressing high potential value traits.

D^2 Analysis for identification of diverse groups

Further to identify the diverse group/accessions within these accessions. The pod, seed and seedling traits data was subjected to D^2 statistics. The accessions from Chhattisgarh were grouped into seven clusters and those from Rajasthan into four groups (Table 4a and 4b). Cluster II was comprised of maximum number of 45 accessions collected from Chhattisgarh and the rest of the six clusters comprised of two accessions each, whereas for Rajasthan, Cluster IV had 15 accessions and rest of three clusters comprised of two accessions each. The inter and intra cluster distances for the accessions from Chhattisgarh and Rajasthan have been provided in Table 5a and 5b. The maximum inter cluster distances in Chhattisgarh was recorded in between cluster VI (57) and VII (432) followed by Cluster I (3.7) and VII (319), whereas the intra cluster distance was maximum in cluster VII. The maximum inter cluster distance for Rajasthan was recorded between I and IV and the maximum intra cluster distance was recorded in cluster IV. Selection of genetically divergent parents such as the accessions from clusters having maximum genetic distance would facilitate their exploitation by hybridization. The cluster means for each of the traits (Table 6a and 6b) revealed that cluster VII in Chhattisgarh and cluster IV from Rajasthan were conspicuous by recording higher 100-seed weight. Further, among the traits studied 100-seed weight emerged as the key character contributing to divergence both in Chhattisgarh (77%) and Rajasthan (75%).

Conclusions

The findings of the present study by phenotypic traits were in line

Table 4 A and B: Grouping of accessions collected from Chhattisgarh and Rajasthan into D^2 clusters.
Chhattisgarh

Cluster No.	Accessions
I	**VNKR-09**-24, 30
II	**VNKR-09**-1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,25,26,27,28,29,31,32, 34,35,36,37,38,39
III	**VNKR-09**-42, 44
IV	**VNKR-09**-47,48
V	**VNKR-09**-45,50
VI	**VNKR-09**-41, 49
VII	**VNKR-09**-40, 43

Rajasthan

I	**SNP-09**-11,12
II	**SNP-09**-8,9
III	**SNP-09**-6,14
IV	**SNP-09**-1,2,3,4,5,7,10,13,15,16,17,18,19,20,21

Table 5 A and B: Estimates of inter and intra cluster D^2 values for pod, seed and seedling traits for accessions from Chhattisgarh and Rajasthan.
Chhattisgarh

	I	II	III	IV	V	VI	VII
I	3.7	90.0	101.0	32.0	122.0	38.0	319.0
II		94.0	60.0	70.0	88.0	155.0	195.0
III			8.9	53.0	26.0	174.0	100.0
IV				16.0	68.0	90.0	216.0
V					31.0	185.0	119.0
VI						57.0	432.0
VII							151.0

Rajasthan

	I	II	III	IV
I	2.8	29.0	38.0	114.0
II		3.7	17.0	111.0
III			13.0	94.0
IV				166.0

Table 6 A and B: Cluster means for the pod, seed and seedling traits for accessions from Chhattisgarh and Rajasthan.
Chhattisgarh.

Trait / **Cluster No.**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
I	4.5	1.9	1.0	2.3	1.3	0.7	3.7	15.7	9.1	110.0
II	4.9	2.0	1.1	2.2	1.4	0.7	4.0	22.0	11.6	150.0
III	5.1	2.3	1.1	2.3	1.5	0.7	4.4	25.5	15.5	168.5
IV	4.3	2.0	1.2	2.3	1.3	0.8	5.0	19.0	15.8	138.0
V	4.7	2.6	1.1	2.1	1.6	0.7	4.4	29.5	17.5	165.5
VI	4.6	2.1	1.0	2.0	1.3	0.6	4.5	21.0	14.6	91.0
VII	5.3	2.4	1.1	2.1	1.7	0.7	4.3	27.0	15.0	211.0

Rajasthan

Trait / **Cluster No.**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
I	5.0	2.0	1.0	1.4	1.3	0.7	5.3	25.3	15.0	155.0
II	5.2	2.4	1.0	1.7	1.6	0.7	4.3	23.0	15.0	148.0
III	5.7	2.5	0.9	1.9	1.4	0.6	4.7	25.8	11.5	154.0
IV	5.4	2.3	1.0	2.0	1.5	0.7	4.7	29.0	15.0	169.0

with those by Thudi et al. [26], who using AFLP molecular markers grouped 48 accessions of *Pongamia*, sourced from six different states into four clusters, they also reported an average genetic similarity of 0.61 among the accessions although the range recorded was from 0.28 to 0.90. This would imply that the variation was less than 40% among these accessions. Although a high level of polymorphism is expected in an out-crossing species like *Pongamia*. However, the diversity reported using the above phenotypic traits may not be comprehensive. In this regard, the findings of Sharma et al. [29], who used microsatellite markers successfully in the diversity analysis

of 24 genotypes of *Pongamia* and Biswas et al. [30] who opined that use genetic and genomic tools to unravel the diversity and use of phenotype associated link markers may be the way forward for understanding the extent of diversity in the native population and tapping the diversity crop improvement.

Acknowledgement

The authors are thankful to the Director, NBPGR, and New Delhi for all the necessary support for undertaking the present study. The financial assistance provided by Rain shadow Areas Development (RSAD) of the erstwhile Andhra Pradesh government is gratefully acknowledged.

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Rasool et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00065.3

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Survey and Status of Rice Blast Caused by Magnaporthe Oryzae B.C. Couch in Commercial Rice Growing Areas of Kashmir

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Abstract

Four commercial rice growing districts of Kashmir valley *viz.,*Bandipora, Kulgam, Anantnag and Pulwama were surveyed for incidence and intensity of leaf and neck blast of rice. The survey was carried out at tillering and around flowering stages. The highest leaf and neck blast incidence of 80.67 and 19.36 per cent was observed in Anantnag, whereas it was the lowest (18.33 and 1.03 per cent) in Kakapora in district Pulwama. The leaf blast intensity ranged from 10.82 per cent in district Bandipora to 29.59 per cent in district Kulgam. The highest neck blast intensity (4.83%) was recorded at Duroo Shahabad, whereas the lowest (0.77%) was observed at Ajus in district Bandipora. The rice blast showed the highest occurrence in south Kashmir as it is the hot spot for the disease having the ideal predisposing conditions for the disease.

Keywords

Magnaporthe oryzae; Kashmir; Rice blast; Neck blast; Leaf blast; Incidence; Intensity

Introduction

Among the major constraints in boosting the production and productivity of rice in the state, the onslaught of blast disease is believed to be a major bottleneck, and takes a heavy toll of the produce [1]. Rice blast caused by *Magnaporthe oryzae* is endemic to most rice-growing areas of Kashmir valley due to prevailing blast-conducive environments during the crop season [2].The pathogen manifests itself at the seedling, tillering and flowering stages of crop growth causing losses on account of leaf-, node- and neck-blast in the state [3]. Frequent epiphytotics of the disease in the state for the last about fifteen years have been inflicting heavy qualitative and quantitative losses to the growers. The disease emerged as a major problem in the Jammu and Kashmir state prior to 1950's [4]. With the evolution and wide spread cultivation of blast tolerant varieties, the blast incidence in Kashmir remained under check till suddenly the disease surfaced in late 1990's . In order to provide a baseline information on the status of the disease in the major rice growing areas in the valley and its relation with edaphic factors and effect on productivity, the present investigation was taken up to analyse the status of incidence and intensity of rice blast.

Materials and Methods

Three random rice growing blocks/locations in each district and five fields representing each block/location were selected to record the incidence and intensity of rice blast at, tillering and around flowering stages.

The leaf blast incidence was recorded by assessing upper three leaves of each random tiller from each of the ten random hills from each field and expressed as per cent for each location [5].

$$\text{Disease incidence (\%)} = \frac{\text{No of diseased leaves}}{\text{Total No. of leaves assessed}} * 100$$

The following 0 to 9 scale [6] was adopted for recording the blast intensity of leaves in each field (Figure 1 ; Table 1).

The leaf blast disease intensity was calculated using the following formula:-

$$\text{Per cent leaf blast intensity (PDI)} = \frac{\Sigma nv}{N \times \text{maximum grade value}} * 100$$

Where

PDI = Per cent disease intensity

Σ = Summation

V = Disease score

n = Number of leaves showing a particular score

N = Total number of leaves examined/assessed

Neck blast incidence

One random tiller from each of the ten hills in each field was assessed for the neck blast and expressed as per cent. Neck blast incidence was calculated using the following formula:-

$$\text{Neck blast incidence (\%)} = \frac{\text{No. of panicles with severe neck blast}}{\text{Total No. of panicles observed per location}} * 100$$

Neck blast intensity

The extent of neck blast was further quantified by scoring it using the following scale (Table 2).

Neck blast intensity was calculated using the following formula:-

$$\text{Percent neck blast intensity (NBI)} = \frac{\Sigma nv}{N \times \text{maximum grade value}} * 100$$

Where

NBI = Per cent neck blast basnity

Σ = Summation

V = Disease score

n = Number of panicles showing a particular score

N = Total number of panicles examined

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Received: May 07, 2016 **Accepted:** July 12, 2016 **Published:** July 18, 2016

Figure 1: Grading of infected rice leaves on 0-9 scale.

Results and Discussion

An intensive stratified survey of paddy growing areas of four rice growing districts *viz.,* Anantnag, Bandipora, Kulgam and Pulwama, of Kashmir valley, revealed that the disease occurred in all the surveyed areas of Kashmir in varying proportions during all the cropping seasons with maximum leaf and neck blast intensity recorded in district Anantnag (36.89 and 4.83%, respectively) and Kulgam (29.58 and 3.54%,respectively) known as the rice bowl of Kashmir; the leaf blast intensity in Pulwama and Bandipora being lower (12.18 and 10.82%, respectively). Anwar et al. [1] also confirmed high incidence and intensity of leaf and neck blast in these areas. The results (Table 3) revealed that the overall mean leaf blast incidence in all the four districts during 2011, 2012 and 2013 varied from 21.78 per cent in district Bandipora to 61.45 per cent in district Kulgam. The highest mean leaf blast incidence of 80.67 per cent was observed in Anantnag followed by 74.76 per cent in Duroo Shahabad in district Anantnag, whereas it was the lowest (18.33%) in Kakapora in district Pulwama during 2011-13. The pooled leaf blast incidence during 2011, 2012 and 2013 was 45.50, 31.75 and 48.69 per cent, respectively, with a pooled mean of 41.98 per cent.

The average leaf blast intensity ranged from 10.82 per cent in district Bandipora to 29.59 per cent in district Kulgam (Table 4) during 2011, 2012 and 2013. The highest leaf blast intensity (36.89%) was observed at Anantnag followed by Duroo Shahabad (34.04%) in district Anantnag and Khudwani (33.41%) in district Kulgam during 2011, 2012 and 2013. The pooled leaf blast intensity during the years 2011, 2012 and 2013 was 21.12, 15.30 and 23.67 per cent, respectively, with a pooled mean of 20.03 per cent. The average neck blast intensity ranged from 0.97 per cent in district Bandipora to 4.14 per cent in district Anantnag (Table 4) during 2011, 2012 and 2013. The highest neck blast intensity (4.83%) was recorded at Duroo Shahabad

The average neck blast incidence ranged from 1.83 per cent in district Pulwama to 12.57 per cent in district Anantnag during 2011, 2012 and 2013 (Table 3). The highest neck blast incidence (19.36%) was observed in Anantnag followed by Duroo Shahabad (16.53%) in district Anantnag, whereas the lowest neck blast incidence (1.03%) was recorded in Kakapora in district Pulwama. The pooled neck blast incidence during 2011, 2012 and 2013 was 5.88, 4.32 and 7.68 per cent, respectively, with a pooled mean of 5.96 per cent followed by Anantnag (4.50%) in district Anantnag, whereas the lowest neck blast intensity (0.77 %) was observed at Ajus in district Bandipora. The pooled neck blast intensity during the years 2011, 2012 and 2013 was 2.32, 2.64 and 2.32 per cent, respectively, with a pooled mean of 2.43 per cent.

The widespread occurrence of the disease and the introduction and cultivation of different rice genotypes together with their distribution in time and space predispose the pathogen population for co-evolution and emergence of variable isolates and pathotypes/ races. Ascertaining the prevalence, frequency of occurrence and the ultimate status of the disease in length and breadth of rice growing areas of the valley was, therefore, imperative before taking up studies on pathogen variability. The disease has been found occurring with different dimensions in other parts of the globe. Several studies have been made to estimate the incidence and intensity of leaf and neck blast [1,7,8,2,9].Variations in disease intensity observed in different years and different places during the present studies were mostly due to variations in fertilizer dosage, field and seed insanitation and the tolerance levels of rice genotypes cultivated. The rice growing regions which showed higher levels of blast intensity were the hot spots of the disease where the predisposing factors for the disease development and spread were prevalent. High plant density with high relative humidity in the micro-environment, and high inoculum load in fields/areas with history of blast occurrence serve as the main factors for the pathogen proliferation and establishment of infection in the surveyed areas as have also been argued by Rathour et al. [10] and Singh et al. [11].

Table 1: Leaf blast disease score for rice [6].

Leaf blast score	Score description
0	No lesions
1	Small brown specks of pin head size
2	Larger brown specks
3	Small, roundish to slightly elongated, necrotic grey spots about 1-2 mm in diameter, with distinct brown margin
4	Typical blast lesions, elliptical, 1-2 cm long, usually confined to the area of the 2 main veins, infecting less than 2 per cent of the leaf area
5	Typical blast lesions infecting less than 10 per cent of leaf area
6	Typical blast lesions infecting 11-25 per cent of leaf area
7	Typical blast lesions infecting 26-50 per cent of leaf area
8	Typical blast lesions infecting 51-75 per cent of leaf area and many leaves dead
9	All leaves dead

Table 2: Neck blast disease score for rice [6].

Neck Blast Score	*Score description
0	No visible lesions or lesions only on few pedicles
1	Lesions on several pedicles or secondary branches
3	Lesions on few primary branches or the middle part of panicle axis
5	Lesions partially around the panicle base(node) or the uppermost internode neck of the panicle or the lower part of the panicle axis near the base
7	Lesions completely around the panicle base or the uppermost internode or panicle axis near the base with more than 30% of filled grain
9	Lesions completely around the panicle base or the uppermost internode or panicle axis near the base with less than 30% of filled grain

Table 3: Incidence of leaf and neck blast (*Magnaporthe oryzae*) disease of rice at different locations in Kashmir during 2011-2013.

District	Location	Leaf blast incidence (%)*				Neck blast incidence (%)**			
		2011	2012	2013	Mean	2011	2012	2013	Mean
Anantnag	Anantnag	81.00	74.00	87.00	80.67	20.00	15.34	22.75	19.36
	Duroo Shahabad	79.00	65.00	80.29	74.76	17.34	12.25	20.00	16.53
	Larnoo	25.00	22.00	35.00	27.33	0.00	0.00	5.42	1.81
Mean		**61.67**	**53.67**	**67.43**	**60.92**	**12.45**	**9.20**	**16.06**	**12.57**
Pulwama	Kakapora	23.00	10.00	22.00	18.33	1.33	0.00	1.75	1.03
	Pampore	25.00	19.00	38.00	27.33	1.33	1.25	6.27	2.95
	Pinglina	28.00	17.00	32.00	25.67	1.42	0.00	3.12	1.51
Mean		**25.33**	**15.33**	**30.67**	**23.78**	**1.36**	**0.42**	**3.71**	**1.83**
Bandipora	Ajus	31.00	14.00	29.00	24.67	1.75	1.25	4.42	2.47
	Bandipora	21.00	12.00	35.00	22.67	1.42	1.25	3.42	2.03
	Potushai	19.00	11.00	24.00	18.00	3.42	0.00	1.75	1.72
Mean		**23.67**	**12.33**	**29.33**	**21.78**	**2.20**	**0.83**	**3.20**	**2.08**
Kulgam	Khudwani	76.00	59.00	83.00	72.67	10.33	9.15	11.25	10.24
	Kulgam	68.00	36.00	55.00	53.00	7.78	6.00	6.27	6.68
	Yaripora	70.00	42.00	64.00	58.67	4.42	5.33	6.33	5.36
Mean		**71.33**	**45.67**	**67.33**	**61.45**	**7.51**	**6.83**	**7.73**	**7.36**
Pooled mean		**45.50**	**31.75**	**48.69**	**41.98**	**5.88**	**4.32**	**7.68**	**5.96**

*Average of 300 leaves taken per observation

**Average of 100 panicles taken per observation

Table 4: Intensity of leaf and neck blast (*Magnaporthe oryzae*) disease of rice at different locations in Kashmir during 2011-13.

District	Location	Leaf blast intensity (%)*				Neck blast intensity (%)**			
		2011	2012	2013	Mean	2011	2012	2013	Mean
Anantnag	Anantnag	36.66	33.68	40.34	36.89	3.80	5.80	3.90	4.50
	Duroo Shahabad	34.30	32.00	35.82	34.04	4.70	4.60	5.20	4.83
	Larnoo	9.45	7.90	17.25	11.53	3.00	3.70	2.60	3.10
Mean		**26.80**	**24.53**	**31.14**	**27.49**	**3.83**	**4.70**	**3.90**	**4.14**
Pulwama	Kakapora	12.92	4.35	11.46	9.58	1.02	0.70	1.72	1.15
	Pampore	13.15	8.84	20.32	14.10	1.50	1.30	0.80	1.20
	Pinglina	14.26	8.67	15.69	12.87	0.60	1.20	0.60	0.80
Mean		**13.44**	**7.29**	**15.82**	**12.18**	**1.04**	**1.07**	**1.04**	**1.05**
Bandipora	Ajus	15.26	7.19	14.64	12.36	1.00	0.80	0.50	0.77
	Bandipora	9.10	6.46	17.48	11.01	1.60	1.40	0.80	1.27
	Potushai	8.84	5.21	13.46	9.17	0.70	0.90	1.00	0.87
Mean		**11.07**	**6.29**	**15.19**	**10.85**	**1.10**	**1.03**	**0.77**	**0.97**
Kulgam	Khudwani	32.71	18.22	27.99	26.31	4.50	4.20	3.80	4.17
	Kulgam	33.00	22.17	31.98	29.05	2.50	4.00	3.10	3.20
	Yaripora	33.78	28.82	37.64	33.41	2.90	3.10	3.80	3.27
Mean		**33.16**	**23.07**	**32.54**	**29.59**	**3.3**	**3.77**	**3.57**	**3.55**
Pooled mean		**21.12**	**15.30**	**23.67**	**20.03**	**2.32**	**2.64**	**2.32**	**2.43**

*Figures based on observations on 300 leaves

**Figures based on observations on 100 panicles

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Rajappa et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00066.5

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Identification of Conserved Orthologous Set Markers in Cultivated *Vigna radiata* (L.) *Wilczek*

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Abstract

FWith an aim to develop widely applicable conserved gene markers to identify and tag orthologous genes from related *Vigna* species to reveal phylogenetic relationships and the nature of genes conserved in genus *Vigna* across the evolution, low copy nuclear Conserved Ortholog Set (COS) genes were tested to explore the interspecific genetic relationship of *Vigna radiata* (L.) Wilczek with other species such as *V. mungo* (L.), *V. umbellata Thunb*., *V. angularis* Wild and *V. unguiculata* (L.) Walp. To detect COS regions, computational approach was followed utilizing the available expressed sequence tags (EST) database of *Vigna radiata* and its related species. The ESTs were processed to eliminate sequence repeats, contaminants and low-complexity sequences. Upon alignment with Soybean genome, only high quality ESTs were grouped into clusters from which consensus sequences representing putative genes are generated for each *Vigna* species. From 6443 ESTs, 2550 contigs were acquired to develop 230 primer pairs to amplify conserved orthologous sequences across *Vigna* species. Among 14 primer pairs used for validation with genotypes of *V. radiata*, 3 gave double bands (paralogs) and 9 produced single bands indicating single copy orthologs. This infers that DNA sequences identified by this comparative genomics approach would be of great use in analyzing genomic information of related, unexplored crop genomes to augment gene discovery and plant breeding in other legumes.

Keywords

Comparative genomics; Conserved orthologous set (COS) markers; ESTs; *Vigna*

Introduction

Legumes provide protein-rich food for a large part of the world's population hence the research on legumes is essential for the establishment of extensive genetic and genomic resources, which can accelerate the discovery of critical genes. Cultivated *Vigna* species are an important protein source in countries where people have limited access to food rich in protein [1]. Globally, *Vigna* species are cultivated as three main types such as Asian beans, African beans and American *Vigna*. The Asian *Vigna* is: moth bean (*V. aconitifolia* (Jacq.) Marechal), adzuki bean (*V. angularis* (Willd.) Ohwi and Ohashi), black gram (*V. mungo* L.), mungbean (*V. radiataL.*), rice bean (V. *umbellata* Thunb.); two African beans; bambara ground nut (*V. subterranean* L.) and cowpea (*Vigna unguiculata* (L.) Walp). One of the prerequisites for increasing yield of these legumes is the study on comparative genomics for better understanding of genome structure [2] which is essential for the establishment of extensive genetic and genomic resources to accelerate the discovery of critical to genes for crop improvement. Genetic improvement of *Vigna* species through conventional breeding has been slow due lack of exploitable genetic variability within the cultivated germplasm and also due to limited gene pool and sexual-incompatibility with wild and related species, the reservoir of desirable genes. Genome research in mungbean is still far behind the other major legume crops such as soybean, cowpea, and common bean, or even their relative but less important, adzuki bean. The genome study in mungbean and related *Vigna* species has been made possible by using genetic markers from other related legumes, and this trend will continue since only limited genetic resources are available for further study in this crop. The utility of marker assisted selections in improvement of *Vigna* species is limited largely due to limited marker polymorphism within the species and hence there is a scope to look for diversity in related crop species. Efforts are being made to develop high-throughput markers with greater resolution [3]. A high degree of similarity in the nucleotide sequences among green gram and other *Vigna* species reported in earlier studies by performing comparative genome analysis using DNA markers [4,5]. To alleviate the use of the wide genetic diversity present in wild relatives and landraces of crops, more information is needed on the organization and structure of their genes and genomes. Molecular markers linked to loci with important effects can facilitate the introgression of those traits into adapted germplasm. Agriculturally important traits captured during domestication are often coded by very limited number of loci with major phenotypic effects. These loci possibly have putative orthologous counterparts in other species [6] and therefore molecular markers, such as Conserved Orthologus Set (COS) markers are of great use in comparing genomic information between the phylogenetically related species within the genus or family. They are extremely useful for the analysis of genome evolution among closely and distantly related species within Leguminosae family. For a given group of species, a COS is formed by identifying a gene from each species that is orthologous to all other genes in the set. These markers are apparent single-copy evolutionary conserved genes in two or more species that share common ancestry (are orthologous) [7]. Information from the comparative genomic analyses within the genus *Vigna* will elucidate the genetics of domestication and enable the isolation of novel genes for use in breeding of mungbean germplasm in particular and *Vigna* in general. Generation of molecular level genetic data in *Vigna* has led to search for ways and means to utilize the existing plant genetic resources to support breeding challenges aiming at gainful applications in crop improvement. Molecular markers such as RAPD, AFLP [8] RFLP, ISSR [9,10], SSRs [11] and sequence tagged microsatellite site [12] have been used in mungbean to test their usefulness in genetic diversity among cultivars. During domestication, species of *Vigna* have got the agriculturally important traits coded by very limited number of loci with major phenotypic effects. It is common to find that these loci have putative orthologous counterparts in other species within

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Received: May 29, 2016 **Accepted:** July 18, 2016 **Published:** July 25, 2016

the same genus and therefore molecular markers, such as Conserved Orthologous Set (COS) markers, are powerful in comparing genomic information across species. Orthologous genes are called so as they are related by common ancestry sharing the same function or activity and acquire homologous relationship across the speciation event.

COS sequences were first described by Tanksley et al. with identification of a subset of plant genes that have remained relatively stable in both sequence and copy number since the radiation of flowering plants from their last common ancestors [7]. This is the landmark work to enunciate utility of COS sequences in comparative genomics and phylogenetic studies. In a further refinement, Wu et al. [13] identified and annotated a large set of conserved, single-copy, putatively orthologous genes using a set of approaches of computational and phylogenetic algorithms to demonstrate the use of these new ortholog resources to elucidate issues related to comparative genomics, molecular systematics, and gene evolution studies in the euasterid clade. Mingai Li [14] developed widely applicable COS markers (pCOS) for phylogenetic reconstructions at low taxonomic level and found that these markers are highly informative in phylogenetic reconstruction of congeneric species. An understanding of conservation of genome structure among legume species is a prerequisite, to use existing wide genetic diversity present in landraces and wild relatives of legumes [2]. Since the last three decades, we have seen large advancement in linking plant genomes through comparative genetic maps, especially for species belonging to the same family [15]. In the present study, computational approach of finding COS markers in *Vigna* species, we have used ESTs for finding conserved regions as molecular markers constructed from ESTs since they are contained within an exon region of genes that are actually expressed. The present study was undertaken with the objectives of mining the large EST collections of *Vigna* species to identify non-redundant ESTs, design primer pairs across all four species for putative COS markers across the *Vigna* species and finally to test some of these markers in *V. radiata*. To define conserved genic regions between *V. radiata* and other related *Vigna* species with soybean genome (which is the most sequenced and is also a legume) orthologous groups that share among them were identified. Validation is done using some of the newly designed primers from contigs of *Vigna* species and proven their amplification in *V.radiata*.

We demonstrate here the use of this new Ortholog resource to shed light on issues related to comparative genomics, molecular systematics and gene evolution studies in the legumes especially in *Vigna* genus. The present study was undertaken with the objectives of mining the large EST collections of *Vigna* species to identify non-redundant ESTs, design primer pairs across all four species for putative COS markers across the *Vigna* species and finally to test some of these markers in *V. radiata*. To define conserved genic regions between V. radiata and other related *Vigna* species with soybean genome (which is the most sequenced and is also a legume) orthologous groups that share among them were identified

Materials and Methods

EST datasets

The ESTs of all *Vigna* species in the study were searched and retrieved from NCBI (ftp://ftp.ncbi.nih.gov/blast/db/). Also the genome sequence data set of Soybean was downloaded and used as reference genome for identifying conserved genomic regions in *Vigna* species. EST

Processing and assembly

The repetitive and ambiguous sequences in the downloaded ESTs were first trimmed. Subsequently, ESTs with sequences <30 bp were omitted from the final data set. The processed EST sequence files were combined and assembled into contigs using the CAP3 program at both high and low stringency levels. The steps followed while EST analyses [16] are depicted in Figure 1.

Primer designing

The Conserved Primer 2.0 pipeline was implemented and the command line made it possible to design intron-flanking primer pairs or marker candidates for polymorphism discovery in a high-throughput manner and to use any genome size of the model species and any number of the ESTs as inputs without memory and speed restrictions. Processed ESTs are input for designing primers in batch (Tables 1-3) and got custom synthesized.

DNA Isolation

Genomic DNA was isolated using the CTAB isolation method from young leaves collected from plants of 40 mungbean varieties. DNA quantity is estimated by Nanodrop method and quality by running 2uL of genomic DNA solution mixed with 1 uL loading buffer on a 1% agarose gel. The DNA was then diluted to a concentration of 10ng per uL for PCR amplification.

PCR Amplification and marker analysis

To test the feasibility of using *Vigna* COS markers in mungbean, genomic DNA fragments from 40 varieties were amplified using 14 COS markers. PCRs were conducted in a 25-ml reaction volume each reaction consisted of 10 mM Tris-HCl (pH 9.0 at room temperature), 1.5 mM $MgCl_2$, 100 mM each of dNTPs, 0.1 mM each primer, 10 ng of genomic DNA template, and 1unit of Taq DNA polymerase. Reactions were heated at 94^{0}C for 4 min followed by 35 cycles of 1 min at 94^{0}C, 1 min at specific annealing temperature for each primer pairs and a 1-min extension at 72^{0}C. Final reactions were extended at 72^{0}C for 5 min. Amplification was performed in a programmable thermal controller. Following the amplification reactions, the PCR products were separated on 1.8% agarose gel and visualized using ethedium bromide staining (Figure 2). Successful amplification of COS markers are mentioned in table 2.

Results and Discussion

Approximately 6443 ESTs were processed and analyzed for the searching of COS in *Vigna* genus for redundancy minimization and assembling of sequences. The non-redundant ESTs analysed (Figure 1) were used for the development of specific intron-based markers. Soybean genomic sequence database as reference is used to predict intron positions in the EST sequences and then designed a pair of primers flanking the intron position. The Multiple sequence alignments, with the Soybean genomic sequence inferred intron position, facilitate design of PCR primers that anneal to conserved exon sequences and amplify across more diverged introns. A query EST was considered to be homologous to a subject-coding sequence only if there were at least 100 bp overlapping and 80% similarity between them. Only high-quality ESTs are grouped into clusters based on sequence similarity and assembly of clusters which would represent a putative gene, likewise consensus sequences representing putative genes are generated for each *Vigna* species in the study. We found four contigs in *Vigna* radiata from 829 processed ESTs, 33

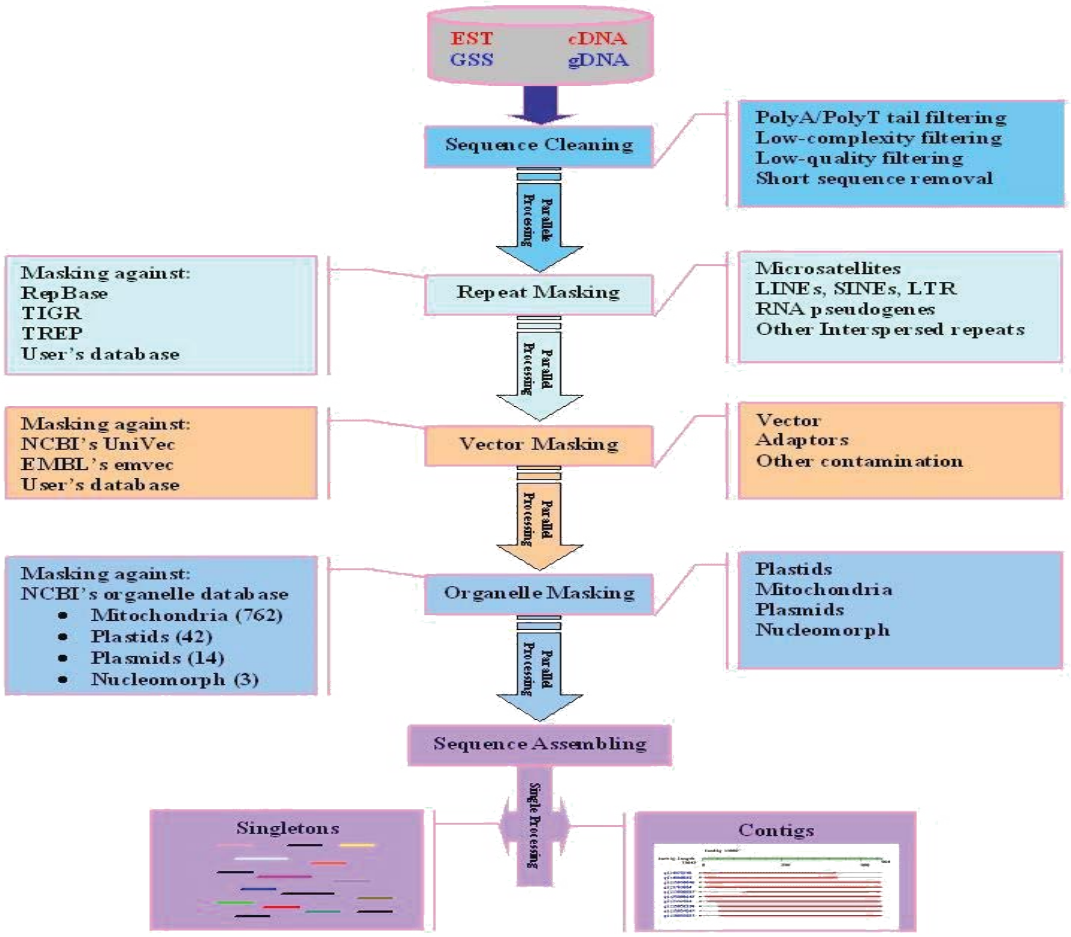

Figure 1: EST processing and Analysis.

Table 1: Statistics of EST analysis for *Vigna* spp.

Species	No. of ESTs	Contigs	No. of Singletons	Predicted genes from contigs	No. of conserved markers found from ESTs
V.radiata	829	4	807	3	2
V.mungo	299	33	198	14	2
V.umbellata	3006	268	1946	95	78
V.unguiculata	2309	175	403	168	134

Table 2: List of conserved primers designed from contigs obtained using available ESTs of *Vigna* species and used for amplification in *V.radiata* genotypes.

SN	SEQ. ID	OLIGO	FORWARD PRIMER	REVERSE PRIMER	REFRENCE	SIZE(bp)	REMARKS
1	XET	47-FR	ACAAACGGTTGCATTTCA	ATGTTCCTTCCCATTAGGAT	Soybean chr03	298	No amplification
2	CSD	48-FR	ACAAACGGTTGCATTTCA	ATGTTCCTTCCCATTAGGAT	Soybean chr08	725	Double bands
3	AGA	49-FR	TTGAATGGAATTGCTGAGA	GTGAATTGACTTCAGCACAA	Soybean chr05	255	Single band
4	SP	50-FR	ACATGCCTAAAGTTGGTTCT	ATTTGGCTTTCCCTCTTT	Soybean chr05	407	Single band
5	CGGCS	51-FR	TTGAATGGAATTGCTGAGA	AATCCAATTCCCATTTCC	Soybean chr08	560	Single band
6	PK	52-FR	TCTTGAAAGAAATGCAGCAC	AAACCAGATACAAACGCTTC	Soybean chr03	732	Single band
7	angularis_CA908726	53-FR	AAGCGATTGCTGAATTGGAT	TCGGTGCTGCTTCTTTAATTT	Soybean chr08	239	Single band
8	CA908739_angularis	54-FR	TTGCTGAATTGGATACATTGG	GCGGCTTCTTTAATTTCATCA	Soybean chr08	236	Double bands
9	DY637441_angularis	55-FR	TTGCTGGAAATGCTTTGTGA	AACGGCATCGCTAACATAAA	Soybean chr05	899	No amplification
10	AB037239_angularis	56-FR	TATGCCGAAGAAATGCAATG	TGTTGGGTGCCATATAATGAA	Soybean chr08	695	Single band
11	mungo.Contig12	57-FR	AAGTCATTTGCACCAGGAA	GAAATTTATCCAAGCGCACA	Soybean chr03	383	Single band
12	umbellata_contig10	58-FR	ATCTTCAATTGCCCTCCATT	TCTTCATGAATGCAAACCAG	Soybean chr08	202	Single band
13	umbellata_contig16	59-FR	TGGTGTTTGCAAGGGAATTT	TTCTTCCCAATAGCATCAACA	Soybean chr08	604	Double bands
14	umbellata_contig44	60-FR	ACCCAAACAATGAGAAAGCA	CACAATCCTTGACAAATCCAAA	Soybean chr05	466	Single band

Table 3: List of Conserved markers identified in the study.

Index	Seq ID	Orientation	Len	Seq	Prod Size	Seq Length	Reference	E-value
							G.max	2e-25/2e-16/1e-
1	FJ896375.1	Fwd	18	ACAAACGGTTGCATTTCA	298	1065	chr03	24
2	FJ896375.1	Rev	20	ATGTTCCTTCCCATTAGGAT				
							G.max	4e-28/e-147/e-
3	FJ896374.1	Fwd	18	ACAAACGGTTGCATTTCA	298	1451	Chr08	162
4	FJ896374.1	Rev	20	ATGTTCCTTCCCATTAGGAT				
							G.max	8e-25/1e-60/2e-
5	EU570914.1	Fwd	18	TTCCATTTGTGCTCTTGA	725	4890	Chr05	45
6	EU570914.1	Rev	18	TTTGGGTGCAATTCTTCA				
							G.max	6e-23/7e-17/8e-
7	HQ999996.1	Fwd	19	TTGAATGGAATTGCTGAGA	255	1202	Chr05	25
8	HQ999996.1	Rev	20	GTGAATTGACTTCAGCACAA				
							G.max	8e-25/1e-60/5e-
9	HQ999996.1_1	Fwd	20	ACATGCCTAAAGTTGGTTCT	407	4584	Chr08	43
10	HQ999996.1_1	Rev	18	ATTTGGCTTTCCCTCTTT				
							G.max	1e-63/4e-47/5e-
11	HQ999996.1_2	Fwd	19	TTGAATGGAATTGCTGAGA	560	6330	Chr05	61
12	HQ999996.1_2	Rev	18	AATCCAATTCCCATTTCC				
							G.max	8e-60/7e-46/6e-
13	AA080680_1	Fwd	18	TTGAAAGAAATGCAGCAC	732	1926	Chr08	51
14	AA080680_1	Rev	20	AAACCAGATACAAACGCTTC				
							G.max	
15	radiata.Contig4_1	Fwd	21	AAATGGACAACCCATTTACCA	862	1133	Chr08	1e-14/4e-11
16	radiata.Contig4_1	Rev	21	CATTCAAATGCTGAGATCGAA				
							G.max	3e-42/2e-27/3e-
17	mungo.Contig12	Fwd	20	AAAGTCATTTGCACCAGGAA	383	525	Chr09	39
18	mungo.Contig12	Rev	20	GAAATTTATCCAAGCGCACA				
							G.max	3e-34/1e-18/2e-
19	umbellata.Contig10	Fwd	20	ATCTTCAATTGCCCTCCATT	202	619	Chr08	16
20	umbellata.Contig10	Rev	21	TCTTCATGAATGCAAACCAGA				
							G.max	5e-27/2e-66/3e-
21	umbellata.Contig10_1	Fwd	20	ATCTTCAATTGCCCTCCATT	202	621	Chr04	59
22	umbellata.Contig10_1	Rev	21	TCTTCATGAATGCAAACCAGA				
							G.max	
23	umbellata.Contig16	Fwd	20	TGGTGTTTGCAAGGGAATTT	604	868	Chr03	8e-32/9e-44
24	umbellata.Contig16	Rev	21	TTCTTCCCAATAGCATCAACA				
							G.max	
25	umbellata.Contig16_1	Fwd	20	TGGTGTTTGCAAGGGAATTT	522	781	Chr07	6e-11/2e-11
26	umbellata.Contig16_1	Rev	21	TTCTTCCCAATAGCATCAACA				
							G.max	8e-16/1e-36/2e-
27	umbellata.Contig29	Fwd	22	AAGCATCTGAAATGGCAGAATA	1056	1088	Chr07	16
28	umbellata.Contig29	Rev	20	ATGTAAGGGCGTCAAATCAA				
							G.max	
29	umbellata.Contig33	Fwd	20	TGTGTTGCTGACAAATCCAA	1038	1263	Chr04	6e-13/1e-84
30	umbellata.Contig33	Rev	22	TTCATCCAATGTATCTGGCAAT				
							G.max	
31	umbellata.Contig44	Fwd	20	ACCCAAACAATGAGAAAGCA	319	466	Chr04	4e-33/1e-73
32	umbellata.Contig44	Rev	22	CACAATCCTTGACAAATCCAAA				
							G.max	
33	umbellata.Contig44_1	Fwd	20	ACCCAAACAATGAGAAAGCA	325	472	Chr07	2e-50/3e-98
34	umbellata.Contig44_1	Rev	22	CACAATCCTTGACAAATCCAAA				

							G.max	
35	umbellata.Contig48	Fwd	21	TCAATTGCATTTGTGGAAGAA	371	420	Chr01	4e-13/5e-25
36	umbellata.Contig48	Rev	21	CGAATATATTGCCAACCCAAA				
							G.max	3e-20/7e-15/5e-
37	umbellata. Contig48_1	Fwd	21	TCAATTGCATTTGTGGAAGAA	352	406	Chr01	28
38	umbellata. Contig48_1	Rev	21	CGAATATATTGCCAACCCAAA				
							G.max	4e-13/3e-11/8e-
39	umbellata. Contig48_2	Fwd	21	TCAATTGCATTTGTGGAAGAA	351	415	Chr04	24
40	umbellata. Contig48_2	Rev	21	CGAATATATTGCCAACCCAAA				
							G.max	
41	umbellata. Contig48_3	Fwd	21	TCAATTGCATTTGTGGAAGAA	351	407	Chr02	2e-12/3e-23
42	umbellata. Contig48_3	Rev	21	CGAATATATTGCCAACCCAAA				
							G.max	
43	umbellata. Contig48_5	Fwd	21	TCAATTGCATTTGTGGAAGAA	367	416	Chr08	8e-13/1e-24
44	umbellata. Contig48_5	Rev	21	CGAATATATTGCCAACCCAAA				
							G.max	
45	umbellata.Contig70	Fwd	21	AATGTTCCCGAAGATGACATT	670	844	Chr03	2e-19/3e-43
46	umbellata.Contig70	Rev	22	TTGTTCTTGGACAAGCATGTTA				
							G.max	
47	umbellata.Contig86	Fwd	20	ATGTTGGCATTCTTGGGATT	213	862	Chr04	2e-75/2e-87
48	umbellata.Contig86	Rev	22	TCATGCCCAAATCTATCTCAAA				
							G.max	
49	umbellata. Contig86_1	Fwd	20	ATGTTGGCATTCTTGGGATT	213	827	Chr09	7e-39/8e-11
50	umbellata. Contig86_1	Rev	22	TCATGCCCAAATCTATCTCAAA				
							G.max	5e-32/4e-23/1e-
51	umbellata.Contig89	Fwd	20	TTTGACGGCATTCAAAGTGA	221	293	Chr08	51
52	umbellata.Contig89	Rev	20	TCACCAACATTTCCAACGAT				
							G.max	
53	umbellata.Contig91	Fwd	22	TTCAATTGGTTTAATGCCAGAA	1008	1083	Chr03	7e-16/8e-34
54	umbellata.Contig91	Rev	22	TTCCACCATCATCAAATAATGC				
							G.max	
55	umbellata.Contig94	Fwd	22	TTTGTTTATGTTGTTGCTCGAA	436	596	Chr09	3e-20/3e-42
56	umbellata.Contig94	Rev	22	TTCAATATTTGCAGCTTCCTTG				
							G.max	1e-20/2e-34/2e-
57	umbellata. Contig94_1	Fwd	22	TTTGTTTATGTTGTTGCTCGAA	428	513	Chr03	43
58	umbellata. Contig94_1	Rev	22	TTCAATATTTGCAGCTTCCTTG				
59	umbellata. Contig110	Fwd	20	TCCAATCGGAAAGTGAACAA	878	977	*G.max*	2e-19/4e-17/1e-
							Chr01	38
60	umbellata. Contig110	Rev	21	TTCTCCATAAACGGAACCAAA				
							G.max	
61	umbellata. Contig110_1	Fwd	20	TCCAATCGGAAAGTGAACAA	805	904	Chr05	1e-36/5e-11
62	umbellata. Contig110_1	Rev	21	TTCTCCATAAACGGAACCAAA				
							G.max	
63	umbellata. Contig117	Fwd	20	ATTTGCGATATGGTGCGATT	446	662	Chr05	e-114/1e-18
64	umbellata. Contig117	Rev	20	TGCACCAATCAAACGTGAAA				

							G.max	
65	umbellata. Contig117_1	Fwd	20	ATTTGCGATATGGTGCGATT	446	645	Chr04	1e-61/4e-15
66	umbellata. Contig117_1	Rev	20	TGCACCAATCAAACGTGAAA				
							G.max	e-102/2e-97/e-
67	umbellata. Contig121	Fwd	20	AAAGACCATTTGCTGCCATT	261	998	Chr05	101
68	umbellata. Contig121	Rev	21	CAAATTTGTCTGCAATCACCA				
							G.max	e-110/2e-91/e-
69	umbellata. Contig122	Fwd	22	TTTCTTGTCCAATCCATAAGCA	739	913	Chr05	101
70	umbellata. Contig122	Rev	21	TCATTGGGATTGATCTTGGAA				
							G.max	e-107/1e-86/4e-
71	umbellata. Contig122_1	Fwd	22	TTTCTTGTCCAATCCATAAGCA	735	1992	Chr08	99
72	umbellata. Contig122_1	Rev	21	TCATTGGGATTGATCTTGGAA				
							G.max	e-102/2e-97/e-
73	umbellata. Contig122_2	Fwd	22	TTTCTTGTCCAATCCATAAGCA	740	1927	Chr08	103
74	umbellata. Contig122_2	Rev	21	TCATTGGGATTGATCTTGGAA				
							G.max	
75	umbellata. Contig122_3	Fwd	22	TTTCTTGTCCAATCCATAAGCA	710	887	Chr07	e-115/1e-18
76	umbellata. Contig122_3	Rev	21	TCATTGGGATTGATCTTGGAA				
							G.max	
77	umbellata. Contig125	Fwd	21	TTTCAATGGCCTTGATTTCAG	341	489	Chr04	1e-21/3e-22
78	umbellata. Contig125	Rev	20	TCGGGATAATTCTGCATTTG				
							G.max	8e-13/4e-27/6e-
79	umbellata. Contig127	Fwd	21	TTCATCATTCATTCTCCTCCA	204	866	Chr08	23
80	umbellata. Contig127	Rev	22	TGTGCACAATTTCTCTGTTTGT				
							G.max	
81	umbellata. Contig130	Fwd	20	TTGCAACGATGAAGAAAGGT	753	2237	Chr08	1e-40/4e-16
82	umbellata. Contig130	Rev	22	TCCAATGATACATTTGGAGGAA				
							G.max	6e-12/8e-36/1e-
83	umbellata. Contig130_2	Fwd	20	TTGCAACGATGAAGAAAGGT	740	886	Chr05	13
84	umbellata. Contig130_2	Rev	22	TCCAATGATACATTTGGAGGAA				
							G.max	
85	umbellata. Contig137	Fwd	20	TCCAGGCATTCTTTGTGAAA	498	969	Chr01	2e-14/e-107
86	umbellata. Contig137	Rev	21	TTTGGATTCACACCATGAACA				
							G.max	4e-14/2e-19/2e-
87	umbellata. Contig137_1	Fwd	21	TGCAATGTTCATGGTGTGAAT	203	762	Chr03	12
88	umbellata. Contig137_1	Rev	22	TTTCCAAACACATCCAACTTGA				
							G.max	1e-44/1e-20/2e-
89	umbellata. Contig140	Fwd	20	AAACCACCAATGTTCCACAA	329	2108	Chr07	34
90	umbellata. Contig140	Rev	21	TTTCAATGGAGGCTTTCTTCA				

							G.max	
91	umbellata. Contig148	Fwd	20	CATTTCATCGAACAGTGCAA	414	2945	Chr02	9e-46/2e-28
92	umbellata. Contig148	Rev	22	AGCTCAACATCGGATTCAATTA				
							G.max	
93	umbellata. Contig152	Fwd	22	TGGAACCTTTGATGTTTCCATA	848	1195	Chr02	3e-28/4e-43
94	umbellata. Contig152	Rev	20	TCACATCCAACAGCAACAAA				
							G.max	
95	umbellata. Contig171	Fwd	21	TCCATGTGATTGCTTGTTTGA	430	810	Chr05	3e-44/2e-14
96	umbellata. Contig171	Rev	20	TTCATCGGTTCTTGGAGAAA				
							G.max	8e-38/e-119/1e-
97	unguiculata. Contig8	Fwd	21	TTAATCCCAAGGCCAAATCTT	820	1561	Chr06	67
98	unguiculata. Contig8	Rev	22	GGATGAGAATCATTCCAACAAA				
							G.max	
99	unguiculata. Contig8_1	Fwd	21	TTAATCCCAAGGCCAAATCTT	1117	1827	Chr01	4e-43/4e-15
100	unguiculata. Contig8_1	Rev	22	GGATGAGAATCATTCCAACAAA				
							G.max	
101	unguiculata. Contig8_2	Fwd	21	TTAATCCCAAGGCCAAATCTT	1081	1401	Chr02	4e-18/3e-22
102	unguiculata. Contig8_2	Rev	22	GGATGAGAATCATTCCAACAAA				
							G.max	
103	unguiculata. Contig9	Fwd	22	TTCCTGGATCGTCAAATTTCTT	1163	1250	Chr03	8e-63/4e-15
104	unguiculata. Contig9	Rev	20	AAACCATGTTTCGTCAACCA				
							G.max	
105	unguiculata. Contig13	Fwd	21	GCTTTGGTTTGAGGAATTTCA	467	1435	Chr04	2e-42/2e-54
106	unguiculata. Contig13	Rev	22	TGAAGAAATGGATTCATTGTGG				
							G.max	3e-22/2e-36/3e-
107	unguiculata. Contig14	Fwd	21	TTTCACTGCCAAGAAACTTGA	640	999	Chr06	13
108	unguiculata. Contig14	Rev	21	CAAGAAACAACCAACACGAAA				
							G.max	
109	unguiculata. Contig14_2	Fwd	21	TTTCACTGCCAAGAAACTTGA	1463	2134	Chr01	3e-22/8e-20
110	unguiculata. Contig14_2	Rev	22	CCAATATCCTTCAAAGCACAAA				
							G.max	
111	unguiculata. Contig14_3	Fwd	21	TTTCACTGCCAAGAAACTTGA	1080	1153	Chr02	2e-17/1e-15
112	unguiculata. Contig14_3	Rev	22	CCAATATCCTTCAAAGCACAAA				
							G.max	6e-67/2e-30/3e-
113	unguiculata. Contig16_1	Fwd	21	AAATTGGTGCGAGATTTCAAG	257	764	Chr04	16
114	unguiculata. Contig16_1	Rev	20	TTTCGGATTTCGATGGATTT				
							G.max	5e-58/2e-23/8e-
115	unguiculata. Contig16_2	Fwd	21	TCTTCCGAAACGATGAACATT	338	796	Chr06	20
116	unguiculata. Contig16_2	Rev	20	TTTCGGATTTCGATGGATTT				

							G.max	1e-46/9e-26/3e-
117	unguiculata. Contig16_3	Fwd	21	TCTTCCGAAACGATGAACATT	338	783	Chr06	22
118	unguiculata. Contig16_3	Rev	20	TTTCGGATTTCGATGGATTT				
							G.max	5e-49/9e-26/8e-
119	unguiculata. Contig16_4	Fwd	21	AAATTGGTGCGAGATTTCAAG	257	758	Chr06	20
120	unguiculata. Contig16_4	Rev	20	TTTCGGATTTCGATGGATTT				
							G.max	
121	unguiculata. Contig16_5	Fwd	21	AAATTGGTGCGAGATTTCAAG	257	756	Chr07	2e-11/5e-21
122	unguiculata. Contig16_5	Rev	20	TTTCGGATTTCGATGGATTT				
							G.max	4e-65/2e-70/5e-
123	unguiculata. Contig19	Fwd	20	CATGCCATGGAAATCATTCA	1500	1843	Chr02	15
124	unguiculata. Contig19	Rev	20	ATCAGCCAAACATTCAGCAA				
							G.max	
125	unguiculata. Contig19_1	Fwd	22	AAGACAATTGCTGAATGTTTGG	200	2037	Chr04	6e-67/4e-31
126	unguiculata. Contig19_1	Rev	21	CCTTGGCAACTCTTTCAATTT				
							G.max	
127	unguiculata. Contig24	Fwd	20	TCTTTGCCATTCACATGCTT	234	1603	Chr08	2e-14/8e-14
128	unguiculata. Contig24	Rev	20	TGTCGGATTTGATTGCTTGA				
							G.max	5e-53/1e-84/e-
129	unguiculata. Contig24_1	Fwd	20	TCAAGCAATCAAATCCGACA	1224	1247	Chr09	139
130	unguiculata. Contig24_1	Rev	22	CAATTTCAATCTTGGCATTCAA				
							G.max	
131	unguiculata. Contig25	Fwd	21	TGAATTTGCCTCCACTTTCAT	1460	1795	Chr02	4e-31/0.0
132	unguiculata. Contig25	Rev	20	TTCAATTGGGATCACAGCAT				
							G.max	
133	unguiculata. Contig33	Fwd	21	TCGTGGCAAACTTATGTTGAT	1478	1728	Chr08	9e-26/4e-65
134	unguiculata. Contig33	Rev	21	CATCATAAATGCCAATGACCA				
							G.max	6e-30/2e-29/e-
135	unguiculata. Contig33_1	Fwd	21	TCGTGGCAAACTTATGTTGAT	1145	1285	Chr05	103
136	unguiculata. Contig33_1	Rev	20	TTGTGCAGAAGCCAATGAAA				
							G.max	1e-24/1e-43/1e-
137	unguiculata. Contig33_2	Fwd	21	TGGTCATTGGCATTTATGATG	252	1811	Chr08	77
138	unguiculata. Contig33_2	Rev	22	CAAGCATCAAACACTTTCTTCA				
							G.max	
139	unguiculata. Contig37	Fwd	20	AATCGGCTCAAAGGTGAAAT	1097	1630	Chr07	2e-11/2e-11
140	unguiculata. Contig37	Rev	20	TTGCTTCCTGAAACGAAATG				
							G.max	1e-77/2e-23/2e-
141	unguiculata. Contig47	Fwd	20	AGCATTGCCAACGACAATAA	265	780	Chr06	17
142	unguiculata. Contig47	Rev	20	TTCGATATCGTTTCCATCCA				

							G.max	4e-80/2e-23/8e-
143	unguiculata. Contig47_1	Fwd	20	AGCATTGCCAACGACAATAA	267	782	Chr06	20
144	unguiculata. Contig47_1	Rev	20	TTCGATATCGTTTCCATCCA				
							G.max	
145	unguiculata. Contig47_2	Fwd	20	AGCATTGCCAACGACAATAA	267	780	Chr04	4e-80/2e-23
146	unguiculata. Contig47_2	Rev	20	TTCGATATCGTTTCCATCCA				
							G.max	
147	unguiculata. Contig47_3	Fwd	20	AGCATTGCCAACGACAATAA	270	624	Chr07	2e-14/2e-11
148	unguiculata. Contig47_3	Rev	20	TTCGATATCGTTTCCATCCA				
							G.max	
149	unguiculata. Contig47_4	Fwd	20	AGCATTGCCAACGACAATAA	246	324	Chr08	6e-48/2e-42
150	unguiculata. Contig47_4	Rev	20	TTCGATATCGTTTCCATCCA				
151	unguiculata. Contig55	Fwd	20	AAATTGCATCATGCTCGTGT	1236	1279	*G.max*	5e-86/7e-82
							Chr04	
152	unguiculata. Contig55	Rev	22	TGATCTATAAATGGCAGCAACA				
							G.max	
153	unguiculata. Contig56	Fwd	20	TTTCAGCGAATTGTTGGAGT	1257	1544	Chr02	0.0/1e-24
154	unguiculata. Contig56	Rev	20	TTTGCTTCTTTCGATTCCTG				
							G.max	1e-31/1e-18/4e-
155	unguiculata. Contig63	Fwd	21	AAGGATGCCAAGAAGAAGAAA	289	1706	Chr08	16
156	unguiculata. Contig63	Rev	21	TCATTCCTGCCTTCAAAGAAA				
							G.max	4e-34/4e-25/6e-
157	unguiculata. Contig63_2	Fwd	21	AAGGATGCCAAGAAGAAGAAA	284	2261	Chr05	18
158	unguiculata. Contig63_2	Rev	21	TCATTCCTGCCTTCAAAGAAA				
							G.max	2e-39/9e-17/1e-
159	unguiculata. Contig63_3	Fwd	21	AAGGATGCCAAGAAGAAGAAA	289	1409	Chr05	15
160	unguiculata. Contig63_3	Rev	21	TCATTCCTGCCTTCAAAGAAA				
							G.max	1e-24/9e-26/5e-
161	unguiculata. Contig63_4	Fwd	21	AAGGATGCCAAGAAGAAGAAA	287	1242	Chr04	18
162	unguiculata. Contig63_4	Rev	21	TCATTCCTGCCTTCAAAGAAA				
							G.max	
163	unguiculata. Contig70	Fwd	20	TTCAAGGCAGGCAAATACAA	402	1776	Chr01	4e-22/e-132
164	unguiculata. Contig70	Rev	22	CAAAGCAACAGCATTGTCATAA				
							G.max	e-135/3e-13/1e-
165	unguiculata. Contig70_1	Fwd	20	TTCAAGGCAGGCAAATACAA	402	1304	Chr03	21
166	unguiculata. Contig70_1	Rev	22	CAAAGCAACAGCATTGTCATAA				
							G.max	
167	unguiculata. Contig72	Fwd	22	AAAGCCATGTTCTTCATCTTGA	298	598	Chr09	6e-39/1e-18
168	unguiculata. Contig72	Rev	20	TCCAAGCAAAGTCCCAATTT				

							G.max	2e-66/3e-16/5e-
169	unguiculata. Contig87	Fwd	22	TTTATTCGAGGAAGCAAAGTCA	255	2168	Chr05	27
170	unguiculata. Contig87	Rev	20	TGCATCCAAACGCTTAAACA				
							G.max	e-157/6e-19/6e-
171	unguiculata. Contig87_1	Fwd	22	TTTATTCGAGGAAGCAAAGTCA	254	1666	Chr07	28
172	unguiculata. Contig87_1	Rev	20	TGCATCCAAACGCTTAAACA				
							G.max	e-157/4e-26/2e-
173	unguiculata. Contig89	Fwd	21	AAAGTGCTTTGGGTTTGTGAA	1361	2190	Chr02	25
174	unguiculata. Contig89	Rev	20	CAAAGAAATTTGGCACAGGA				
							G.max	4e-37/e-114/5e-
175	unguiculata. Contig89_1	Fwd	21	AAAGTGCTTTGGGTTTGTGAA	1327	2157	Chr04	55
176	unguiculata. Contig89_1	Rev	20	CAAAGAAATTTGGCACAGGA				
							G.max	6e-27/e-106/3e-
177	unguiculata. Contig90	Fwd	20	ATTTACAAAGCCGTCCATGA	726	1547	Chr06	50
178	unguiculata. Contig90	Rev	20	TTCAAACCGACCAATGTGTT				
							G.max	
179	unguiculata. Contig90_1	Fwd	20	ATTTACAAAGCCGTCCATGA	711	1504	Chr09	1e-24/0.0
180	unguiculata. Contig90_1	Rev	20	TTCAAACCGACCAATGTGTT				
							G.max	
181	unguiculata. Contig91	Fwd	21	TTTGAAATGGCTTTGTCACTG	830	1151	Chr02	e-108/e-132
182	unguiculata. Contig91	Rev	22	TTTCAAGATCTTTGCACTGGTT				
							G.max	7e-39/2e-48/1e-
183	unguiculata. Contig98	Fwd	20	TTCCATTGTTCGTTTGACCA	439	1941	Chr05	58
184	unguiculata. Contig98	Rev	22	TGGTTTGTTGGCTTACCATAAA				
							G.max	5e-86/6e-30/5e-
185	unguiculata. Contig98_1	Fwd	20	TTCCATTGTTCGTTTGACCA	1134	1675	Chr02	61
186	unguiculata. Contig98_1	Rev	22	TGGTTTGTTGGCTTACCATAAA				
							G.max	
187	unguiculata. Contig105_1	Fwd	22	ATGAAATTCAAGGATGGGTACA	278	478	Chr07	3e-13/1e-15
188	unguiculata. Contig105_1	Rev	20	TTTAGGATCCCAATCAAGCA				
							G.max	2e-76/5e-21/2e-
189	unguiculata. Contig113	Fwd	20	AGCATTGCCAACGACAATAA	266	780	Chr06	17
190	unguiculata. Contig113	Rev	22	GTTCGATATCATTTCCATCCAA				
							G.max	7e-79/5e-21/8e-
191	unguiculata. Contig113_1	Fwd	20	AGCATTGCCAACGACAATAA	268	760	Chr06	20
192	unguiculata. Contig113_1	Rev	22	GTTCGATATCATTTCCATCCAA				
							G.max	
193	unguiculata. Contig113_2	Fwd	20	AGCATTGCCAACGACAATAA	268	758	Chr04	1e-37/5e-12
194	unguiculata. Contig113_2	Rev	22	GTTCGATATCATTTCCATCCAA				

							G.max	4e-46/3e-22/6e-
195	unguiculata. Contig113_4	Fwd	20	AGCATTGCCAACGACAATAA	271	624	Chr05	36
196	unguiculata. Contig113_4	Rev	22	GTTCGATATCATTTCCATCCAA				
							G.max	2e-14/e-108/2e-
197	unguiculata. Contig121	Fwd	20	TTTGCGAAGAAGGACTTCAA	201	1532	Chr05	35
198	unguiculata. Contig121	Rev	20	AGGAACAATTTCGGAAGGAA				
							G.max	
199	unguiculata. Contig121_1	Fwd	20	TTTGCGAAGAAGGACTTCAA	201	1783	Chr01	2e-14/5e-70
200	unguiculata. Contig121_1	Rev	20	AGGAACAATTTCGGAAGGAA				
							G.max	
201	unguiculata. Contig121_2	Fwd	20	TTTGCGAAGAAGGACTTCAA	201	397	Chr07	2e-22/6e-23
202	unguiculata. Contig121_2	Rev	20	AGGAACAATTTCGGAAGGAA				
							G.max	5e-27/1e-49/3e-
203	unguiculata. Contig126	Fwd	20	TTTGGGTTTGAGATTCCTGA	253	1596	Chr03 (	53
204	unguiculata. Contig126	Rev	20	TGAAGTTCGGTTATGCCAAT				
							G.max	3e-47/7e-48/e-
205	unguiculata. Contig138	Fwd	22	AATGAAGTCAACGAAATCTCCA	1253	1585	Chr03	144
206	unguiculata. Contig138	Rev	22	ATTCTCCTCATCCAAAGGATTT				
							G.max	1e-31/2e-33/e-
207	unguiculata. Contig138_1	Fwd	22	AATGAAGTCAACGAAATCTCCA	1039	1765	Chr02	116
208	unguiculata. Contig138_1	Rev	22	ATTCTCCTCATCCAAAGGATTT				
							G.max	9e-23/4e-34/e-
209	unguiculata. Contig138_2	Fwd	22	AATGAAGTCAACGAAATCTCCA	1047	1381	Chr02	135
210	unguiculata. Contig138_2	Rev	22	ATTCTCCTCATCCAAAGGATTT				
							G.max	9e-17/e-123/e-
211	unguiculata. Contig138_3	Fwd	22	AATGAAGTCAACGAAATCTCCA	883	1218	Chr07	111
212	unguiculata. Contig138_3	Rev	22	ATTCTCCTCATCCAAAGGATTT				
							G.max	
213	unguiculata. Contig142	Fwd	20	AAACGGCTTCAAACATTGGT	874	956	Chr02	6e-14/1e-33
214	unguiculata. Contig142	Rev	21	CCATAATCATTTGGGTTTCCA				
							G.max	1e-58/4e-43/2e-
215	unguiculata. Contig167	Fwd	20	TTTGGTTTGCCACTTCGTAA	255	1393	Chr05	42
216	unguiculata. Contig167	Rev	20	CAATGCCCGATTAATCTCAA				
							G.max	
217	unguiculata. Contig167_1	Fwd	20	TTTGGTTTGCCACTTCGTAA	250	1531	Chr04	3e-25/2e-11
218	unguiculata. Contig167_1	Rev	20	CAATGCCCGATTAATCTCAA				
							G.max	2e-23/e-114/2e-
219	unguiculata. Contig170	Fwd	21	TTAATCCCAAGGCCAAATCTT	819	1530	Chr06	51
220	unguiculata. Contig170	Rev	22	GATGAGAATCATTCCAACGAAA				

							G.max	
221	unguiculata. Contig170_1	Fwd	21	TTAATCCCAAGGCCAAATCTT	1116	1791	Chr01	5e-40/9e-20
222	unguiculata. Contig170_1	Rev	22	GATGAGAATCATTCCAACGAAA				
							G.max	
223	unguiculata. Contig170_2	Fwd	21	TTAATCCCAAGGCCAAATCTT	1080	1392	Chr02	3e-29/6e-18
224	unguiculata. Contig170_2	Rev	22	GATGAGAATCATTCCAACGAAA				
							G.max	
225	unguiculata. Contig175	Fwd	20	ATGCAGGTCACAATTGCTTT	323	685	Chr05	3e-19/1e-30
226	unguiculata. Contig175	Rev	22	TTGACTATTGATGGTTGGGTTT				
							G.max	
227	unguiculata. Contig175_1	Fwd	20	ATGCAGGTCACAATTGCTTT	911	1114	Chr04	2e-69/3e-40
228	unguiculata. Contig175_1	Rev	22	TTGACTATTGATGGTTGGGTTT				
							G.max	
229	unguiculata. Contig175_2	Fwd	20	ATGCAGGTCACAATTGCTTT	490	854	Chr04	2e-69/3e-40
230	unguiculata. Contig175_2	Rev	22	TTGACTATTGATGGTTGGGTTT				

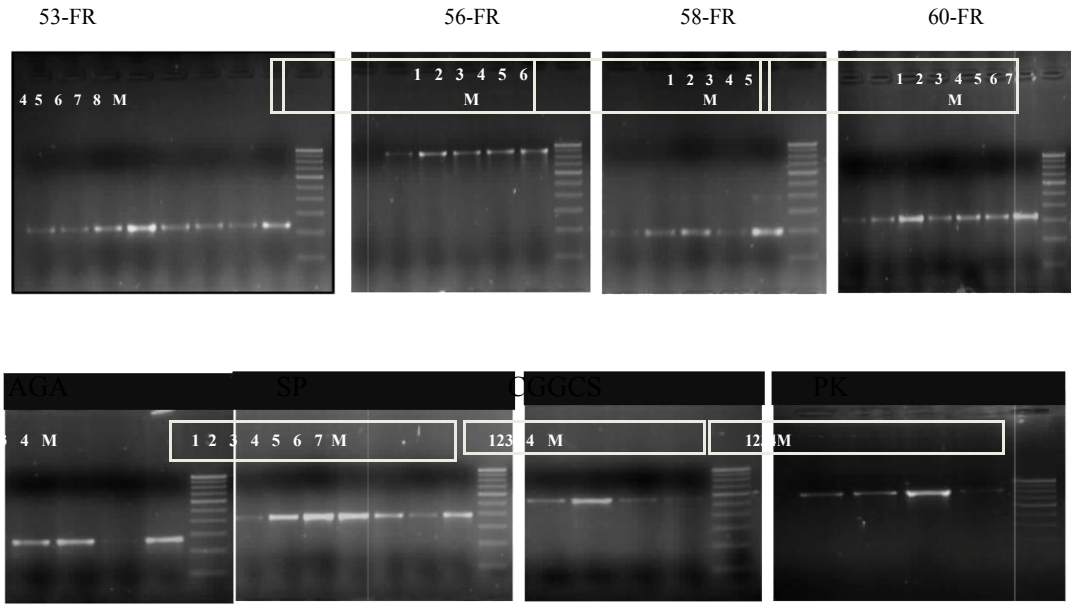

Figure 2: Representative patterns of DNA Amplification of single copy genes in *V.radiata*.

contigs for *V. mungo* from 299 processed ESTs, 268 contigs out of 3006 ESTs of *V. umbellata,* and 175 contigs from 2309 ESTs belonging to *V. unguiculata* (Table 1). These high-quality ESTs are grouped into 'clusters' based on sequence similarity. The maximum informative consensus sequences generated by assembling these clusters represent a putative gene. The output of all *Vigna* EST processing pipeline is the list of putative genes belonging to respective *Vigna* species providing list of 230 conserved primers. Of these, 14 markers were used to validate the COS markers generated with amplification of genomic DNA from 40 genotypes of *Vigna radiata* in Indian collections.

The successful amplification revealed the mixed type of result such as single band and multiple bands. To confirm the amplification, repeated reactions were carried for multiple/double banded amplicons and there was no change in such reactions even after changing the required components and thermal cycling conditions of PCR reaction. Three primer pairs (CSD-48FR, CA908739-54FR and umbellata_contig16-59FR) produced double or multiple bands which indicates that they probably multi gene loci and hence were not considered. Primer–genotype combinations that gave what appeared to be single bands on agarose gels were noted which are likely to be noted as single copy genes are considered as orthologs. Primers that produced single bands with genotypes of *V. radiata* varieties were 53-FR, 56-FR, 58-FR, 60-FR, AGA, SP, CGGCS and PK (Figure 2). Of these, the primer pair 57-FR was derived from contig derived from

ESTs belonging to *Vigna mungo*, whereas 58-FR and 60-FR were from contig of *V. umbellata*. This infers that the there is a possibility of these kind of markers derived from available genomic information (ESTs) of related species can amplify in *V.radiata.* Finally we have found that amplification of single copy genes attained were from 11 primer pairs (Figure 2).

Molecular (COS) markers in comparative genomic studies within *Vigna*

Though much progress has been made in the genomics of *Vigna* species, yet it is still far behind that in other grain legumes like common bean and soybean. Most of the cultivated *Vigna* species have a narrow genetic base resulting in limited marker polymorphism within the germplasm. Due to this major limitation, most of the genetic linkage maps in *Vigna* species have been constructed using inter-specific or inter-sub specific crosses to increase the level of polymorphism. The use of COS markers as a starting point for marker development was motivated by their expected low copy number in the genomes of various legume species for which genomic information is not abundant. A major challenge for comparative legume genomics is to translate information gained from model species into improvements in crop legumes. The complexity of that challenge may well be defined by the structural and functional similarities and dissimilarities among these very fascinating genomes. Agriculturally important traits captured during domestication are often coded by very limited number of loci with major phenotypic effects. It is common to find that these loci have putative orthologous counterparts (Orthologous Set markers) in other species and therefore such molecular markers are powerful in comparing genomic information across species.

The present study aimed at identifying the COS markers through computational approach and validating some of them in wet-lab screening lead us to use of this new ortholog resource can shed light on issues related to comparative genomics, molecular systematics, and gene evolution studies in the *Vigna* genus. COS markers thus selected can further be taken for characterization to test their applicability for phylogenetic studies in Asiatic *Vigna* species as COS markers are evolutionary conserved single-copy genes of great use in constructing syntenic genetic maps among species. The COS markers reported here will be useful for comparative mapping at the family level and may help to establish the syntenic relationship between genomes of different *Vigna* species, allowing a picture of chromosome evolution. In this study, a set of 230 COS markers were identified using ESTs of four *Vigna* species and some amplified in *V.radiata* can serve as anchor markers for a syntenic map of other *Vigna* species. This study forms the basis for a number of significant outcome for genomics of *Vigna* in general: (1) the genic markers developed here may be used across *Vigna* species to determine patterns of chromosomal evolution, as argued previously for markers with defined utility [17], and to characterize syntenic relationships between V. radiata and other related species under cultivation; (2) with the aid of shared anchor markers, the *Vigna* map created may be integrated with all existing legume maps containing various important domestication traits; (3) the high levels of syntenic relationships if detected between these species will enable the future identification of tightly linked markers for direct marker-assisted trait selection and future map-based isolation of candidate genes.

COS markers conserved in legume species can be used in phylogenetic studies and in the identification of conserved non-coding regions or to make comparative maps of major crop species. Although the amount of genetic data available for plants is increasing exponentially, most of the work is being done in just a few species. The identification of a set of such markers common to a variety of species within genus would allow researchers studying less well-characterized plant species to take advantage of gains being made in legume species such as Medicago, soybean and other model or reference plant species. Because ESTs give an idea of the genes expressed in an organism, and because EST data are abundant for a variety of species, ESTs are ideal starting material for the identification of genes conserved among species.

Acknowledgement

The authors are thankful to the Director, NBPGR, New Delhi, for providing the facilities for the research work. The scholarship provided to the author1 by the Indian Council of Agricultural Research, New Delhi, India, for pursuing Ph.D. research from Indian Agricultural Research Institute, New Delhi is gratefully acknowledged.

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Seth et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00091.4

Vegetos- An International Journal of Plant Research

Research Article

A SCITECHNOL JOURNAL

Evidence of Economic Heterosis and Genetic Control of Fruit Yield and Yellow Vein Mosaic Virus Disease Severity Traits of Okra

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Abstract

Ten diverse genotypes of cultivated and wild species were crossed in half diallel fashion to produce 45 F_1 hybrids to determine mode of gene action, extent of economic heterosis and dominance effect, and to estimate combining ability for eight quantitative traits. Predictability ratio revealed overwhelming response of non-additive gene action for controlling fruit length, number of fruits per plant, and fruit yield per plant; additive gene effects for days to 50% flowering, while both additive and non-additive genetic control for node number at first flowering, fruit weight, fruit girth, and PDI of YVMV disease. Appropriate breeding strategies for improvement of studied traits are highlighted here. Significant standard heterosis over two commercial hybrids, Shakti and Abantika was comparatively lower in magnitude for fruit yield per plant (4.62 % and 17.59 %, respectively) and higher in magnitude for PDI of YVMV disease (-71.28 % and -72.28 %, respectively). Partial- to over-dominance effects were involved in the inheritance of the studied traits. BCO-1 and 11/RES-6 were identified as potential donors for future use. The study could able to identify an outstanding hybrid (BCO-1 × Arka Anamika) having high tolerance both under field and artificial conditions. This hybrid would definitely make a room in okra growing zones of the tropics after critical testing.

Keywords

Combining ability; Dominance effect; Gene action; Heterosis; Okra; YVMV disease

Introduction

Being the leading producer of okra, India got major setback in recent times to attain the optimum productivity of this crop as compared to other leading countries like Ghana, Egypt etc. in spite of its well acceptability among growers and consumers and the maximum range of available genetic resources [1]. One of the major bottlenecks to get optimum productivity is high incidence of yellow vein mosaic virus (YVMV) disease that infects crop at all growth stages [2], and plants of 20, 50 and 65 days old reported to be suffered a yield loss of 94 %, 84 % and 49 %, respectively [3,4]. The hybrids so far developed show variable YVMV tolerance level in the hot spots of the tropics and sometimes having unacceptable fruit quality. Usually, the resistance mechanism of these hybrids has been broken within 2-3 years of cultivation which might be due to pathogenic variability, use of parents which are supposed to be symptomless carrier and emergence of B biotype of whiteflies having wide host range. Number of wild species (*Abelmoschus manihot ssp. manihot, A. tetraphyllus, Abelmoschus caillei, A. tuberculatus, A. pungens, A. crinitus, A. panduraeformis, and A. vitifolius*) has been reported to be of resistant to YVMV disease [5,6,7,8]. Attempts made in the past to study the inheritance pattern of resistance to YVMV disease is rather variable, complex and confusing [9,10,11]. Therefore, development of okra hybrids having high, consistent and durable tolerance against this menace is badly needed in the okra growing zone of the country.

Hybrid breeding offers an immense scope to increase in yield, reproductive ability, adaptability, disease resistance, and fruit quality of okra [12,13]. There seems to be an optimal level of genetic diversity beyond which heterosis does not increase or may even decrease due to unfavourable interaction of co-adopted gene complexes or physiological incompatibility [14]. The mean (*per se*) performance of genotype is not always a reliable indicator for their superior combining ability. Genetic analysis provides a guide line for the assessment of relative breeding potential of the parents or identifies best combiners in crops [15], which could be utilized either to exploit heterosis in F_1 or to accumulate fixable genes to evolve variety. The information about the relative contribution of components of variation *viz*., additive and non-additive, is essential for effective crop improvement programme [16]. The analysis of diallel cross by the method proposed by Griffing [17] which partition the total genetic variation into general combining ability (GCA) of the parents and specific combining ability (SCA) of the crosses have been widely used. Such studies also simultaneously demonstrate the nature and magnitude of gene action involved in the expression of desirable traits and to predict the performance of the progenies.

Thus, the main aim of the present study was to determine the magnitude of economic heterosis and to estimate the dominance effect for fruit yield and its components, and YVMV disease severity, and to assess the nature of gene action for these traits in order to identify good combiners, as well as to formulate the breeding strategy for the genetic improvement of such traits.

Materials and Methods

Breeding material and procedure

Eight optimally diverse genotypes viz. BCO-1, VNR Green, VRO-6, 11/RES-6, 10/RES-6, 10/RES-4, Pusa Sawani, Arka Anamika belonging to *Abelmoschus esculentus* and two wild genotypes of *Abelmoschus manihot* (IC-140950) and *Abelmoschus caillei* (IC-433483) were selected on the basis of fruit characters, yield potentiality and YVMV disease severity as per our previous study [18]. Two standard private bred commercial okra hybrids (Shakti and Abantika) showing high tolerance against YVMV disease for the last couple of years under the Gangetic plains of eastern India were also taken to study the standard heterosis.

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Received: July 27, 2016 **Accepted:** August 23, 2016 **Published:** August 29, 2016

Homozygous seeds of ten parents were sown in well-prepared plot having sandy loam soil (pH 6.5) during the third week of February, 2013 to raise 45 cross combinations in 10 × 10 half diallel mating design as per our previous study [12]. Seeds of 10 parents, 45 hybrids and 2 standard checks (Shakti and Abantika) were sown at a spacing of 60 cm (row to row) x 30 cm (plant to plant) in 3.6 m × 2.7 m plot during third week of July, 2014 following randomized complete block design with 3 replications in the research plots of All India Coordinated Research Project on Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India, situated at 23.5°N latitude and 89°E longitude at a mean sea level of 9.75 m. The period from July to September has been selected for screening of parents/hybrids against YVMV disease because of high population of viruliferous whiteflies which spread the maximum virus disease. Standard cultural practices were followed as per Chattopadhyay et al. [19,20]. No plant protection measures against sucking insect pests of okra were done in and around the experimental area to build up a reasonable amount of whitefly population. At the same time, one row of infected plants of local susceptible variety was sown after every plot of parents and hybrids to ensure sufficient virus inoculums.

Data recording

Data on days to 50% flowering, node number at first flowering, fruit length (cm), fruit diameter (cm), fruit weight (g), number of fruits per plant, and fruit yield per plant (g) were recorded from 20 randomly selected plants of each plot in each replication. Fifteen randomly selected fruits of marketable maturity (7 days after anthesis) were sampled from the selected plants per replication to record the observations on the following fruit characters. All harvested fruits of each plant were counted and weighed to determine average number of fruits per plant and total weight of fruits per plant which was recorded as fresh fruit yield per plant (g).

Monitoring of white fly population

The incidence of YVMV disease depends on the population build up of the vector (*Bemisia tabaci)* and the presence of virus source. Whitefly populations were monitored from July to September and were recorded on five leaves, two each from lower, middle and one from upper canopy of the plants between 5.30 a.m. and 6 a.m. from 5 randomly selected tagged plants of each plot at 10-days interval starting from 20 days after sowing.

Estimation of yellow vein mosaic virus disease severity

Ten parents, forty hybrids and two standard checks were grown without any protective cover of insecticides to take data on percent disease index (PDI). PDI was recorded replication wise at five stages at an interval of 15 days starting from 30 days after sowing (DAS) to 90 DAS. Vein clearing symptom of any form in the plant was treated as disease incidence. The PDI was expressed as percentage taken from all the 54 plants in a replication by using self-made disease severity scale (0-4) for single plant through visual evaluation. The rating of disease severity scale is mentioned below.

Scale	Description of symptom
0	No disease
1	Up to 20% leaf area affected of a plant
2	21 - 40% leaf area affected of a plant
3	41 - 60% leaf area affected of a plant
4	> 60% leaf area affected of a plant

Numbers of plants infected in each parent and hybrid was recorded and PDI (%) was calculated at 90 DAS

Physiological basis of YVMV resistance

Tolerance to YVMV was confirmed by feeding of viruliferous whitefly as suggested by Nariani and Seth [18] in related species of okra. Artificial inoculation by viruliferous whitefly for screening of virus free germplasm and confirmation of presence and/or absence of the virus was done under poly house condition. A wooden frame measuring 45 x 45 x 30 cm was fixed with glass and muslin cloth and the frame was fit on a wooden rectangular base of 45.5 x 45.5 x 10 cm. Twenty whiteflies were released on the twenty okra plants of two most field tolerant hybrids and one most susceptible hybrid grown inside the insect proof rearing cages and subsequently maintained by introducing the young plants into rearing cage. Whiteflies were collected from YVMV infected plants by sucking with the help of the aspirator by slowly turning the leaves slightly upwards. Whiteflies were starved for 1-2 hours and then they were subjected to artificial inoculation feeding for 4-5 hours using YVMV infected leaves. After the acquisition period, the flies were released on the seedlings inside the cages and the feeding of vector on the plants was ensured since they were protecting inside the cages.

Statistical analysis and estimation of genetic parameters

Data of all the eight characters were analyzed statistically using the standard methods of the randomized complete blocks design [21]. The magnitude of heterosis was estimated in relation to standard hybrids and was calculated as percentage increase or decrease of F_1s over standard hybrids (SH) values as per the formula suggested by Wynne et al. [22]. The dominance estimate (D.E.) usually referred to as "potence ratio" was computed using the following formula as suggested by Smith [23].

$$D.E.=F_1 - MP/0.5 \times P_2 - P_1$$

Where, F_1=mean of the hybrid; MP=mid-parent; P_2=mean of the highest parent; P_1=mean of the lowest parent. Over dominance is considered when D.E. exceeds ± 1; Complete dominance is realized when D.E.=+1; while partial dominance is indicated when D.E. is between −1 and +1; D.E.=0 suggests absence of dominance. The '+' and '–' signs indicate the direction of dominance of either parent.

Combining ability variances and effects were worked out according to Griffing's [17] Model 1 and Method 2 as parents and one set of non-reciprocal F'$_1$s were included. Statistical analyses were done using software SPSS Professional Statistics version 7.5 (SPSS Inc., Chicago, IL).

Results and Discussion

Genetic effects for different characters

The analysis of variance for combining ability based on Griffing's Model 1 and Method 2 illustrated that components of gca and sca mean squares were highly significant for fruit yield per plant along with all other quantitative traits in F_1 generation (Table 1), indicated equal importance of both additive and non-additive gene actions in the inheritance of studied traits. The relative importance of genetic effects for quantitative traits is generally ascertained by the predictability ratio [24]. Preponderance of additive gene effects for days to 50% flowering was reflected as their predictability ratios were approaching unity (more than 0.80). In contrast, node number at first flowering, fruit diameter, fruit weight and PDI of YVMV disease

were controlled by both additive and non-additive gene action as their predictability ratios were between >0.50 and <0.80. On the other hand, the ratios <0.50 for fruit length, number of fruits per plant, and fruit yield per plant were indicative of non-additive genetic control of these traits (Table 2). In the present study, the GCA effects could not be considered to predict the performance of the parents, because most of the values of predictability ratio were much lower than the unity. Low predictability ratio highlighted the importance of SCA variance, and hence late selection would be practised based on better heterotic combinations rather than the performance of the parents involved in crossing programmes. Overwhelming response of non-additive gene action for the control of fruit length, number of fruits per plant and fruit yield per plant has been observed, hence selection will bring no or slow genetic improvement. In such case, heterosis breeding could be used to harness it by producing and marketing hybrids affordable to the resource-poor farmers of tropics for increasing okra productivity. The effects of non-additive gene actions for such traits are in a harmony with the findings of Das et al. [12]. On the other hand, selection for traits, such as node at first flowering, fruit weight, fruit diameter, and PDI of YVMV disease, that were governed by both additive and non-additive type of gene actions, might be deferred to later generations to allow a decrease in dominance, additive × dominance, and dominance × dominance effects [25]. The use of reciprocal recurrent selection could improve these traits [26]. Preponderance of both additive and non-additive gene action for the control of node number at first flowering was earlier reported by Jindal et al. [11]. The greater importance of additive gene effects (α^2a) in case of days to 50% flowering suggested the use of breeding systems that emphasize mainly α^2a. The amount of α^2aa contribution to the non-additive variance estimated for such traits is not known. However, if additive × additive epistatic variance was of importance, breeding system would change very little because additive × additive epistatic variance can be exploited by pedigree method. This type of epistatic variance increases during the selfing process so that selection of traits governing earliness of the crop in early generations should be handled accordingly as suggested by Singh et al. [25]. Previous studies also suggested additive genetic control for days to 50% flowering [27].

Standard heterosis and dominance effect of F_1 hybrids

The number of F_1 hybrids displaying either significantly positive or negative heterosis over two standard commercial hybrids (Shakti and Abantika) is presented in Table 3. The cross BCO-1 × Arka Anamika expressed the maximum standard heterosis for fruit yield per plant and PDI of YVMV disease over Shakti (4.62%, -71.28%, respectively) and Abantika (17.59%, -72.28%, respectively). However, the hybrid VRO-6 × 11/RES-6 also exhibited desired significant standard heterosis for fruit yield per plant and PDI of YVMV disease over Abantika (12.00%, -55.82%, respectively) and also showed heterosis for PDI of YVMV disease over Shakti (-54.24 %). In general, high yielding crosses exhibited low severity of YVMV disease and less population density of whiteflies as revealed from the Figures 1-2. The correlation study also depicted that fruit yield expressed strong inverse relations with PDI of YVMV disease and average whitefly population per leaf (Table 4). The highest mean (*per se*) performance for fruit yield per plant along with low severity of YVMV disease was recorded in BCO-1 followed by 11/RES-6 (Table 2). Thus, two promising crosses involved at least one parent having high yield potential with low disease severity. The hybrids with negative estimates of heterosis for days to 50% flowering, node number at first flowering and PDI of YVMV disease are desirable and could always be exploited. Our results are in well comparable with Jagan et al. [28] for node number at first flowering. Significant negative standard heterosis for PDI of YVMV disease has also been reported [29]. Fruit yield of crosses were highly influenced by YVMV disease severity and whitefly population density. The inverse relationships between yield and disease causing factors have also been reported from the Gangetic plains of eastern India [30]. On the other hand, positively significant standard heterosis for fruit length, fruit diameter, fruit weight, number of fruits per plant and fruit yield per plant found in our study, have also been reported [12,13,29,31,32] and could be useful for selection of high yielding hybrids. No problem of cross compatibility has been observed between cultivated (*A. esculenta*) and two wild species (*A. manihot* and *A. caillei*) as well as between two wild species. However, the expression of two wild species in a series of hybrid combinations with parents of cultivated species (*A.esculenta*) did not show any promise with regard to fruit yield and YVMV disease tolerance. These wild species might have acted as symptomless carrier of YVMV disease as reported earlier by Nariani and Seth [18]. Therefore, utilization of wild parent belonging to these species may be discouraged in hybrid development programme of okra.

The values of dominance estimates illustrated in 45 F_1 hybrids are presented in Table 5. Preponderance of partial dominance was reflected in most of hybrids in days to 50% flowering and fruit diameter. Overwhelming response of over dominance in majority of the hybrids was evident in conditioning of characters like node at first flowering, fruit length, number of fruits per plant, fruit yield per plant and PDI of YVMV disease. Thus the present study reflected various degrees of dominance; i.e., complete, partial to over-dominance or absence of dominance which involved in the inheritance of characters studied. To the best of our knowledge no previous works have been documented so far in okra to support our findings.

Table 1: Analysis of variance (mean square) for combining ability of eight characters in okra.

Source of variation (d.f.)	D50F	NFF	FL	FD	FW	NFPP	FYPP	PDI
GCA (9)	433.64**	24.67**	5.80**	0.53**	45.40**	65.08**	7659.30**	1501.19**
SCA (45)	4.30**	1.75**	0.64**	0.02**	3.16**	15.88**	1133.74**	109.41**
Error (108)	1.98	0.02	0.0014	0.0007	0.11	0.09	6.24	6.61
α^2a	35.97	2.05	0.48	0.04	3.77	5.42	637.76	124.55
α^2na	2.32	1.73	0.64	0.02	3.05	15.80	1127.50	102.80
Predictability ratio $\alpha^2a/(\alpha^2a + \alpha^2na)$	0.94	0.54	0.43	0.73	0.55	0.26	0.36	0.55

D50F= Days to 50% flowering, NFF= Node at 1st flowering; FL= Fruit length (cm), FD=Fruit diameter (cm),

FW= Fruit weight (g), NFPP= Number of fruits per plant, FYPP= Fruit yield per plant (g),

PDI= Percent Disease Index (%) of YVMV disease.

** Significant at 1% level

Table 2: Mean (*per se*) performance of 10 parents for eight characters of okra.

Parents	D50F	NFF	FL	FD	FW	NFPP	FYPP	PDI
BCO-1	47.33	6.50	8.35	1.45	9.01	25.07	177.53	9.56
VNR Green	49.00	5.03	6.93	1.46	9.90	12.17	113.88	17.25
VRO-6	46.67	4.07	7.86	1.52	9.02	13.33	111.09	60.23
11/RES-6	49.00	5.07	5.87	1.28	5.58	16.80	170.00	14.40
10/RES-6	46.00	4.50	7.72	1.60	9.59	16.40	111.80	45.86
10/RES-4	47.00	6.50	7.90	1.46	8.21	18.33	108.79	55.40
Pusa Sawani	48.00	6.03	9.00	2.20	8.75	5.63	48.46	74.29
Arka Anamika	49.67	6.00	8.20	1.42	7.40	6.47	55.18	64.02
A. manihot	75.00	9.00	6.16	1.46	5.61	27.57	32.64	27.72
A. caillei	79.67	11.03	12.00	2.73	21.02	7.67	95.43	25.46

D50F= Days to 50% flowering, NFF= Node number at 1st flowering; FL= Fruit length (cm),
FD= Fruit diameter (cm), FW= Fruit weight (g), NFPP= Number of fruits per plant,
FYPP= Fruit yield per plant (g), PDI= Percent Disease Index (%) of YVMV disease.

Table 3: Selected crosses with high standard heterosis (%), their corresponding gca and sca effects, and type of cross combinations.

Characters	Cross (es) with high standard heterosis (%) over Shakti	Cross (es) with high standard heterosis (%) over Abantika	Parents with gca effects	Sca effects of crosses with *per se* performance	Type of combinations
Days to 50% flowering	BCO-1 × VNR Green (-10.39%**)	BCO-1 × VNR Green (-9.80*)	BCO-1 (-2.64**), VNR Green (-2.69**), Arka Anamika (-2.19**)	-1.88 (46.00)	H x H
	BCO-1 × Arka Anamika (-9.87%*)	BCO-1 × Arka Anamika (-9.28*)		-2.32* (46.27)	H x H
Node number at first flowering	VRO-6 × Pusa Sawani (-53.33%**)	VRO-6 × Pusa Sawani (-36.36%**)	VRO-6 (-0.97**), Pusa Sawani (-1.07**), 10/ RES-4 (-0.77**)	-1.33** (3.50)	H x H
	VRO-6 × 10/RES-4 (-40.00 %**)	VRO-6 × 10/RES-4 (-18.18 %**)		-0.62** (4.50)	H x H
Fruit length (cm)	None	*A. caillei* × Arka Anamika (25.24% **)	*A. caillei* (1.07**). Arka Anamika (0.37**), BCO-1 (0.39**)	0.52 (10.07)	H x H
		BCO-1 × *A. caillei* (20.93%**)		0.15 (9.73)	H x H
Fruit diameter (cm)	10/RES-4 × *A. caillei* (44.70 %**)	10/RES-4 × *A. caillei* (72.70 %**)	10/RES-4 (-0.08**), *A. caillei* (0.57**), Pusa Sawani (0.08**)	0.19 (2.32)	L x H
	Pusa Sawani × *A. caillei* (43.66 %**)	Pusa Sawani × *A. caillei* (71.46 %**)		0.01 (2.30)	H x H
Fruit weight (g)	BCO-1 × *A. caillei* (4.94 %)	BCO-1 × *A. caillei* (125.69 %**)	BCO-1 (0.50**), *A. Caillei* (3.84**)	2.09** (16.37)	H x H
Number of fruits per plant	BCO-1 × Arka Anamika (48.41 %**)	BCO-1 × Arka Anamika (23.03 %**)	BCO-1 (2.83**), Arka Anamika (-0.64**), VRO-6 (0.55**), 11/RES-6 (1.29**)	8.61** (23.40)	H x H
	VRO-6 × 11/RES-6 (43.55%**)	VRO-6 × 11/RES-6 (25.55%**)		8.21** (22.63)	H x H
Fruit yield per plant (g/plant)	BCO-1 × Arka Anamika (4.62 %**)	BCO-1 × Arka Anamika (17.59 %**)	BCO-1 (34.44**), Arka Anamika (2.15*), VRO-6 (14.61**), 11/ RES-6 (21.44**)	108.42** (257.21)	H x H
	VRO-6 × 11/ RES-6 (2.35 %)	VRO-6 × 11/ RES-6 (12.00 %**)		96.73** (245.0)	H x H
PDI (%) of YVMV disease	BCO-1 × Arka Anamika (-71.28 %**)	BCO-1 × Arka Anamika (-72.28 %**)	BCO-1 (-19.20**), Arka Anamika (6.54**), VRO-6 (10.82**), 11/ RES-6 (-14.28**)	-22.39** (7.23)	H x L
	VRO-6 × 11/ RES-6 (-54.24 %**)	VRO-6 × 11/ RES-6 (-55.82 %**)		-27.30** (11.52)	L x H

Data in parentheses indicate *per se* values.

* Significant at 5% level, ** Significant at 1% level.

Choosing of good general and specfic combiners

Two parents, BCO-1 and 11/RES-6 exhibited significant gca effects in desired direction in most of the heterotic hybrids for fruit yield per plant followed by number of fruits per plant, fruit weight, fruit length, days to 50% flowering, node number at first flowering and PDI of YVMV disease (Table 3). They also exhibited the highest *per se* performance for fruit yield per plant along with number of fruits per plant. Thus, these two parental lines were found most promising as general combiner because they produced the maximum frequency of high yielding hybrids with appreciable YVMV disease tolerance when crossed with other parents. Significantly positive gca effects for fruit yield per plant and other yield component characters have also been reported by previous workers [12,31].

Two crosses, BCO-1 × Arka Anamika and VRO-6 × 11/RES-6 expressed the maximum significant sca effects for fruit yield per plant along with number of fruits per plant, days to 50% flowering, and PDI of YVMV disease in desired directions (Table 3). Among the forty five hybrids, the *per se* performance of the hybrid BCO-1 × Arka Anamika was found to be the highest for fruit yield per plant and could be identified as potential specific combiner for certain important traits. The negative SCA effects observed in some of the crosses for different characters might be due to the presence of unfavourable gene combinations in the parents for the respective traits. These best specific combiners having the highest magnitude of significant SCA effects in desired direction are recommended for heterosis breeding. The inter-crossing of these materials could, therefore, generate a population with a large gene pool, where genetic linkages and genetic blocks could be broken [33]. Significant sca effects in desired direction

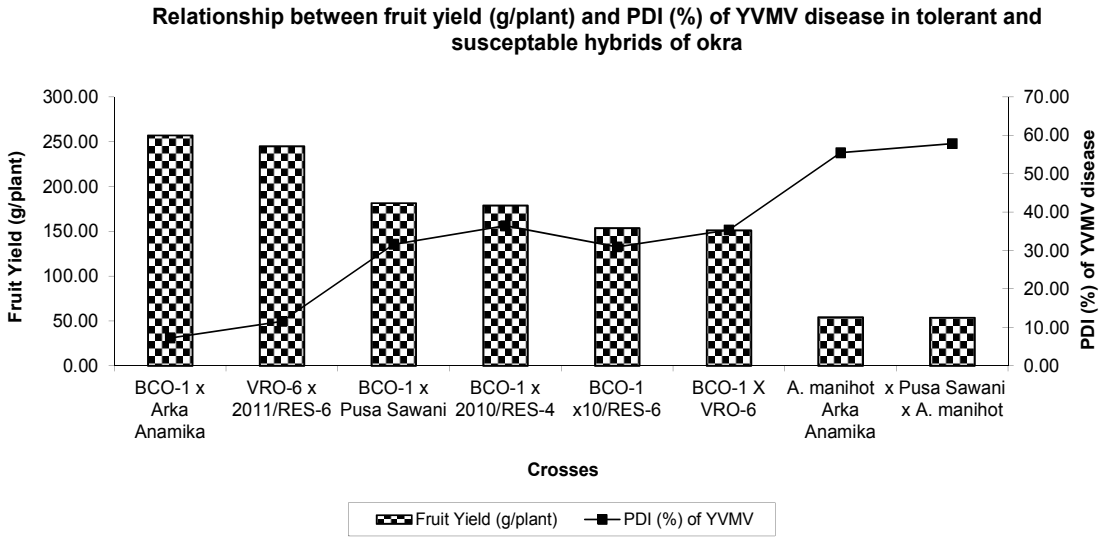

Figure 1: Relationship between fruit yield (g/plant) and PDI (%) of YVMV disease in tolerant and susceptible hybrids of okra.

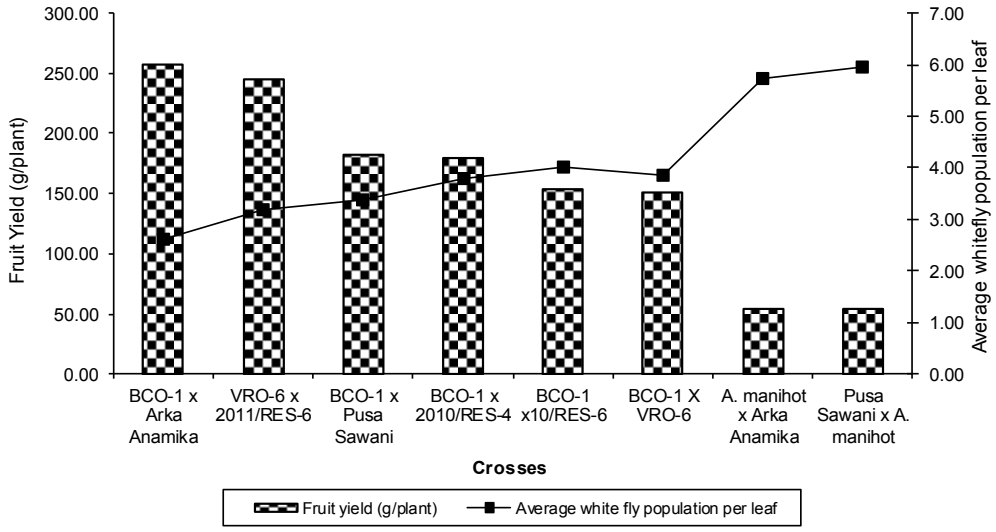

Figure 2: Relationship between fruit yield (g/plant) and average whitefly population per leaf in tolerant and susceptible hybrids of okra.

for node at 1st flowering [34] number of fruits per plant [35-37]; fruit length, fruit diameter, fruit weight and fruit yield per plant [10]; PDI of YVMV disease [12] involving various combinations of gca effects of the parents have also been reported. These two promising hybrids along with one most susceptible hybrid (BCO-1 × VRO-6) were grown under artificial inoculation condition to confirm the tolerance against this virus. Out of twenty plants inoculated with whiteflies none of the plants of the hybrid (BCO-1 × Arka Anamika) were developed any symptom of YVMV even after 30 and 45 days of inoculation and grow normally even after feeding by the vectors. Only one plant after 60 days of inoculation showed 5.00 % disease incidence in BCO-1 × Arka Anamika as compared to another promising hybrid VRO-6 × 11/RES-6 which showed 15.00 % disease incidence. However, the susceptible hybrid BCO-1 × VRO-6 exhibited 80.00 % disease incidence after 60 days of inoculation (Table 6).

The perusal of different cross combinations revealed that the crosses involved three types of combinations namely, H × H type; H × L type and L × H type, where H stands for significant gca effect and L for non-significant gca effect in desired direction of the parents (Table 3). The result depicting the type of cross combinations for the genetic control of the characters under study, both additive as well as additive × additive type of epistatic interactions were involved in H × H type cross combinations and thus, can be exploited effectively for the improvement of the traits through pedigree method of selection [38]. On the other hand, crosses of H × L type or L × H type involved one parent having either positive or negative significant gca effect indicated that predominantly additive effect was present in good combiner and possibly complementary epistatic effect in poor combiner and these two gene actions acted in complementary fashion to maximize the expression as suggested by Salimath and Bahl [26].

Table 4: Dominance estimate (DE) of F_1 hybrids for eight traits of okra.

Crosses	D50F	NFF	FL	FD	FW	NFPP	FYPP	PDI
BCO-1 × VNR Green	2.60	0.32	0.43	7.67	1.42	-1.12	-1.01	1.29
BCO-1 × VRO-6	-5.00	-0.64	2.11	-0.30	367.67	-0.90	0.20	-0.02
BCO-1 ×11/RES-6	1.40	-0.49	0.23	2.47	1.30	-2.27	-16.98	1.36
BCO-1 × 10/RES-6	-1.00	0.47	4.73	-0.06	8.69	-1.77	0.27	-0.18
BCO-1 × 10/RES-4	-1.00	0.00	6.66	3.00	8.52	-2.02	1.04	-0.17
BCO-1 × Pusa Sawani	1.00	7.29	0.51	-0.87	13.36	0.18	1.06	0.32
BCO-1 × *A. caillei*	-0.09	-1.44	-0.25	0.31	0.23	-1.26	-1.18	-1.22
BCO-1 × *A. manihot*	-0.20	0.20	-0.97	13.50	-1.35	-11.72	-0.64	-1.22
BCO-1 x Arka Anamika	1.91	3.13	14.78	4.43	3.56	0.82	2.30	1.09
VNR Green × VRO-6	0.14	-3.00	4.80	-0.60	5.88	-1.63	21.83	-0.68
VNR Green × 11/RES-6	0.00	-27.00	1.08	0.62	0.85	-1.80	-1.57	-0.21
VNR Green × 10/RES-6	0.33	-2.75	4.36	0.77	8.61	-0.46	32.38	-1.13
VNR Green × 10/RES-4	0.33	-0.36	2.97	3.00	2.39	-0.57	14.79	1.17
VNR Green × Pusa Sawani	1.67	-1.93	0.10	-0.98	0.48	1.73	1.86	-0.29
VNR Green × *A. caillei*	0.09	-0.32	-0.43	0.20	-0.22	-1.95	-2.87	-3.53
VNR Green × *A. manihot*	0.15	-0.53	-0.48	0.00	-0.94	-1.26	-0.43	-3.96
VNR Green × Arka Anamika	2.00	-3.14	2.35	1.40	1.89	1.19	1.93	-0.19
VRO-6 × 11/RES-6	0.71	-2.93	1.69	0.97	2.03	4.37	3.55	1.13
VRO-6 × 10/RES-6	-1.00	-3.62	-3.14	-1.33	6.19	-1.78	62.01	-1.97
VRO-6 × 10/RES-4	1.00	0.64	-3.83	-2.16	2.08	-1.15	12.38	-7.46
VRO-6 × Pusa Sawani	1.50	1.58	-2.06	-1.28	-0.60	1.36	1.60	-1.77
VRO-6 × *A. caillei*	0.19	-0.71	-0.82	-0.30	-0.60	-1.45	-3.80	0.14
VRO-6 × *A. manihot*	0.08	-0.39	-0.64	7.95	-0.77	-1.51	-0.44	-0.46
VRO-6 ×Arka Anamika	0.33	-2.10	1.26	-2.21	1.48	1.22	1.78	1.34
11/RES-6 × 10/RES-6	0.11	0.88	2.14	0.60	2.20	-25.00	-0.05	-0.80
11/RES-6 × 10/RES-4	1.00	1.79	1.38	0.58	1.91	-3.26	0.08	-0.40
11/RES-6 × Pusa Sawani	2.33	4.24	0.88	-0.54	2.81	0.21	0.59	0.21
11/RES-6 × *A. caillei*	0.15	-1.99	0.14	-0.01	-0.09	-1.27	-1.41	-2.80
11/RES-6 × *A. manihot*	0.03	-1.03	-1.28	3.60	-1.00	-1.33	-0.69	-2.86
11/RES-6 × Arka Anamika	1.00	-2.14	0.82	-0.43	2.79	0.72	0.45	0.40
10/RES-6 × 10/RES-4	-7.00	-0.57	5.33	-1.23	1.01	-5.34	4.57	-1.00
10/RES-6 × Pusa Sawani	0.67	1.00	-0.08	-0.63	11.71	-0.37	1.45	0.62
10/RES-6 × *A. caillei*	-0.15	-1.62	-0.64	0.42	0.05	-1.47	-1.97	-1.45
10/RES-6 × *A. manihot*	0.08	-0.59	-1.11	1.47	-1.62	-1.56	-0.41	-1.75
10/RES-6 × Arka Anamika	0.09	-2.42	1.60	-1.15	1.03	0.18	1.23	0.23
10/RES-4 × Pusa Sawani	2.33	7.57	1.52	-0.66	14.53	-0.43	1.21	1.18
10/RES-4 × *A. caillei*	-0.41	0.32	-0.70	0.35	-0.41	-1.31	-4.43	-1.32
10/RES-4 × *A. manihot*	0.21	0.60	-0.91	0.00	-1.11	-0.93	-0.38	-1.10
10/RES-4 × Arka Anamika	1.00	-3.27	6.73	4.09	8.68	-0.42	1.16	1.18
Pusa Sawani × *A. caillei*	0.22	-0.59	-1.50	-0.62	-0.29	-1.13	0.01	-0.23
Pusa Sawani × *A. manihot*	0.04	-0.66	-1.04	0.09	-2.49	-0.02	1.66	-0.29
Pusa Sawani × Arka Anamika	3.40	57.00	-1.61	-1.09	0.92	5.16	4.10	1.64
A. caillei × *A. manihot*	4.00	-1.46	-0.70	-0.19	-0.94	-0.56	0.26	2.67
A. caillei × Arka Anamika	-0.02	-0.01	-0.01	-0.05	-0.31	0.20	0.04	-0.30
A. manihot × Arka Anamika	0.00	0.02	0.00	5.18	-0.23	-0.71	0.90	-0.53

D50F= Days to 50% flowering, NFF= Node number at 1st flowering, FL= Fruit length (cm), FD= Fruit diameter (cm), FW= Fruit weight (g), NFPP= Number of fruits per plant, FYPP= Fruit yield per plant (g), PDI= Percent Disease Index (%) of YVMV disease.
* LSD at 5% and ** LSD at 1%.

Table 5: Correlation comparison matrix between disease causing variables and fruit yield among tolerant and susceptible crosses of okra.

Parameter	Fruit yield per plant (g)	PDI (%) of YVMV disease	Average whitefly population per leaf
Fruit yield per plant (g)	1.000	-0.976**	-0.972**
PDI (%) of YVMV disease		1.000	0.940**
Average whitefly population per leaf			1.000

** LSD at 1%.

Table 6: Per cent infection of YVMV disease in tolerant/susceptible hybrids after cross inoculation.

Tolerant/Susceptible Crosses	Number of plants inoculated with whiteflies	Plants infected at 30 days after inoculation	Plants infected at 45 days after inoculation	Plants infected at 60 days after inoculation	Percentage of plants infected at 60 days after inoculation
BCO-1 x Arka Anamika	20	0	0	1	5.00
VRO-6 x 11/RES-6	20	0	1	3	15.00
BCO-1 × VRO-6	20	5	11	16	80.00

Conclusion

The present study demonstrated the prevalence of non-additive gene effects in governing traits like fruit length, number of fruits per plant, and fruit yield per plant which could be improved through heterosis breeding. Pedigree method of selection is suggested for the improvement of days to 50% flowering controlled by additive gene effects. Reciprocal recurrent selection is ascertained to improve node number at first flowering, fruit weight, fruit diameter, and PDI of YVMV disease influenced by both additive and non-additive gene effects. Two parents, BCO-1 and 11/RES-6 could be utilized as promising donors in future okra breeding programme for improvement in fruit yield and YVMV disease tolerance. Exploitation of parents belonging to wild species (*A. manihot* and *A. caillei*) for the development of desirable hybrids should not be encouraged. The cross BCO-1 × Arka Anamika emerged as outstanding hybrid in respect of fruit yield and level of tolerance against YVMV disease, and could be exploited at commercial level in the tropics after its critical evaluation. The dominance effects clearly demonstrated partial- to over-dominance reactions for the inheritance of fruit yield and other economically important traits in okra.

Acknowledgements

First author wishes to acknowledge the Department of Science and Technology, Government of India for financial help in the form of Inspire Fellowship to conduct the entire study and ICAR-NBPGR, New Delhi, India for providing wild species of okra.

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Kaur et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00067.7

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Assessment of Genetic Diversity in Mungbean Genotypes using ISSR Markers

Gunnjeet Kaur, Arunabh Joshi, Devendra Jain*, Deepak Rajpurohit and Divya Vyas

Abstract

Molecular assessment of genetic diversity was done for 23 mungbean (*Vigna radiata* (L.) Wilczek) genotypes using ISSR marker system. Twenty ISSR primers were used in the study out of which thirteen primers enabled DNA amplification in all genotypes. A total of 75 amplified and reproducible amplicons were obtained from 13 primers, out of which 60 were found polymorphic. The total number of amplified bands varied between 2 (UBC-813) and 11 (UBC-810) with an average of 6.5 per primer. The overall size of PCR amplified products ranged between 200 to 2500 bp. PIC values ranged from 0.07 to 0.35 with an average value of 0.208 across all genotypes. Four unique bands were detected in four genotypes with 3 (UBC-818, UBC-820 and UBC-826) out of 13 ISSR primers. The genotype IC-393407 gave maximum number of distinct bands. The similarity indices between the 23 genotypes ranged from 0.38 to 0.94. The extent of diversity among genotypes was also estimated in relation to their source and a set of genotypes with narrow genetic bases developed from various region were identified. Based on a dendrogram generated through UPGMA method and PCA, most of the genotypes could be divided into two main clusters. Cluster I included twenty genotypes, while cluster II included three genotypes. The genotype GM-9925 lay apart from all the two clusters. A minimum similarity co-efficient of 0.38 was observed between genotypes GM-9925 and EC-398885 thereby indicating maximum genetic divergence. The Mantel statistical analysis (r = 0.92) also supported cluster analysis.

Keywords

Dendrogram; ISSR; Mantel test; Similarity coefficient

Introduction

Mungbean contributes to about 14% of total pulses cultivation area and 7% of total pulses production in India. Maharashtra, Rajasthan, Madhya Pradesh, Bihar, Punjab and Andhra Pradesh are the leading producers of mungbean. Pulses are grown in nearly 25.4 million hectare area in the country with production status of nearly 19.66 million tones, on an average productivity level of 770 kg/ha [1]. As of the 2012-13 cultivation statistics, green gram was grown on 2.75 million hectares with a production status of 1.19 million tonnes and yield of 436 kg/ha [1]. In Rajasthan, it was grown on 796.9 million hectares with a production status of 241.2 million tonnes and yield of 303 kg/ha [1].

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Received: June 14, 2016 **Accepted:** July 19, 2016 **Published:** July 25, 2016

Mungbean belongs to the family *Fabaceae (Leguminoceae)*. The roots of this legume has the ability to fix atmospheric nitrogen @30-50 kg/ha. It is a diploid with the chromosome number, 2n= 22 [2]. It is grown primarily as an intercrop with wheat, maize, potato, etc during *kharif* and as monocultures during rabi and zaid. On account of its short duration, photo-insensitivity and dense crop canopy, it assumes special significance in crop intensification, diversification, and conservation of natural resources as well as sustainability of the production system. Mungbean is an excellent source of high quality protein [3]. Its seed contains 24.2% protein, 1.3% fat and 60.4% carbohydrate. The seeds are also rich in Vitamin C, Vitamin B, folate and mineral nutrients eg. calcium, magnesium, phosphorous, potassium etc. Although germplasm collection within India is quite large, much diversity has not been reported on the basis of morphological characters. Therefore, an urgent need to identify genetic divergence based on molecular analysis is an essential pre-requisite for further utilization in crop improvement programmes.

The PCR based ISSR marker system allows DNA amplification at regions located between two closely spaced, oppositely oriented SSR sequences, resulting in a reproducible pattern of genomic fragments. Thus, ISSR markers show high level of polymorphism, bearing high potential for genetic diversity evaluation at inter and intra-specific levels [4]. Since knowledge of genetic diversity is essential for crop improvement programmes, molecular marker based analyses are in rapidly using in many related research areas [5]. The ISSR technique is useful in studies on genetic diversity, phylogenetic studies, gene tagging and genome mapping in a wide range of legume crops. In the present the genetic diversity of 23 genotypes of green gram were estimated using ISSR.

Material and Method

Plant material and DNA isolation

In the present investigation seeds of 23 genotypes of green gram were procured from Agriculture Research Station (ARS), Durgapura, Jobner Agriculture University, Jaipur. Source details of the materials used are given in Table 1. Genomic DNA was isolated and purified from young leaves of 21-28 day old seedlings [6] and stored at -20^{0}C.

ISSR-PCR amplification

A total of 20 primers identified by the University of British Columbia (UBC) were synthesized from Bangalore Genei Pvt. Ltd., Bangalore, India and used for ISSR-PCR optimization trials.Thirteen primers, which gave the best amplification results with the sample DNA, were selected for final ISSR-PCR analysis. PCR amplification was carried out in a 20-µL reaction volume containing 200 µM of dNTP mix, 1U of *Taq* polymerase, 1.5 mM $MgCl_2$, 1X reaction buffer, 0.5 µM of primer and double distilled water with 25 ng genomic DNA. The amplification was performed in a Eppendrof Master cycler, with reaction conditions programmed as initial predenaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 42.9-60°C for 1 min, and extension at 72°C for 2 min with a hold temperature of 4 °C. A final extension was done for 10 min at 72°C. Amplicons were separated by electrophoresis on 1.2% agarose gels stained with ethidium bromide run at constant voltage

Table 1: The origin, latitude, longitude and city of 23 genotypes.

S.No.	Origin	Latitude	Longitude	Genotype	City
1	Delhi	28.38	77.12	1. PUSA-672 2. IC- 393407	IARI NBPGR
2	Maharashtra	20.00	76.00	1. AKM- 962	PKV, Akola
3	Utrakhand	30.15	79.15	1. UPM-02-18 2. GBPAU	GBPAU, Pantnagar GBPAU, Pantnagar
4	Punjab	30.4	75.5	1. ML-729 2. MG- 331	PAU, Ludhiana Gurdaspur, Punjab
5	Taiwan	23.50	121.00	1. EC-398885 2.PRATEEKSHA-NEPAL	AVRDC, Taiwan AVRDC, Taiwan
6	Haryana	30.30	74.60	1. ASHA	HAU, Hisar
7	Gujarat	23.00	80.00	1.GM- 9925	S.K. Nagar
8	Rajasthan	27.00	74.60	1. RMG-62 2. RMG- 353	RAU, Durgapura RAU, Durgapura
9	U.P.	27.40	80.00	1. IPM- 02-01 2. IPM-02-3 3. IPM-02-14 5. IPOI-1539 6. PDM-288 7. MEHA (IPM -99-125) 8. SAMRAT (PDM-139) 9. HUM-1 10. HUM-12	IIPR, Kanpur IIPR, Kanpur IIPR, Kanpur IIPR, Kanpur IIPR, Kanpur IIPR, Kanpur IIPR, Kanpur BHU, Varanasi BHU, Varanasi

(3V/cm of gel) till bromophenol blue/loading dye migrated to other end of the gel [7]. The gel was visualized on a UV-transilluminator and photographed using gel documentation system (Alpha Innotech Corporation). Only clear, reproducible and unambiguous bands were scored. The size of the amplified bands of ISSR were observed based on its migration and confirmation relative to standard molecular size markers (100 bp DNA ladder and 1 kb DNA ladder obtained from Bangalore Genei Pvt. Ltd., Bangalore, India).

Data analysis

Amplified bands generated from ISSR -PCR amplification were scored based on the presence (1) or absence (0) of bands for each primer and were used to calculate a genetic similarity matrix employing the SMC using NTSYS-pc version 2.1 [8]. Cluster analysis was performed on both morphological and molecular data using the unweighted pair group method using arithmetic means algorithm (UPGMA), from which dendrograms depicting similarity amongst the genotypes were drawn and plotted using NTSYS-pc. The cophenetic correlation was calculated to find the degree of association between the original similarity matrix and the tree matrix in both morphological and molecular analyses. Mantel test [9] was performed for these genotypes by calculating the correlation between data sets in NTSYS-pc. Using the same software, PCA was also calculated to identify any genetic association amongst the genotypes.

Polymorphism information content (PIC)

To measure the polymorphism information of ISSR marker system the PIC was calculated according to following formula: PIC = 1-Σpi^2 where, N= total number of allele detected for a locus of a marker, Pi= frequency of the 1st allele [15].

Results and Discussion

Thirteen out of the twenty ISSR primers used in the present investigation, thirteen showed amplification in all genotypes. A total of 75 amplified bands were obtained from them, out of which 60 were found polymorphic. The total number of amplified bands varied between the second (UBC-813) and eleventh (UBC-810) with an average of 6.5 bands per primer (Table 2). The polymorphism percentage ranged from 37.5% (UBC-836) to 100% for six primers (UBC-813, UBC-815, UBC-818, UBC-820, UBC-826 and UBC-878) used. Average polymorphism across all the 23 genotypes of *V. radiata* L. was found to be 80%. The overall size of PCR amplified products ranged between 250 to 2500 bp. The PCR amplification using ISSR primers resulted in reproducible amplification products. The number of potential ISSR markers depends on the genotypes and the frequency of microsatellites, which tends to change with species and the SSR motifs that are targeted [10]. Minimum polymorphic frequency ranged between 0.00-0.38 found with UBC-836 where as maximum between 0.08-0.47 with UBC-810. The average PIC was 0.208 ranging from 0.07 to 0.35. The lowest and highest PIC values were recorded for primers UBC-836 and UBC-810, respectively (Table 2). Similar results were shown by Das et al. [11] who used ten primers that amplified a total number of 353 bands under 93 loci across five genotypes of mungbean with an average of 9.3 loci / primer, exhibiting an overall polymorphism of 52.7%. Singh et al. [12] also studied ISSR markers that were used to study DNA polymorphism in elite mungbean genotypes. They found that percentage polymorphism had ranged from 25% (UBC844) to 85% (UBC846, UBC864, UBC895), with an average value of 58.3% across all the genotypes. Tantasawat et al [13] measured genetic diversity and relatedness in 17 mungbean (Vigna radiata (L.) Wilczek) and 5 blackgram (Vigna mungo (L.) Hepper) genotypes by ISSR analysis.. The 18 ISSR primers had produced 341 scorable fragments of which 309 fragments were found to be polymorphic (90.6%). Percentage polymorphism ranged from 66.7% (ISSR 856) to 100% (ISSR 811, 836, 885), with an average value of 89.51%, across all the genotypes.

Four unique bands were detected in three genotypes *viz.,* IC-393407, PUSA-672 and GM-9925 with 3 ISSR primers (UBC-818, UBC-820 and UBC-826). The genotype IC-393407 gave maximum number of distinct bands *i.e.,* 2. The size of these unique bands ranged from 300-950 bp (Table 2). Amplification profiling of 23 genotypeswith two ISSR primers viz., UBC-810 and UBC-822 is shown in Figure 1.

Table 2: Characteristics of DNA profiles generated in *V. radiata* using 13 ISSR primers.

Primer Code	Squence	Annealing Temp (°C)	Molecular weight range (bp)	Total number of bands amplified (x)	Number of polymorphic bands (y) (%polymorphism)	Unique band	Frequency of polymorphic bands	PIC
UBC-810	$(GA)_8T$	42.9	1800-200	**11**	10(90.90)	**0**	**0.08-0.47**	**0.35**
UBC-813	$(CT)_8T$	43.3	1000-400	**02**	2(100)	0	0.15-0.28	0.22
UBC-815	$(CT)_8G$	44.9	2200-900	03	3(100)	0	0.08-0.34	0.19
UBC-817	$(CA)_8A$	52	1000-400	04	2(50)	0	0.00-0.49	0.14
UBC-818	$(CA)_8G$	52	700-350	04	4(100)	1	0.08-0.28	0.13
UBC-820	$(GT)_8T$	50	1000-450	06	6(100)	2	0.08-0.42	0.24
UBC-822	$(TC)_8A$	45	2500-300	08	7(87.5)	0	0.00-0.47	0.23
UBC-826	$(AC)_8C$	52	1400-300	07	7(100)	1	0.08-0.49	0.34
UBC-836	$(AG)_8YA$	43.3	1600-250	08	3(37.5)	**0**	**0.00-0.38**	**0.07**
UBC-840	$(GA)_8YT$	45	1100-300	05	3(60)	0	0.00-0.22	0.12
UBC-848	$(CA)_8RG$	55.5	1500-250	07	4(57.14)	0	0.00-0.42	0.17
UBC-873	$(GACA)_4$	45	2100-600	07	6(85.71)	0	0.00-0.42	0.25
UBC-878	$(GGAT)_4$	60	900-400	03	3(100)	0	0.22-0.34	0.26
Total				**75**	**60(80%)**	**4**		**0.208**

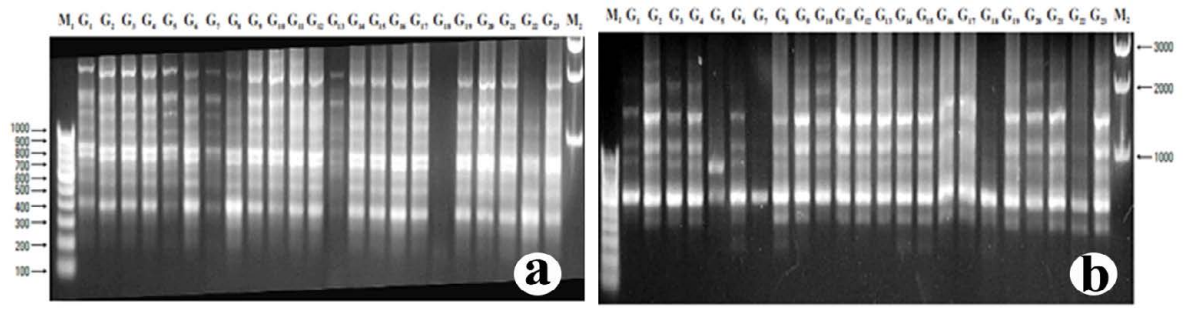

Figure 1: ISSR profiles of *Vigna radiata* genotypes generated through (a) UBC-810 (b) UBC-822. G1-G23 represents G1-PUSA672; G2-AKM962; G3-UPM02-18; G4-ML-729; G5-EC-398885; G6-IPM-02-1; G7- IPM-02-03; G8-IPM02-14; G9-IPOI-1539; G10-RMG-62; G11-PDM-288; G12-RMG-353; G13-PRETEEKHSA-NEPAL; G14-MEHA; G15-PANT; G16-ASHA;G17-MG331;G18-GM9925, G19-IC-393407; G20-DRA-24; G21-SAMRAT; G22-HUM-1 and G23-HUM-12. M1-100 bp DNA Ladder and M2- 1000 bp DNA Ladder.

Similarity Matrices based on ISSR markers

ISSR similarity matrix values of 23 *V. radiata* L. genotypes revealed a close relationship amongst them. The similarity indices between genotypes ranged from 0.38 to 0.94 *i.e.* 38-94% or the genetic diversity ranged from 6 to 62% (Table 3). The average similarity across all the 23 genotypes was found out to be 0.66, showing that all genotypes were moderately similar to each other. Maximum similarity value of 0.94 was observed between genotypes SAMRAT and DRA-24 , HUM-12 and DRA-24 ,MG-331 and ASHA , ASHA and RMG-232,DRA-24 and PDM-288 ,HUM-12 and PDM-288 followed by RMG-62 and IPOI-1539, SAMRAT and RMG-62, PANT and RMG-535 and ASHA and PANT, MG331 and PANT with similarity coefficient of 0.93. Genotypes GM-9925 and EC-398885 were found to be genetically diverse with a minimum similarity value of 0.38 followed by GM-9925 and AKM-962, GM-9925 and IPM99-125, MEHA and IPM 99-125 with a similarity value of 0.53.

Similar findings are reported by Das et al. [11] in mungbean cultivars. The value of Jacaard's similarity coefficient ranged from 0.566 to 0.793. Singh et al. [12] found similar results in 30 mungbean genotypes through Dice analysis, the similarity coefficient ranging from 0.65 to 0.85 with an average of 0.69.

According to Jaccard's similarity coefficient genetic similarity between groups of genotypes of common geographical origin has also been evaluated (Table 4). Genotypes from Rajasthan state showed highest range of similarity 0.90. This revealed high diversity amongst genotypes of Rajasthan origin. Similarity coefficient values of 0.84 have been observed in genotypes from Punjab state. A maximum of 10 genotypes have also been evolved from Uttar Pradesh showing similarity (range 0.62 to 0.94) with a mean value of 0.78 which are moderate mean values of similarity coefficient. Lowest similarity coefficient of 0.64 was found between genotypes of Taiwan. A maximum of 45 numbers of paired combinations was found in genotypes grouped under genotypes of Uttar Pradesh of origin (Table 4).

ISSR Marker based cluster tree analysis

The ISSR data based derivation of similarity matrix shown in Table 3 reveal the similarity values lay between 0.60-0.95. The 23 genotypes could be divided into two major clusters at a similarity coefficient of 0.74 (Figure 2). Cluster I was the main one that included twenty genotypes *viz.,* PUSA-672, UPM-02-18, PDM-288, IPOI-1539, RMG-62, RMG-353, DRA-24, HUM-12, SAMRAT, PRATEEKSHA-NEPAL, ASHA, MG-331, PANT, IC-393407, ML-729,IPM-02-01, MEHA , HUM-1, AKM-962 and IPM-02-14 at a similarity coefficient of 0.72. It could be further divided into six sub-clusters. Subcluster I included two genotypes UPM-02-18 and PDM-288 are similar to each other at a similarity coefficient of 0.90. Subcluster II included

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Table 3: Jaccard's similarity coefficient values for ISSR pattern as generated by agarose gel electrophoresis.

Genotypes	PUSA-672	AKM-962	UPM-02-18	ML-729	EC-398885	IPM- 02-01	IPM -02-3	IPM-02-14	IPOI-1539	RMG-62	PDM-288	RMG- 353	P. NEPAL	MEHA	PANT	ASHA	MG- 331	GM- 9925	IC-393407	DRA-24	SAMRAT	HUM-1	HUM-12
PUSA-672	1.0																						
AKM- 962	0.72	1.0																					
UPM-02-18	0.82	0.78	1.0																				
ML-729	0.77	0.76	0.89	1.0																			
EC-398885	0.70	0.54	0.65	0.69	1.00																		
IPM- 02-01	0.78	0.76	0.78	0.86	0.70	1.00																	
IPM-02-3	0.72	0.65	0.70	0.73	0.76	0.76	1.00																
IPM-02-14	0.68	0.68	0.70	0.73	0.64	0.74	0.72	1.00															
IPOI-1539	0.78	0.73	0.88	0.85	0.64	0.78	0.64	0.74	1.00														
RMG-62	0.77	0.74	0.89	0.86	0.65	0.78	0.68	0.76	0.93	1.00													
PDM-288	0.78	0.76	0.90	0.82	0.62	0.77	0.69	0.73	0.86	0.85	1.00												
RMG- 353	0.76	0.73	0.85	0.85	0.62	0.78	0.66	0.78	0.92	0.90	0.86	1.00											
P. NEPAL	0.76	0.76	0.84	0.81	0.62	0.78	0.68	0.74	0.89	0.89	0.85	0.92	1.00										
MEHA	0.70	0.77	0.70	0.70	0.54	0.72	0.53	0.69	0.81	0.77	0.74	0.82	0.80	1.00									
PANT	0.72	0.74	0.82	0.82	0.62	0.82	0.66	0.76	0.90	0.88	0.86	0.90	0.93	0.84	1.00								
ASHA	0.76	0.80	0.84	0.84	0.60	0.84	0.70	0.76	0.89	0.89	0.85	0.92	0.94	0.80	0.93	1.00							
MG- 331	0.70	0.76	0.84	0.84	0.58	0.78	0.68	0.72	0.89	0.86	0.85	0.89	0.92	0.80	0.93	0.94	1.00						
GM- 9925	0.56	0.53	0.56	0.56	0.38	0.58	0.53	0.62	0.58	0.64	0.60	0.61	0.69	0.54	0.62	0.66	0.64	1.00					
IC- 393407	0.70	0.69	0.78	0.76	0.56	0.73	0.62	0.70	0.81	0.81	0.77	0.84	0.86	0.77	0.85	0.89	0.86	0.61	1.00				
DRA-24	0.73	0.73	0.88	0.85	0.61	0.76	0.69	0.76	0.92	0.93	0.86	0.94	0.92	0.77	0.90	0.92	0.92	0.64	0.86	1.00			
SAMRAT	0.78	0.72	0.85	0.85	0.66	0.77	0.69	0.76	0.89	0.93	0.86	0.92	0.90	0.78	0.89	0.90	0.88	0.65	0.85	0.94	1.00		
HUM-1	0.66	0.65	0.70	0.73	0.73	0.73	0.70	0.72	0.77	0.76	0.72	0.80	0.78	0.64	0.77	0.84	0.78	0.69	0.73	0.80	0.80	1.00	
HUM-12	0.73	0.76	0.88	0.82	0.60	0.76	0.66	0.76	0.92	0.90	0.89	0.94	0.92	0.77	0.90	0.92	0.89	0.61	0.84	0.94	0.92	0.80	1.00

Table 4: Jaccard's similarity coefficient among 23 genotypes implies genotypes to variety of origins.

Origin	No of genotypes	Paried combination	Similarity Coefficient range
Delhi	2	1	0.70
Maharastra	1	-	-
Utrakand	2	1	0.82
Punjab	2	1	0.84
Taiwan	2	1	0.62
Rajasthan	2	1	0.90
Haryana	1	-	-
Gujarat	1	-	-
U.P.	10	45	0.62-0.94

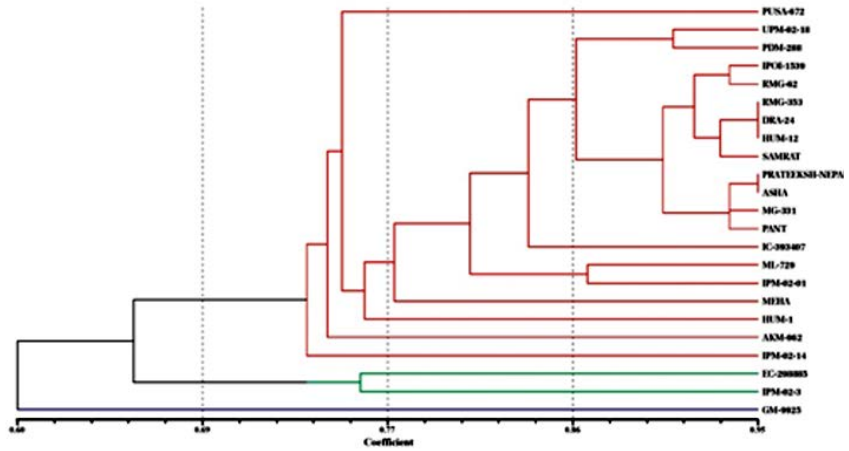

Figure 2: Dendrogram constructed with UPGMA clustering method among 23 genotypes of *V. radiata* L. using ISSR markers.

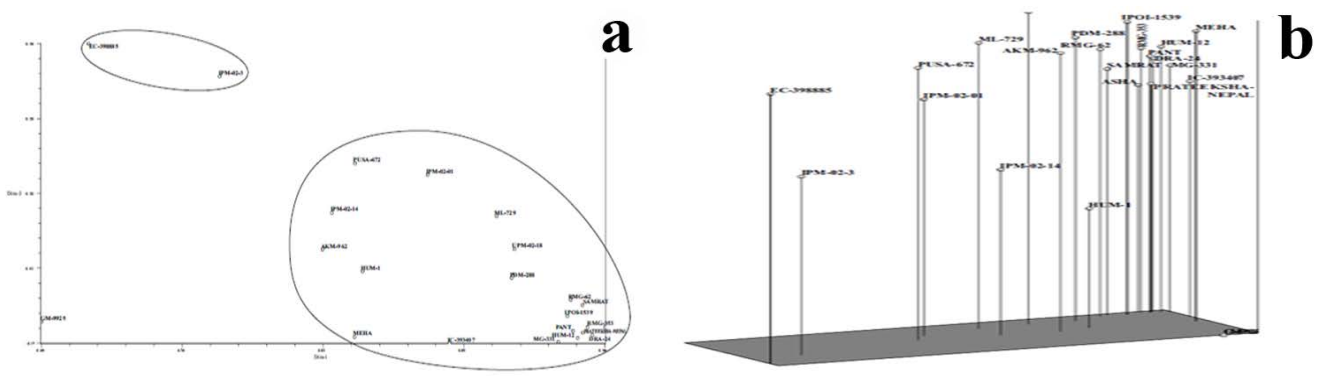

Figure 3: a. Two Dimensional Principal Component Analysis. **b:** Three Dimensional Principal Component Analysis based on Euclidean Cluster Analysis Using ISSR Markers.

two genotypes, IPOI-1539 and RMG-62 found similar to each other at a similarity coefficient of 0.93. Subcluster III included three genotypes *viz.,* RMG-353, DRA-24 and HUM-12 identified found at a similarity coefficient of 1.00. Subcluster IV included two genotypes, *viz.,* PRATEEKSHA-NEPAL and ASHA again found similar to each other at a similarity coefficient of 1.00. Sub-cluster V included two genotypes viz., MG-331 and PANT that were similar to each other at a similarity coefficient of 0.93. Finally subcluster VI included two genotypes, *viz.,* ML-729 and IPM-0201, at a similarity coefficient of 0.86. Cluster II included two genotypes EC-398885 and IPM-02-3 which were similar to each other at a similarity coefficient of 0.74. Genotype GM-9925 could be out grouped from both major clusters at a similarity coefficient of 0.60.

Similar results have been reported by Singh et al. [12]. The UPGMA distributed the 30 genotypes into five main clusters; clusters with Dice's analysis indicated similarity coefficient values ranging from 0.65 to 0.8. One genotype namely, ML-818 forms an out-group by not falling in any cluster. The variety PDM-139 used as standard check was grouped separately. The genetic variation amongst advanced lines of diverse crosses could be useful for selecting parents for crossing so as to yield populations required for breeding for yield and related agronomic traits.

Mantel test (Z) was done between cophentic matrix computed from marker based tree matrix and the original similarity data. This resulted in significant correlation (r=0.92) which revealed a good fit for the cluster analysis performed.

Principal component analysis based on ISSR markers

Two and three dimension principal component analysis based on ISSR data (Figure 3) showed similar clustering of 23 genotypes as evident from cluster tree analysis. Dice's similarity coefficients ranged from 0.69 to 0.96, indicative of an average degree of variation among the genotypes. As visible in the dendrogram, the genotypes that were closer were more similar than those that were lying apart. Similar observations were recorded with PCA as well. Most of the genotypes tended to associate mainly into two clusters. Cluster I was the major one that included seventeen genotypes *viz.* ,PUSA-672, UPM-02-18, PDM-288, IPOI-1539, RMG-62, RMG-353, DRA-24, HUM-12, SAMRAT, PRATEEKSHA-NEPAL, ASHA, MG331, PANT, IC-393407, ML-729, IPM-02-01, MEHA, HUM-1, AKM-962 and IPM-02-14 while cluster II included only 2 genotypes, *viz*., EC-398885 and IPM-02-3 that lay closer to each other. Genotype GM-9925 lay apart from both two clusters. Similar finding have been reported in Tantasawat et al. [13]

ISSR markers are easy, fast, inexpensive, accurate, reliable, and simultaneous in detection of polymorphisms at multiple loci in the genome using small quantities of DNA sample. These properties have made the ISSR markers useful for the genetic analysis of various plants [14-16]. Thus, this investigation may be useful for selecting the diverse parents and observing genetic diversity in breeding trails towards better genotype collection of mungbean.

Acknowledgement

Dr. Gunjeet Kaur thanks to UGC, New Delhi for Senior Research Fellowship under MANF. Authors are gratefully acknowledged the financial assistance from RKVY project "validation of important crop varieties through DNA fingerprinting".

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Gazal et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00068.9

Vegetos- An International Journal of Plant Research

Research Article

A SCITECHNOL JOURNAL

Genetic Diversity of Maize (*Zea mays* L.) Genotypes Assessed by SSR Markers under Temperate Conditions

Asima Gazal[1*], Dar ZA[1], Lone AA[1], Shikari AB[2] and Habib M[1]

Abstract

A set of twenty four maize lines comprising fifteen drought promising inbred lines, four drought susceptible inbred lines and five drought tolerant checks were analyzed for molecular characterization. These inbred lines belong to AICRP (All India Co-ordinated Research Programme) Maize Srinagar Centre and the checks were selected from CIMMYT (International Maize and Wheat Improvement Centre) material.. Molecular characterization was done using a set of 45 SSR markers having genome wide coverage. The marker data was analyzed using Power Marker Software (Version 3.25). The markers detected a total of 271 alleles with an average of 8.46 alleles per locus in twenty four maize lines. The average polymorphism information content (PIC) ranged from 0.56 and 0.89 with a mean of 0.78. The level of heterozygosity in the inbred panel was significantly low. The mean value of heterozygosity was 0.05 implying that most of the loci attained homozygosity. Dendrogram derived from UPGMA cluster analysis showed presence of two major clusters, one of which had many subgroups. Phenotyping of these inbreds was done by using morphological, maturity, physiological, yield and yield attributing traits. Genotyping data complemented by phenotyping data would be used to identify a number of pair-wise combinations for the development of mapping populations for drought tolerance related traits and potential heterotic pairs for the development of drought tolerant hybrids.

Keywords

Zea mays; Simple sequence repeats; Diversity; Inbreds; Drought tolerance

Introduction

Maize is an important crop under temperate conditions next to rice and is gaining more popularity due its multiple uses. It is grown mainly in Kharif season and under rainfed conditions facing erratic precipitation which leads to yield losses. Due to global warming the most important abiotic stress affecting maize crop production and productivity worldwide is moisture stress which in turn is becoming one of the topmost priorities in maize research programmes [1]. Assessment of genetic diversity in every crop species is important to understand variability and the pattern of genetic and evolutionary relationship between crop germplasm which further guides to sample genetic resources in a more systematic fashion for conservation and plant improvement [2]. In maize germplasm genetic relationships were morphologically characterized since 1970's [3]. But as morphological characters are more influenced by environment and have low heritability, mostly having stage specific expression, are less in number and less polymorphic [4] therefore, DNA markers are preferred in genetic characterization and diversity studies [5]. Among many marker systems, simple sequence repeats (SSRs) are considered as the markers of choice because of their abundance in the eukaryotic and prokaryotic genome, reproducibility, high variability, and uniform distribution in the genome. SSRs are PCR-based, codominant in inheritance and multiallelic marker system [6]. Several studies have extensively used molecular markers to assess the genetic relationship of genotypes in maize [7-17]. In maize many mapped SSR markers are available which can be utilized for molecular characterization. The objective of this study was to perform molecular characterization of a set of elite maize inbred lines using SSR markers. The purpose of this study was to identify inbred lines to develop a trait specific mapping populations for drought tolerance and later on to develop drought tolerant hybrids which would be able to cope up with the climate change conditions prevailing in the valley especially erratic rainfall pattern mainly during the critical crop stage (flowering stage) of the maize crop which results in appreciable yield losses.

Materials and Methods

Plant material – The experimental material comprised of a set of twenty four maize lines comprising fifteen drought promising inbred lines, four drought susceptible inbred lines and five drought tolerant checks. These inbred lines belong to AICRP (All India Co-ordinated Research Programme) Maize Srinagar Centre and the checks were selected from CIMMYT (International Maize and Wheat Improvement Centre) Mexico, AAU, Anand and MPUAT, Udaipur (Table 1).

DNA isolation

The extraction of plant DNA was carried out by CTAB (Cetyl-Tri Methyl Ammonium Bromide) method as described by Murray and Thompson (1980) with minor modifications from a pool sample of leaves from 15 seedlings. Fresh leaf samples (1000 mg) ground to fine powder in liquid nitrogen, were transferred to 50 ml centrifuge tubes containing 10 ml pre warmed CTAB extraction buffer (1M Tris-Cl, 0.5M EDTA, 2% CTAB, pH 8.0). Sample tubes were incubated in water bath at 65°C for 1 h with occasional swirling. Equal volume of chloroform: isoamyl alcohol (24:1 v/v) was added to the tubes and mixed thoroughly followed by centrifugation at 10,000 rpm for 10 min at 20°C. Aqueous phase was pipetted out and pre-chilled iso-propanol @ 0.6 volume of the content in tube was added followed by gentle inversion until fibrous mass was visible. Tubes were shifted at 4°C for overnight. Tubes were then centrifuged at 5000 rpm for 10 minutes at 4°C to obtain a precipitate. The supernatant was drained by gently inverting the tubes. The tubes were left inverted with lids open on blotting paper to drain the residual iso-propanol. After a while, the DNA pellet was washed twice with 70% ethanol, and kept overnight at room temperature for drying the pellet. 100-200 µl of TE buffer (pH 8.0) was added to dissolve the pellet. After 6-8 h RNase (10 mg/ml) was added to the DNA voil @ of 2 µl/100 ul of crude DNA. Mixture was incubated in a water-bath for 1 hour at 37°C with intermittent mixing.

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Received: March 11, 2016 **Accepted:** July 21, 2016 **Published:** July 28, 2016

Table 1: List of inbred lines used in the investigation.

S. No.	Line	Institution
1.	KDM-361A	SKUAST-K, Srinagar
2.	KDM-932A	SKUAST-K, Srinagar
3.	KDM-961	SKUAST-K, Srinagar
4.	KDM-717	SKUAST-K, Srinagar
5.	KDM-463	SKUAST-K, Srinagar
6.	KDM-912A	SKUAST-K, Srinagar
7.	KDM-343A	SKUAST-K, Srinagar
8.	KDM-1051	SKUAST-K, Srinagar
9.	KDM-402	SKUAST-K, Srinagar
10.	KDM-918A	SKUAST-K, Srinagar
11.	KDM-1156	SKUAST-K, Srinagar
12.	KDM-1236	SKUAST-K, Srinagar
13.	KDM-372	SKUAST-K, Srinagar
14.	CM-129	IIMR, New Delhi
15.	KDM-331	SKUAST-K, Srinagar
16.	KDM-969	SKUAST-K, Srinagar
17.	KDM-741	SKUAST-K, Srinagar
18.	KDM-344	SKUAST-K, Srinagar
19.	KDM-375	SKUAST-K, Srinagar
20.	GM-6	AAU, Anand
21.	MahiDhawal	MPUAT, Udaipur
22.	H17	SKUAST-K, Srinagar
23.	CML-440	CIMMYT, Mexico
24.	CML-470	CIMMYT, Mexico

Primers and PCR amplification

A set of forty SSR primers (four for each chromosome), distributed uniformly on both arms of all the 10 chromosomes was used in the study (Table 2). The marker sequences were retrieved from www.maizegdb.org. After the initial screen, eight SSR markers which did not amplified were rejected from the experiment. Rest thirty-two SSR markers amplified and generated good and reproducible products for all the lines.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) was performed in a thermal cycler (Eppendorf, Hamburg, Germany) with thermal regimes given in Table 3. PCR reaction mix contained 25 ng of DNA, 10X PCR Hi Buffer A (100 mM Tris-HCl, pH 9.1, 500 mM KCl, 25 mM $MgCl_2$), 10 mM dNTP solution set (MolBio HIMEDIA), 5 pmol each of forward and reverse primer and 3 U/ µl of Taq DNA polymerase (MolBio HIMEDIA) in a reaction volume of 10 µl (Table 4).

Resolution of amplified PCR products

Gel electrophoresis is a widely used technique for the analysis of nucleic acids and proteins. Here we used the technique for size separation of amplified DNA. A concentration of 3.5% (w/v) agarose gel was prepared by dissolving 7 g of weighed agarose powder in 200 ml of 1XTAE [196 ml double distilled water + 4 ml of 50x TAE buffer (242.2g Tris base: Mwt. 121.14; 100 ml of 0.5M EDTA: PH 8.0; 57.1ml Glacial acetic acid: Molecular weight: 61.83 g; make volume to 1000 ml using de-ionized Milli-Q water)] in a conical flask. The suspension was heated in microwave oven for 6 minutes at 600 watt till clear solution was obtained. The solution was allowed to cool down and to this was added 8 µl (0.05 µl/ml of 1X TAE) of Ethidium Bromide stock solution (10 mg/ml of double distilled water). After gentle shaking, the gel was poured onto gel casting tray. After 15-20 minutes the gel was immersed inside the gel tank filled with 1X TAE (PH 8.0). To each PCR product of 10 µl was added 1 µL 6 loading dye (0.25% bromophenol blue; 0.25% xylene cyanol FF; 40% sucrose). With the help of 10 µl pipettes samples were loaded in individual wells. In parallel, was also loaded 50 bp size reference ladder (MolBio HIMEDIA). The power pack was adjusted at 5 Volts/cm of run and the total duration of electrophoresis varied from 1.5 to 2.5 hours. After optimum run of samples the gel slabs were visualized under UV trans-illuminator and documented in gel documentation system (Bio-Rad Laboratories Inc., USA). This was followed by scoring of bands with the help of 50 bp DNA size standard (MolBio HIMEDIA).

SSR data analysis

Scoring of the SSR alleles was performed manually in terms of positions of the bands relative to the ladder sequentially from the smallest to the largest-sized bands. Diffused bands or bands revealing ambiguity in scoring were considered as missing data and designated as '999' in comparison with the base pair reading in the data matrix. Genotypes showing two allelic bands with equal intensity were considered as heterozygous for the locus. Polymorphism information content (PIC) values for each of the primer was estimated using formula given by Goodman and Bird [18] i.e., PIC=1- , where Pij is the frequency of jth allele in ith primer and summation extends over 'n' patterns. Power Marker version 3.25[19] was also used to calculate the average number of alleles, gene diversity, and polymorphic information content (PIC) values. Based on the electrophoretic banding pattern of 32 SSR markers, pair wise genetic distance amongst genotypes were estimated and a dendrogram was generated using UPGMA clustering. Phylogenetic reconstruction was based on the neighbor joining method was conducted using computer software programme Dissimilarity Analysis and Representation for Windows (DARwin) 5.0 [20].

Results and Discussion

The summary statistics of marker related parameters are presented in Table 5. The 32 SSR markers produced as many as 271 alleles with an average of 8.43 alleles per locus in the 24 genotype panel. The average number of alleles per loci (6 to 13) obtained in the present study was higher considering the number of genotypes examined in this study. The results were in accordance with the previous report [15]. The heterozygosity level in the inbred panel was significantly low. The mean value of heterozygosity was 0.06 revealing that most of the loci attained homozygosity. However, for the loci umc-2372 (Figure 1) the heterozygosity was 0.50. The study indicated instances where the SSR primers for the inbreds displayed clear deviation from the unexpected pattern where inbreds are assumed to be highly homozygous and therefore are expected to reveal only a single amplification product (allele) per locus, at least for a large majority of the loci analyzed for the locus umc-2372. The presence of heterozygosity in the inbred lines might be due to a number of probable causes including residual heterozygosity, pollen or seed contamination, mutation at specific SSR loci, or amplification of similar sequences in different genomic regions due to duplication [21,22]. Since maize is a highly cross-pollinated crop, pollen or seed stock contamination during maintenance could be the most plausible explanation could be the residual heterozygosity which is not uncommon in maize. By virtue of its cross pollinated nature, maize maintains 5 to 10 per cent heterozygosity which is called residual heterozygosity. As a result of this, inbreds tend to segregate for a few loci/characters despite repeated cycles of selfing over many generations. Mutations at specific SSR loci, and

Table 2: SSR primers selected for the study.

S.No.	SSR		Primer Sequence (5'-3')	Bin location	Annealing Temperature (˚C)
1	Umc2383	F R	CATAGACGTGCCCCTTGTCATC CTCGCAACTGCGCTTCTAGATACT	1.02-1.03	57.7 58.6
2	Umc1664	F R	AATTGTTTACTGCGCTGAAACTCC CCTCTTTGCCTGTACCGTGTATTC	1.06	57.5 56.3
3	Umc1147	F R	GAGAAACCATCGACCCTTCCTAAC TTCCTATGGTACAGTTCTCCCTCG	1.07	57.1 57.5
4	Umc2100	F R	AAAGGCATTATGCTCACGTTGATT TGACGTGCAAACAACCTTCATTAC	1.12	56.2 55.6
5	Umc2245	F R	GCCCTGTTATTGGAACAGTTTACG CGTCGTCTTCGACATGTACTTCAC	2.01	56.3 57.4
6	Umc1696	F R	CTAGGGTTTAACCAACGGGGAG TAAGGAGAGGGTCGATGAACACAT	2.1	57.3 57.4
7	Umc1823	F R	AAAGCCTTACTGTTATTAGGCTAGGCA AGAAAACCAGCCCCAGATGTTC	2.03	57.8 57.6
8	Umc1026	F R	TCGTCGTCTCCAATCATACGTG GCTACACGATACCATGGCGTTT	2.04	56.8 54.4
9	Umc2372	F R	ACCCCTTGCGTTCTCTTCTGTT CACCAGGCGTAGTGAGACAGC	2.06	57.6 58.3
10	Umc2144	F R	CCAGCCCCTATCTATTTGCTTGT GAATACTATATCACGGTCGGTCGG	2.08	57.0 56.4
11	Umc1594	F R	GCCAGGGGAGAAATAAAATAAAGC CACTGCAGGCCACACATACATA	3.09-3.1	54.4 57.3
12	Umc2071	F R	ACTGATGGTGTTCTTGGGTGTTTT ATACACGCAGTTACCCGAAGGTT	3.01	57.2 58.4
13	Umc2369	F R	TTCGTCTGATGAAAGGTTCAGAGG GATCCTCATCAAGACCAGCAGAGT	3.02-3.03	56.6 58.2
14	Umc1644	F R	CCATAAACTGTTCCTTTGGCACAC CTTTCACGTGTTAAGGGAGACACC	3.06	56.7 57.6
15	Bnlg1890	F R	ACCGGAACAGACGAGCTCTA GTCCTGCAAAGCAACCTAGC	4.11	57.6 56.6
16	Bnlg1621	F R	CTCTTCGATCTTTAAGAGAGAGAGAG ACACGAGGCACTGGTACTAACG	4.06	53.9 58.8
17	Umc1478	F R	GAAGCTTCTCCTCTCGCGTCTC CAGTCCCAGACCCTAGCTCAGTC	5.01	59.3 60.5
18	Umc1800	F R	TTATGGGTGCTGGTGATGTGTATC GAAAAGCAATCGCTTCTGAGAAAA	5.05	56.8 54.4
19	Umc1766	F R	ACAAGAAGGAATCGAGAGCAAATG CTTCGGGATGGAGTCGTAGTTC	5.01	55.5 56.9
20	Bnlg1306	F R	CACCTTGAAAGCATCCTCGT CAAAAACAAATGGCAGCTGA	5.07	55.2 52.2
21	Umc1918	F R	CACAAGAACATTATGACGACCGAG AAGCAGGAGACATCGTTTAAGTCG	6.03	55.7 57.0
22	Umc1762	F R	CTTACTCCAGGCACTCCATACCAT ATCCAGGTGAATGGTGTTTACGAT	6.06	58.1 56.4
23	Umc1063	F R	AGGCCACTGAGCAGGTGAAG GTGATGGTAGAGGAGTCCTTGGTG	6.07	59.7 58.6
24	Umc1018	F R	GAACGGATATTGGAACCTGTGC GTGCACGGTGTCGTACTTGAAC	6.01	56.1 58.4
25	Phi452693	F R	CAAGTGCTCCGAGATCTTCCA CGCGAACATATTCAGAAGTTTG	6.04	56.7 52.4
26	Umc1424	F R	CCGGCTGCAGGGGTAGTAGTAG ATGGTCAGGGGCTACGAGGAG	6.06	61.0 60.6
27	Phi129	F R	GTCGCCATACAAGCAGAAGTCCA TCCAGGATGGGTGTCTCATAAAACTC	6.05	59.3 58.2
28	Umc1002	F R	AGCTAGCTATACACCGCCAGG TCAGTTTGGAACAGGGAAAAGTA	6	58.6 54.2
29	Phi051	F R	GGCGAAAGCGAACGACAACAATCTT CGACATCGTCAGATTATATTGCAGACCA	7.05	60.3 58.3
30	Umc1036	F R	CTGCTGCTCAAGGAGATGGAGA GACACACATGCACGAGCAGACT	7.02	58.7 59.7
31	Umc1708	F R	GATATGTCGAGCTTCGCTGGAG CGCACACTAAAGCATCCTTAACCT	7.04	57.4 57.3
32	Bnlg1056	F R	ATCGTTGTTGGGTACACGGT ACGGGTAGTGGTGAAGATGC	8.08	56.7 57.1

33	Umc1141	F R	AGAGGAGAAAGAGACAGACAGGCA CAGGAACTGAATGAAAGCAACTCA	8.06	59.2 55.6
34	Umc1415	F R	GTGAGATATATCCCCGCCTTCC AGACTTCCTGAAGCTCGGTCCTA	8.04	56.8 59.1
35	Umc1786	F R	ACCGTGACTTCCTCCTCATAACTG CATTTTTCGCATTTAGGAAATCCA	8.01	58.1 52.5
36	Phi067	F R	CTGCAAAGGTAAGCACTAGGATGCT CATCATTGATCCGGGTGTCGCTTT	9.01	59.0 59.7
37	Phi061	F R	GACGTAAGCCTAGCTCTGCCAT AAACAAGAACGGCGGTGCTGATTC	9.03	58.9 60.4
38	Umc1077	F R	CAGCCACAGTGAGGCACATC CAGAGACTCTCCATTATCCCTCCA	10.04	58.6 57.2
39	Mmc0501	F R	TGCTGAACACTCTAAGCAATAC ATTACTCTACTCGCTGCCTG	10.02	52.8 54.0
40	Bmc1655	F R	ATTAAAATCTTGCTGATGGCG TTCTGTTCCCGCCTGTACTT	10.03	51.6 56.2

Table 3: PCR thermal regimes and conditions.

Step	Reaction	Temperature	Time	Cycle
I	Initial denaturation	94°C	4	1
II	Denaturation	94°C	1	35
	Annealing	55°C	1	
	Extension	72°C	2	
III	Final extension	72°C	7	1

Table 4: The PCR reaction mixture.

Reagent	Stock conc.	Aliquot	Final conc.
DNA	25 ng/µl	1 µl	25 ng
PCR buffer	10x	1 µl	1x
dNTP mix	2mM	1 µl	0.2mM
Forward Primer	5 pM	0.5 µl	
Reverse Primer	5 pM	0.5 µl	
Taq DNA polymerase:	3 U/µl	0.2 µl	0.6 U
Milli-Q water	-	5.8 µl	-
Total		10 µl	-

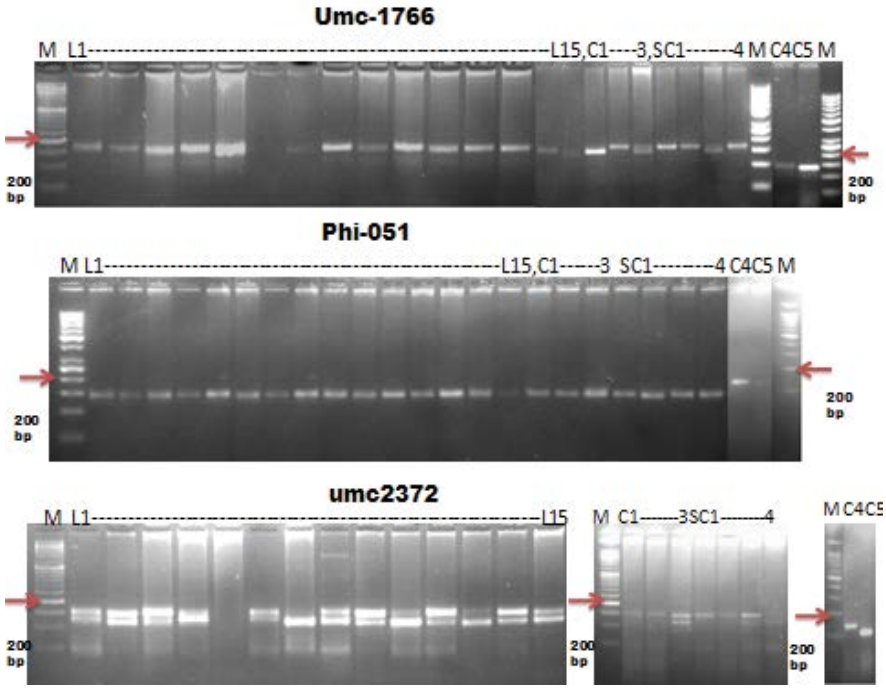

Figure 1: Electrophoretic banding of SSR amplification product resolved on 3.5 % agrose gel for diversity analysis.

Table 5: Allele frequency, alleles5 per locus, gene diversity, heterozygosity and PIC values obtained using SSR locus.

S.No.	Locus	Alleles per locus	Gene Diversity	Heterozygosity	PIC value	Major allelic frequency	Minor allelic frequency
1	Umc2383	7	0.854	0.000	0.837	0.21	0.05
2	Umc1664	10	0.878	0.125	0.779	0.32	0.05
3	Umc1147	8	0.823	0.000	0.835	0.29	0.04
4	Umc1823	10	0.853	0.167	0.802	0.29	0.04
5	Umc1026	7	0.833	0.000	0.820	0.30	0.09
6	Umc2372	10	0.825	0.500	0.563	0.43	0.04
7	Umc2144	12	0.861	0.333	0.735	0.39	0.06
8	Umc1594	9	0.855	0.042	0.843	0.22	0.06
9	Bnlg1621	6	0.798	0.083	0.742	0.31	0.06
10	Umc1478	7	0.778	0.000	0.744	0.36	0.05
11	Umc1766	7	0.799	0.000	0.799	0.33	0.04
12	Bnlg1306	12	0.891	0.083	0.875	0.16	0.05
13	Umc1918	9	0.837	0.250	0.662	0.37	0.05
14	Umc1762	8	0.740	0.042	0.740	0.43	0.07
15	Umc1063	8	0.857	0.042	0.824	0.23	0.05
16	Umc1018	10	0.854	0.000	0.854	0.21	0.04
17	Phi452693	9	0.859	0.042	0.837	0.26	0.04
18	Umc1424	7	0.868	0.000	0.851	0.18	0.09
19	Phi129	7	0.823	0.000	0.796	0.28	0.06
20	Umc1002	7	0.795	0.000	0.761	0.35	0.06
21	Phi051	7	0.701	0.000	0.701	0.42	0.04
22	Umc1036	8	0.766	0.125	0.738	0.33	0.04
23	Umc1708	11	0.889	0.000	0.889	0.17	0.04
24	Bnlg1056	7	0.677	0.000	0.708	0.50	0.08
25	Umc1141	6	0.799	0.000	0.757	0.38	0.05
26	Umc1415	6	0.809	0.000	0.771	0.33	0.05
27	Umc1786	11	0.844	0.000	0.898	0.13	0.06
28	Phi067	6	0.778	0.000	0.744	0.41	0.05
29	Phi061	7	0.819	0.000	0.793	0.32	0.05
30	Umc1077	11	0.855	0.042	0.875	0.18	0.06
31	Mmc0501	13	0.840	0.000	0.891	0.25	0.06
32	Bmc1655	8	0.817	0.042	0.773	0.38	0.05
	Total	271					
	Mean	8.46	0.821	0.060	0.788	0.30	0.05
	Range	6-13	0.891 - 0.677	0.000 – 0.500	0.563 – 0.898	0.13 - 0.43	0.04 - 0.09

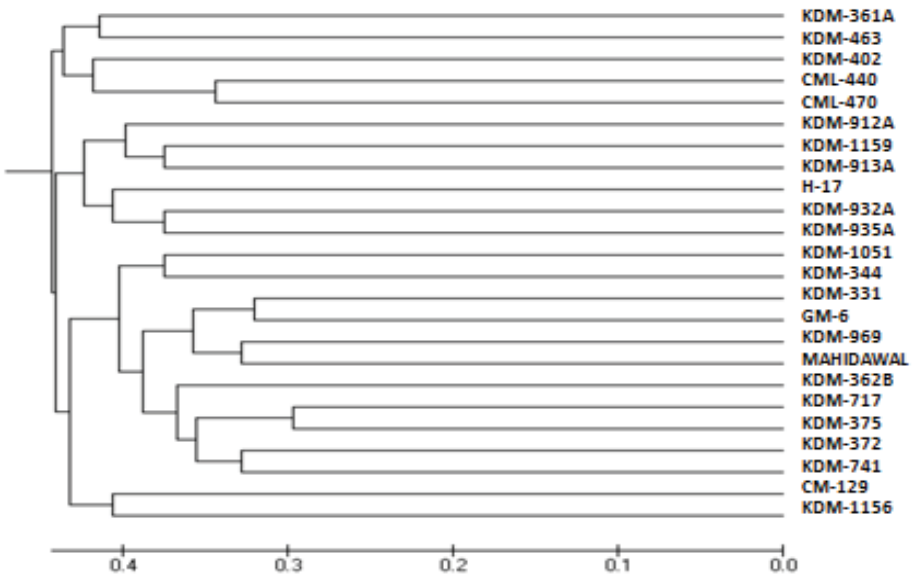

Figure 2: Dendrogram generated using UPGMA analysis showing relationship among maize lines using SSR marker.

amplification of similar sequences in different genomic regions due to duplications possibly explains the occurrence of 'double - bands' [21] when analyzed with locus umc-2372. However, the low level of heterozygosity in the inbred lines revealed that they have been maintained properly and the reported heterozygosity was inherent.

PIC values are influenced by a number of factors *viz*., nature of germplasm, number of SSR loci as well as the inbred lines analyzed, SSR loci assayed, in terms of the nature and type of repeats and methodology employed for allele detection (PAGE versus agrose). The mean PIC value was 0.788 with locus umc-2372 having the lowest PIC value (0.563) whereas the locus umc1786 had the highest PIC value (0.898). Closely related lines would express lower PIC, whereas genetically diverse lines exhibit higher PIC values. Apart from being a different marker system which can detect polymorphisms, the ability to resolve the alleles also plays a crucial role in detecting the number of alleles. The mean PIC value was comparable with the previous findings [15,23], because of the nature of markers were chosen for polymorphism. PIC and alleles per locus indicated that selected primers were highly polymorphic and the degree of diversity among the lines was high and PIC was sufficient to group the population into different clusters. The results were comparable with the findings in Mishra et al. and Wasala et al. [24-26].

Genetic diversity is defined as the probability that two randomly chosen alleles from the population are different among the maize genotypes [19]. The maximum amount of gene diversity was exhibited by the marker Bnlg1306 (0.891) whereas, the minimum gene diversity of (0.677) was recorded for Bnlg1056. The mean value of the gene diversity was 0.821. Since the genetic diversity and PIC values recorded in these studies were within those recorded for genetic diversity studies in maize, these SSR markers are considered useful for genetic purity analysis of maize varieties. Major allelic frequency ranged from 0.13 - 0.43 with a mean of 0.30 whereas the minor allelic frequency ranged from 0.04 to 0.09 with a mean of 0.05 respectively.

The lines got grouped into two major clusters (Figure 2). One group had five lines viz., KDM-361A, KDM-463, KDM-402, CML-440 and CML-470. Another group was further separated into two clusters one having six lines viz., KDM-912A, KDM-1159, KDM-913A, H-17, KDM-932A and KDM-935A and second group had thirteen lines with further classification viz., KDM-1051, KDM-344, KDM-331, GM-6, KDM-969, Mahidhawal, KDM-362B, KDM-717, KDM-375, KDM-372, KDM-741, CM-129 and KDM-1156. The weighted Neighbor Joining (NJ) method (Figure 3) grouped the 24 lines into three major groups (a, b and c). The group A had thirteen lines and it was further divided into two subgroups. The second group (B) had five lines and the third group (C) had six lines. Two lines bred at CIMMYT for drought tolerance were grouped together with elite lines in group A. Group B had lines susceptible to drought stress. This study opened up a lot of possibility of development of hybrids tolerance to drought stress by exploiting the diversity pattern. The SSR-based clustering and the relationship among parental lines can be further used for development of new hybrids. Genetic diversity studies in maize to determine the genetic distance (GD) using various operational taxonomic units (OTUs) are a useful practice. From the genetic diversity analysis results of present study, maize inbreds lacking their pedigree data could be identified based on their GD to make hybridization between them to result in the development of a good hybrid.

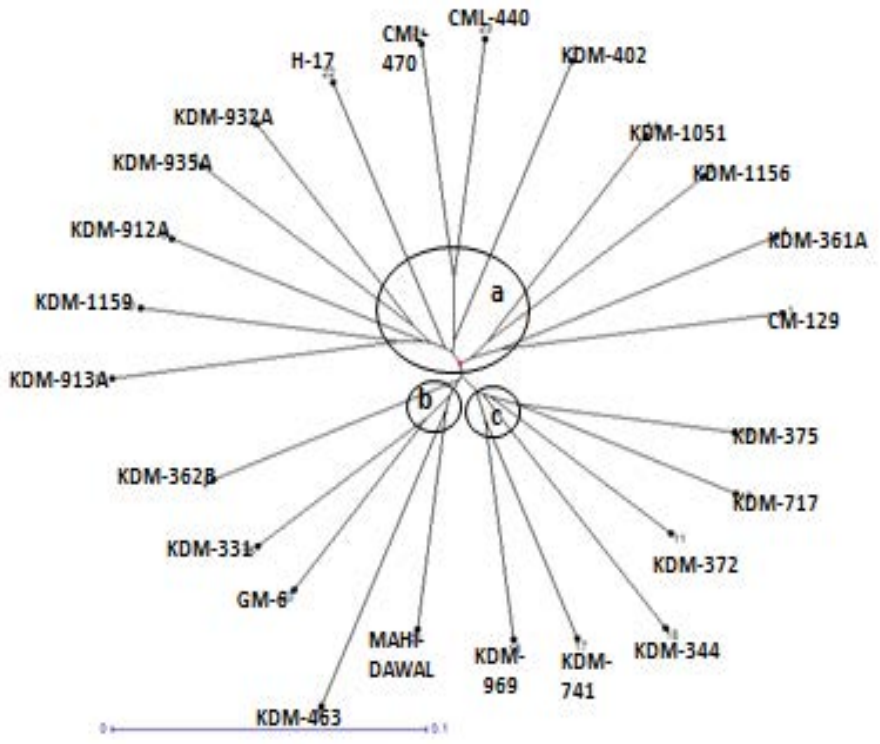

Figure 3: Neighbor joining NJ tree (Radial) generated using DARwin showing relationship among maize inbred lines using SSR marker.

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Upadhyay et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00069.0

Vegetos- An International Journal of Plant Research

Research Article

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Evaluation of Abiotic and Biotic Elicitors for Induction of Defense Related Enzymes in Pea against *U. Viciae–Fabae* (Pers.) J. Schrot

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Abstract

Total of fifteen elicitors tested alone/or in combination for induction of defense related enzymes in pea against *U. viciae–fabae* (Pers.) J. Schrot results in significant induction of total phenols, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in all the treatment as compare to control. Salicylic acid, *Pseudomonas fluorescens*, salicylic acid + *Pseudomonas fluorescens* were found most effective in induction of total phenols and peroxidase at 72 hrs after spray of elicitors. Polyphenol oxidase induction was found significantly high in oxalic acid, *Pseudomonas fluorescens* + *Trichoderma harzianum* and chitosan + *Pseudomonas fluorescens* at 72 hrs after spray of elicitors. Among all the treatments, maximum induction of Phenylalanine ammonia lyase activity was found in oxalic acid, *Trichoderma harzianum* + *Pseudomonas fluorescens* and isonicotinic acid + *Trichoderma harzianum* after 48hrs of spray of elicitors. Effect of different elicitors on percent disease index (PDI) 20 days after inoculation with uredospores of *U. viciae–fabae* showed least PDI in salicylic acid, *Trichoderma harzianum* + *Pseudomonas fluorescens* and chitosan + *Pseudomonas fluorescens* treated plants.

Keywords

Elicitors *U. viciae–fabae*; *Pseudomonas fluorescens*; *Trichoderma harzianum*; Percent disease index

Introduction

A large proportion of Indian population is vegetarian and pulses are the main source of protein for them. The protein content in pulses is about 18-25 per cent. This makes pulse one of the cheapest source of protein for human consumption [1].

India is the largest producer, consumer and importer of pulses in the world. In India pulses are grown about 24-26 million hectares of area producing 17-19 million tonnes of pulses annually. India accounts for over one third of the total world area and over 20 per cent of total world pulse production. Consequently per capita production and availability of pulses in the country has witnessed sharp decline. Per capita net pulse availability has declined from around 60 grams per day in the 1950s to 40 grams in the 1980s and further to around 35 grams per day in 2000s. However, in the past four years, there has been significant increase in consumption averaging around 50 grams due to higher production, under owing to National Food Security Mission (NFSM), with major emphasis on pulses and their imports, mostly of dry peas from Canada and Australia [2].

Major pulses grown in India include chickpea or bengal gram (*Cicer arietinum*), pigeonpea or red gram (*Cajanus cajan*), lentil (*Lens culinaris*), urdbean or black gram (*Vigna mungo*), mungbean or green gram (*Vigna radiata*), lablab bean (*Lablab purpureus*), moth bean (*Vigna aconitifolia*), horse gram (*Dolichos uniflorus*), pea (*Pisum sativum* L.), grass pea or khesari (*Lathyrus sativus*), cowpea (*Vigna unguiculata*), and broad bean or faba bean (*Vicia faba*).

During 2012-13, field pea (*Pisum sativum* L.) occupies an area of 0.76 million hectares with a production 0.84 million tonnes and productivity of 1100 kg/ha in our country. In Uttarakhand, area, production and productivity of pea during 2012-13 was 61.0 thousand hectares, 51.3 thousand tones and 841 kg/ha, respectively [3].

Pea is affected by a number of fungal (rust, powdery mildew, downy mildew, root rot, alternaria blight, aschochyta blight, wilt, anthracnose, cercospora leaf spot, damping off, seedling rot etc.), bacterial (bacterial blight and brown spot), nematode (cyst nematode, lesion nematode and root-knot nematode) and viral diseases (cucumber mosaic virus, pea early browning virus, pea enation mosaic, pea mosaic, pea seed borne mosaic, pea streak and pea stunt). These diseases, under the right conditions, can significantly decrease both yield and quality. Among these, the rust of pea caused by *Uromyces viciae–fabae* [4], *Uromyces fabae* [5] is considered the most important under warm and humid conditions [6]. It has been reported from different parts of the country including eastern India [7,8], central India [9], southern parts of India [10,11] and from Himalayan region of Uttarakhand and Himachal Pradesh [12,13]. In the last few years, disease has been observed in almost epiphytotic form and could cause up to 20-100% losses in yield [13,14].

Plants can be sensitized for a more rapid or more intense mobilization of defence responses leading to enhanced resistance to biotic or abiotic stresses [15]. Many factors such as prior pathogen attack and various chemical and environmental stimuli may act on plants to induce systemic acquired resistance (SAR) to subsequent pathogen attack [5,12,16,17]. SAR has been reported to be effective against a broad spectrum of pathogens including viruses, fungi, bacteria, nematodes and parasitic weeds [15]. Induction of systemic resistance is associated with gene induction, the activation of a wide range of resistance mechanisms and the production of a wide range of defence compounds. It is race non-specific and is often effective against a broad spectrum of pathogenic agents [18,19]. Thus, study on induction of host defence through biotic and abiotic elicitors can be one of the effective sustainable approaches in disease management.

Materials and Methods

Present investigation was carried out both in glass house and Bio-control Laboratory of Department of Plant Pathology in 2014-15 at G.B. Pant University of Agriculture and Technology, Pantnagar. Soil was collected from the upper 0-15 cm layer from NEBCRC and was sterilized by autoclaving at 21lb (121.6˚C) for one hour on three

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Received: July 01, 2016 **Accepted:** July 19, 2016 **Published:** July 25, 2016

consecutive days. The sterilized soil was filled in 5 kg capacity plastic pots and kept in glasshouse. Pots were watered and left for two days to maintain appropriate moisture for proper seed germination. Seeds of highly susceptible pea cultivar 'HFP-4' were washed thoroughly with sterilized distilled water and then treated with sodium hypochlorite for 60-90 seconds and then washed 2-3 times in sterilized distilled water under aseptic condition. Seven seeds were sown in each pot. Three healthy seedlings were maintained in each pot. Pots were watered regularly as and when required to maintain optimum moisture. Experiments were laid out in a completely randomized design with three replications. Recommended concentrations of biotic and abiotic elicitor alone and/or in combination (Table 1) were prepared in sterile water to which Tween-20 (0.03%, v/v) was added. Control plants were treated with sterile water plus Tween-20. Solutions were applied with the help of a paint-brush on 70 days old pea plants. After 24 hours, the top five leaves were collected from all the treatments. Then, on the same day plants were inoculated by spraying with a solution of 1.5×10^6 spores/ml of the local aggressive isolate *U. viciae fabae*. After 24, 46 and 72 hours of inoculation, the top five leaves per treatments were harvested and brought to the laboratory in an ice box. Fresh leaves were weighed and used for determination of activities of enzymes. Disease scoring was also recorded for different treatments on 20 days after inoculation (DAI) using 0-9 rating scale [20] and then disease score data were converted into Per cent Disease Index (PDI) [21].

Per cent disease index = Sum of all numerical rating × 100

Number of leaves examined x Maximum grade

The experimental results were then analyzed statistically. The following enzymatic analysis has been carried out:

Peroxidase (PO) activity

Assay of peroxidase (PO) activity was carried out as per the procedure described by Hammerschmidt et al. [22]. Enzyme extract was prepared by homogenizing one gram of leaf samples in 0.1M sodium phosphate buffer (pH 6.0). It was than centrifuged at 10,000 rpm for 20 min. The reaction mixture consisted of 2.5 ml of a mixture containing 0.25 per cent (v/v) guaiacol in 0.01 M sodium phosphate buffer, pH 6.0 and 0.1 M hydrogen peroxide. Enzyme extract (0.1 ml) was added to initiate the reaction, which was followed colorimetrically at 480 nm. The boiled enzyme preparation served as blank. Activity was expressed as the increase in absorbance at 480 nm min^{-1} mg^{-1} leaf sample.

Polyphenol oxidase (PPO) activity

PPO activity was determined as per the procedure given by Mayer et al. [23]. Leaf samples (1 g) were homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used as the enzyme source. The reaction mixture consisted of 200 µL of the enzyme extract and 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction, 200 µL of 0.01M catechol was added and the activity was expressed as changes in absorbance at 495 nm min^{-1} mg^{-1} leaf sample.

Phenylalanine ammonia lyase (PAL) activity

Enzyme extracted in 0.1 M sodium phosphate buffer (pH 7.0) was used out as per the method described by Ross and Sederoff [24]. About 1 g of leaf sample was homogenized with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4°C. The homogenate was centrifuged for 2.0 min at 10,000 rpm. The supernatant was used as a crude extract for enzyme activity. The assay mixture containing 100 µl of enzyme, 500 µl of 50 mM Tris HCl (pH 8.8) and 600 µl of 1 mM L-phenylalanine was incubated for 60 min and the reaction was arrested by adding, 2 N HCl. Later 1.5 ml of toluene was added, vortexed for 30 sec, centrifuged (1000 rpm, 5 min) and toluene fraction containing trans- cinnamic acid was separated. The toluene phase was measured at 290 nm against the blank of toluene. Standard curve was drawn with graded amounts of cinnamic acid in toluene. The enzyme activity was expressed in µ moles of cinnamic acid min^{-1} mg^{-1} of protein.

Total phenolics

Total phenolics content was determined by following the method of Swain and Hills [25]. One gram leaves were homogenized in 10

Table 1: List of elicitors alone and in combination used for evaluation of defence related enzymes induction in pea against *U. viciae fabae* (Pers.) J. Schrot.

Treatments	Name (source)	Concentration
	Abiotic elicitors	
1.	Salicylic acid (Sigma-Aldrich)	5 mM
2.	Chitosan (Sigma-Aldrich)	1 g/100 ml (1%)
3.	Oxalic acid (Sigma-Aldrich)	20 mM
4.	Isonicotinic acid (Sigma-Aldrich)	100 µg/ml
	Biotic elicitors	
5.	*Pseudomonas fluorescens* (GBPUAT, Pantnagar)	10^8 cfu/ml
6.	*Trichoderma harzianum* (GBPUAT, Pantnagar)	10^6 cfu/ml
7.	*Trichoderma harzianum*+ *Pseudomonas fluorescens*	10^6 cfu/ml+10^8 cfu/ml
	Combinations	
8.	Salicylic acid + *Trichoderma harzianum*	2.5 mM + 10^6 cfu/ml
9.	Salicylic acid + *Pseudomonas fluorescens*	2.5 mM + 10^8 cfu/ml
10.	Chitosan + *Trichoderma harzianum*	0.5 g/100 ml+10^6 cfu/ml
11.	Chitosan + *Pseudomonas fluorescens*	0.5 g/100 ml+10^8 cfu/ml
12.	Oxalic acid + *Trichoderma harzianum*	10 mM + 10^6 cfu/ml
13.	Oxalic acid + *Pseudomonas fluorescens*	10 mM + 10^8 cfu/ml
14.	Isonicotinic acid + *Trichoderma harzianum*	50 µg/ml+ 10^6 cfu/ml
15.	Isonicotinic acid + *Pseudomonas fluorescens*	50 µg/ml + 10^8 cfu/ml
16.	Water	-

ml of 80% methanol and agitated for 15 min at 70°C. One ml of methanolic extract was added to 5 ml of distilled water and 250 µl of Folin-ciocalteu reagent, after this the solution was kept at 25°C. After 3 min, 1 ml of a saturated solution of Na_2CO_3 and 1 ml of distilled water were added, and the reaction mixture was incubated for 1 hr at 25°C. The absorption of the developed color was measured using spectrophotometer at 725 nm. The total soluble phenolic content was calculated by comparison with a standard curve obtained from Folin-Ciocalteu reaction with catechol. Results were expressed as phenol equivalent in µg/mg g^{-1} of fresh weight.

The data was analyzed statistically by Complete Randomized Block design (CRD) [26] using STPR (GBPUA&T statistical software and MS Excel. Data recorded were first transformed (angularly transformed) to make them homogenous before analysis and the treatment were compared by means of critical differences at one per cent level of significance.

Results and Discussion

Induction of different defense related enzymes in pea against *U. viciae–fabae* (Pers.) J. Schrot through abiotic and biotic elicitors were estimated as follows:

Total phenols (mg/gm of fresh leaf)

Data pertaining to effect of different elicitors on activity of total phenols in pea revealed that induction of total phenols was found significantly at 72 hrs after spray of elicitors as compare to control (Table 2).

Between all abiotic elicitors, induction was found maximum in salicylic acid (21.70) followed by oxalic acid (21.66) and isonicotinic acid (21.66). Whereas chitosan showed comparatively less induction (18.47). In biotic elicitors, *Pseudomonas fluorescens* (27.31) was found most effective in induction followed by *Trichoderma harzianum* + *Pseudomonas fluorescens* (27.07) and *Trichoderma harzianum* (20.96). ong all combinations, salicylic acid + *Pseudomonas fluorescens* (24.29) was most effective followed by salicylic acid + *Trichoderma harzianum* (23.22), isonicotinic acid + *Pseudomonas fluorescens* (22.96), chitosan + *Trichoderma harzianum* (22.40), chitosan + *Pseudomonas fluorescens* (21.72), oxalic acid + *Pseudomonas fluorescens* (21.60), oxalic acid + *Trichoderma harzianum* (21.07), isonicotinic acid + *Trichoderma harzianum* (20.27). Lowest induction was observed in control (12.96).

Salicylic acid, *Pseudomonas fluorescens* and salicylic acid + *Pseudomonas fluorescens* were found most effective among all the treatments.

Peroxidase (µmol/min/mg/protein)

Induction of peroxidase in pea by different elicitors was found significantly at 72 hrs after spray of elicitors as compare to control.

Among all abiotic elicitors, induction was found maximum in salicylic acid (52.43) followed by chitosan and isonicotinic acid (49.12) whereas oxalic acid (28.54) showed very less induction. In biotic elicitors, *Pseudomonas fluorescens* (58.41) showed high induction followed by *Trichoderma harzianum* + *Pseudomonas fluorescens* (47.12) and *Trichoderma harzianum* (35.84). Between all combinations, salicylic acid + *Pseudomonas fluorescens* (52.43) was found most effective followed by isonicotinic acid + *Pseudomonas fluorescens* (51.77), oxalic acid + *Pseudomonas fluorescens* (49.78), chitosan + *Pseudomonas fluorescens* (48.45), isonicotinic acid + *Trichoderma harzianum* (42.48), salicylic acid + *Trichoderma harzianum* (39.82), chitosan + *Trichoderma harzianum* (37.17), oxalic acid + *Trichoderma harzianum* (28.54). Lowest induction was observed in control (26.55) (Table 3).

Table 2: Effect of elicitors on total phenols accumulation in pea under controlled condition.

TOTAL PHENOLS (mg/gm of fresh leaf)				
	Time intervals			
Elicitors	**24hr**	**48hr**	**72hr**	**96hr**
Abiotic				
Salicylic acid	9.74 ± 0.24	19.04 ± 0.84	21.70 ± 0.26	14.10 ± 1.13
Chitosan	12.82 ± 0.06	17.10 ± 0.38	18.47 ± 0.49	14.11 ± 0.33
Oxalic acid	17.26 ± 0.63	16.82 ± 0.36	21.66 ± 0.76	11.35 ± 0.90
Isonicotinic acid	9.40 ± 0.18	17.48 ± 1.41	21.66 ± 0.76	12.50 ± 1.00
Biotic				
Pseudomonas fluorescens	13.52 ± 0.01	25.15 ± 1.05	27.31 ± 1.21	18.38 ± 0.49
Trichoderma harzianum	4.96 ± 0.03	16.48 ± 0.33	20.96 ± 0.70	16.85 ± 0.36
Trichoderma harzianum + *Pseudomonas fluorescens*	9.17 ± 0.16	22.00 ± 0.79	27.07 ± 1.23	16.98 ± 0.37
Combinations				
Salicylic acid + *Trichoderma harzianum*	13.99 ± 0.56	16.61 ± 0.34	23.22 ± 0.98	21.38 ± 0.74
Salicylic acid + *Pseudomonas fluorescens*	5.28 ± 0.03	16.82 ± 0.36	24.29 ± 0.89	17.95 ± 0.45
Chitosan + *Trichoderma harzianum*	9.02 ± 0.05	15.53 ± 0.25	22.40 ± 0.82	18.94 ± 0.53
Chitosan + *Pseudomonas fluorescens*	8.17 ± 0.13	15.24 ± 0.22	21.72 ± 0.76	18.30 ± 0.48
Oxalic acid + *Trichoderma harzianum*	11.08 ± 0.29	12.77 ± 0.02	21.07 ± 0.71	13.46 ± 1.08
Oxalic acid + *Pseudomonas fluorescens*	8.58 ± 0.09	14.64 ± 0.17	21.60 ± 0.75	16.49 ± 0.33
Isonicotinic acid + *Trichoderma harzianum*	10.57 ± 0.34	17.15 ± 0.38	20.27 ± 0.64	14.65 ± 1.18
Isonicotinic acid + *Pseudomonas fluorescens*	7.38 ± 0.22	19.43 ± 0.57	22.96 ± 0.87	18.11 ± 0.46
Water (Control)	8.21 ± 0.05	15.82 ± 0.27	12.96 ± 1.03	12.53 ± 1.00
CD at 1%	**a= 0.42****	**b= 0.21****	**a*b= 0.84****	
SEM	**a= 0.11**	**b= 0.57**	**a*b= 0.22**	
CV	**2.42**			

± = Standard error, a= Time interval, b= Elicitors, ** Significant level at the 0.01.

Salicylic acid, *Pseudomonas fluorescens* and salicylic acid + *Pseudomonas fluorescens* were found most effective between all the treatments.

Polyphenol oxidase (µmol/min/mg/protein)

Data pertaining to effect of different elicitors on polyphenol oxidase activity in pea revealed that polyphenol oxidase induction was found at 72 hrs after spray of elicitors as compare to control (Table 4).

Between all abiotic elicitors, induction was found highest in oxalic acid (17.67) followed by isonicotinic acid (16.02), chitosan

Table 3: Effect of elicitors on Peroxidase (PO) activity in pea under controlled condition.

PEROXIDASE (µmol/min/mg/protein)				
	Time interval			
Elicitors	**24hr**	**48hr**	**72hr**	**96hr**
Abiotic				
Salicylic acid	30.65 ± 0.97	35.84 ±0.22	52.43 ± 0.55	22.57 ± 0.20
Chitosan	8.63 ± 0.08	39.82 ± 0.75	49.12 ± 0.73	23.23 ± 0.34
Oxalic acid	21.24 ± 0.71	13.94 ± 0.15	28.54 ± 0.23	21.24 ± 0.11
Isonicotinic acid	35.18 ± 0.55	45.80 ± 0.32	49.12 ± 1.02	24.56 ± 0.97
Biotic				
Pseudomonas fluorescens	22.57 ± 0.04	15.93 ± 0.46	58.41 ± 0.53	17.92 ± 0.24
Trichoderma harzianum	25.22 ± 0.52	24.56 ± 0.44	35.84 ± 0.61	18.58 ± 0.27
Trichoderma harzianum + *Pseudomonas fluorescens*	22.57 ± 0.69	40.49 ± 0.47	47.12 ± 1.27	35.84 ± 1.13
Combinations				
Salicylic acid + *Trichoderma harzianum*	16.59 ± 0.09	15.93 ± 0.67	39.82 ± 1.26	27.88 ± 0.78
Salicylic acid + *Pseudomonas fluorescens*	23.23 ± 0.44	27.88 ± 1.08	52.43 ± 0.65	29.87 ± 0.86
Chitosan + *Trichoderma harzianum*	12.61 ± 0.24	23.89 ± 0.95	37.17 ± 1.17	26.55 ± 0.50
Chitosan + *Pseudomonas fluorescens*	23.23 ± 0.48	35.84 ± 0.94	48.45 ± 0.39	40.49 ± 1.46
Oxalic acid + *Trichoderma harzianum*	43.81 ± 0.71	18.58 ± 0.37	28.54 ± 0.08	24.56 ± 0.11
Oxalic acid + *Pseudomonas fluorescens*	40.49 ± 0.11	30.53 ± 0.25	49.78 ± 0.57	41.81 ± 0.21
Isonicotinic acid + *Trichoderma harzianum*	29.87 ± 0.05	34.51 ± 0.93	42.48 ± 1.03	33.85 ± 0.85
Isonicotinic acid + *Pseudomonas fluorescens*	23.89 ± 1.00	45.13 ± 0.57	51.77 ± 0.21	44.47 ± 0.39
Water (Control)	7.96 ± 0.07	19.25 ± 0.85	26.55 ± 0.02	18.58 ± 0.75
CD at 1%	**a= 0.93****	**b= 0.46****	**a*b= 1.86****	
SEM	**a= 0.25**	**b= 0.12**	**a*b= 0.50**	
CV	**2.78**			

± = Standard error, a= Time interval, b= Elicitors, ** Significant level at the 0.01.

Table 4: Effect of elicitors on Polyphenol oxidase (PPO) activity in pea under controlled condition.

POLYPHENOL OXIDASE (µmol/min/mg/protein)				
	Time interval			
Elicitors	**24hr**	**48hr**	**72hr**	**96hr**
Abiotic				
Salicylic acid	5.73 ± 0.08	5.81 ± 0.25	10.29 ± 0.20	7.77 ± 0.16
Chitosan	6.05 ± 0.24	6.52 ± 0.12	11.93 ± 0.48	3.53 ± 0.03
Oxalic acid	4.71 ± 0.04	12.72 ± 0.37	17.67 ± 0.10	8.17 ± 0.13
Isonicotinic acid	4.71 ± 0.07	10.99 ± 0.15	16.02 ± 0.32	9.81 ± 0.12
Biotic				
Pseudomonas fluorescens	5.65 ± 0.23	5.89 ± 0.18	7.77 ± 0.14	2.36 ± 0.07
Trichoderma harzianum	5.57 ± 0.10	6.83 ± 0.01	10.36 ± 0.02	2.04 ± 0.03
Trichoderma harzianum + *Pseudomonas fluorescens*	6.44 ± 0.08	7.77 ± 0.21	13.82 ± 0.56	8.95 ± 0.24
Combinations				
Salicylic acid + *Trichoderma harzianum*	6.05 ± 0.01	7.62 ± 0.26	14.84 ± 0.44	8.32 ± 0.37
Salicylic acid + *Pseudomonas fluorescens*	6.36 ± 0.24	5.10 ± 0.18	9.34 ± 0.19	8.24 ± 0.19
Chitosan + *Trichoderma harzianum*	2.43 ± 0.07	7.14 ± 0.09	8.17 ± 0.31	6.28 ± 0.04
Chitosan + *Pseudomonas fluorescens*	5.73 ± 0.17	4.95 ± 0.17	16.57 ± 0.40	8.87 ± 0.02
Oxalic acid + *Trichoderma harzianum*	3.61 ± 0.08	7.38 ± 0.22	15.47 ± 0.61	1.65 ± 0.01
Oxalic acid + *Pseudomonas fluorescens*	4.63 ± 0.09	10.68 ± 0.02	15.15 ± 0.52	14.52 ± 0.48
Isonicotinic acid + *Trichoderma harzianum*	4.32 ± 0.05	6.28 ± 0.20	15.00 ± 0.09	12.33 ± 0.27
Isonicotinic acid + *Pseudomonas fluorescens*	5.57 ± 0.17	9.50 ± 0.24	14.21 ± 0.28	8.32 ± 0.25
Water (Control)	2.90 ± 0.05	4.63 ± 0.13	6.60 ± 0.08	3.22 ± 0.03
CD at 1%	**a= 0.24****	**b= 0.12****	**a*b= 0.49****	
SEM	**a= 0.67**	**b= 0.33**	**a*b= 0.13**	
CV	**2.88**			

± = Standard error, a= Time interval, b= Elicitors, ** Significant level at the 0.01.

(11.93) and salicylic acid (10.29). In biotic elicitors, *Trichoderma harzianum* + *Pseudomonas fluorescens* (13.82) showed maximum induction followed by *Trichoderma harzianum* (10.36) and *Pseudomonas fluorescens* (7.77). Among all combinations, chitosan + *Pseudomonas fluorescens* (16.57) was most effective followed by oxalic acid + *Trichoderma harzianum* (15.47), oxalic acid + *Pseudomonas fluorescens* (15.15), isonicotinic acid + *Trichoderma harzianum* (15.00), salicylic acid + *Trichoderma harzianum* (14.84), isonicotinic acid + *Pseudomonas fluorescens* (14.21), salicylic acid + *Pseudomonas fluorescens* (9.34) and chitosan + *Trichoderma harzianum* (8.17). Lowest induction was observed in water (6.60) (Figure 1).

Oxalic acid, *Trichoderma harzianum* + *Pseudomonas fluorescens* and chitosan + *Pseudomonas fluorescens* were found most effective between all the treatments.

Phenylalanine ammonia lyase (PAL) (mg/gm of fresh leaf)

Effect of different elicitors on PAL activity in pea revealed that induction of PAL was found at 48 hrs after spray of elicitors as compare to control (Table 5).

Among all abiotic elicitors, induction was found maximum in oxalic acid (48.54) followed by salicylic acid (47.44), chitosan (38.68) and

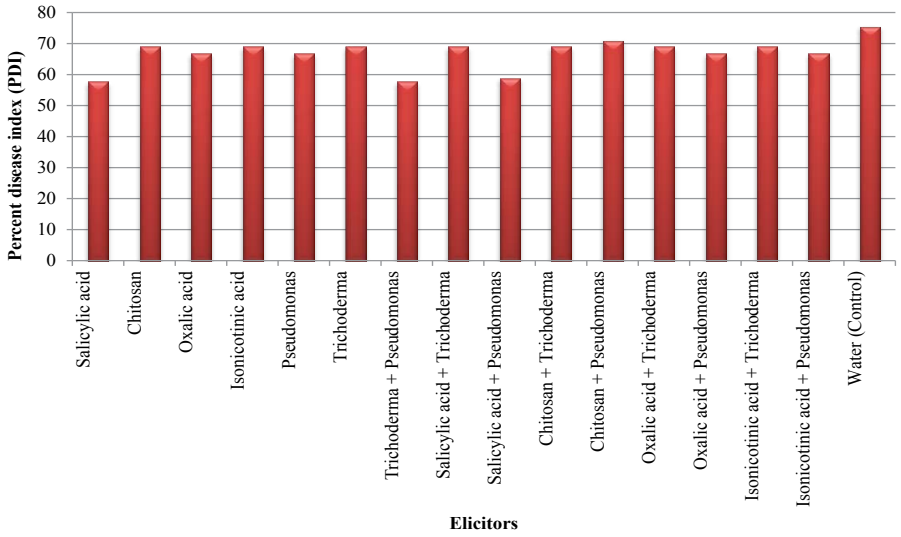

Figure 1: Effect of elicitors on percent disease index (PDI) of rust in pea under controlled condition.

Table 5: Effect of elicitors on Phenylalanine ammonia lyase (PAL) activity in pea under controlled condition.

PHENYLALANINE AMMONIA LYASE (mg/gm of fresh leaf)				
	Time interval			
Elicitors	**24hr**	**48hr**	**72hr**	**96hr**
Abiotic				
Salicylic acid	20.62 ± 0.89	47.44 ± 0.42	17.33 ± 0.26	15.69 ± 0.51
Chitosan	20.25 ± 0.73	38.68 ± 1.01	14.78 ± 0.37	9.85 ± 0.15
Oxalic acid	28.46 ± 0.41	48.54 ± 0.13	37.59 ± 0.43	15.87 ± 0.29
Isonicotinic acid	16.79 ± 0.68	13.50 ± 0.37	17.88 ± 0.47	8.21 ± 0.25
Biotic				
Pseudomonas fluorescens	33.76 ± 0.88	51.45 ± 0.42	33.94 ± 0.18	29.38 ± 0.77
Trichoderma harzianum	10.58 ± 0.30	27.92 ± 1.03	19.89 ± 0.22	9.85 ± 0.43
Trichoderma harzianum + *Pseudomonas fluorescens*	30.11 ± 1.11	52.18 ± 1.21	35.58 ± 0.55	30.11 ± 0.30
Combinations				
Salicylic acid + *Trichoderma harzianum*	6.20 ± 0.06	31.57 ± 0.63	21.53 ± 0.58	15.33 ± 0.29
Salicylic acid + *Pseudomonas fluorescens*	13.50 ± 0.09	25.00 ± 0.23	35.95 ± 1.23	17.70 ± 0.64
Chitosan + *Trichoderma harzianum*	19.52 ± 0.25	36.49 ± 0.48	33.21 ± 0.33	32.48 ± 0.43
Chitosan + *Pseudomonas fluorescens*	19.16 ± 0.19	28.65 ± 0.39	20.80 ± 0.82	10.40 ± 0.12
Oxalic acid + *Trichoderma harzianum*	6.57 ± 0.04	26.27 ± 0.12	19.16 ± 0.74	8.21 ± 0.22
Oxalic acid + *Pseudomonas fluorescens*	14.23 ± 0.40	19.89 ± 0.48	17.70 ± 0.51	9.31 ± 0.25
Isonicotinic acid + *Trichoderma harzianum*	21.17 ± 0.11	44.70 ± 0.37	29.56 ± 0.85	23.54 ± 0.30
Isonicotinic acid + *Pseudomonas fluorescens*	17.15 ± 0.01	31.75 ± 0.18	27.92 ± 1.21	21.90 ± 0.51
Water (Control)	11.86 ± 0.27	15.87 ± 0.29	8.21 ± 0.28	7.91 ± 0.59
CD at 1%	**a= 0.71****	**b= 0.35****	**a*b= 1.43****	
SEM	**a= 0.19**	**b= 0.97**	**a*b= 0.38**	
CV	**2.89**			

± = Standard error, a= Time interval, b= Elicitors, ** Significant level at the 0.01.

isonicotinic acid (13.50). In biotic elicitors, *Trichoderma harzianum* + *Pseudomonas fluorescens* (52.18) was found most effective followed by *Pseudomonas fluorescens* (51.45) and *Trichoderma harzianum* (27.92). Between all combinations, Isonicotinic acid + *Trichoderma harzianum* (44.70) was most effective followed by chitosan + *Trichoderma harzianum* (36.49), Isonicotinic acid + *Pseudomonas fluorescens* (31.75), salicylic acid + *Trichoderma harzianum* (31.57), chitosan + *Pseudomonas fluorescens* (28.65), oxalic acid + *Trichoderma harzianum* (26.27), salicylic acid + *Pseudomonas fluorescens* (25.00), oxalic acid + *Pseudomonas fluorescens* (19.89). Lowest induction was observed in water (15.87).

Oxalic acid, *Trichoderma harzianum* + *Pseudomonas fluorescens* and Isonicotinic acid + *Trichoderma harzianum* were found most effective among all the treatments.

Effect of different elicitors on per cent disease index (PDI) for rust in pea

The data on effect of different elicitors on percent disease index (PDI) for rust in pea showed less PDI in all the elicitors treated plants as compare to control.

Among all abiotic elicitors, PDI was found maximum in chitosan and isonicotinic acid (68.88) followed by oxalic acid (66.66) and salicylic acid (57.77). In biotic elicitors, *Trichoderma harzianum* (68.88) showed highest PDI followed by *Pseudomonas fluorescens* (66.66) and *Trichoderma harzianum* + *Pseudomonas fluorescens* (57.77). Between all combinations, salicylic acid + *Trichoderma harzianum,* chitosan + *Trichoderma harzianum*, oxalic acid + *Trichoderma harzianum*, Isonicotinic acid + *Trichoderma harzianum* showed similar PDI of 68.88 followed by oxalic acid + *Pseudomonas fluorescens* and isonicotinic acid + *Pseudomonas fluorescens* (66.66), salicylic acid + *Pseudomonas fluorescens* (58.88) and chitosan + *Pseudomonas fluorescens* (57.77) (Figure 1).

Salicylic acid, *Trichoderma harzianum* + *Pseudomonas fluorescens* and Chitosan + *Pseudomonas fluorescens* recorded least plant disease index as compare to all the treatments.

Outcome of present investigations revealed that all fifteen elicitors tested alone/or in combination for induction of defense related enzymes in pea against *U. viciae–fabae* (Pers.) J. Schrot results in significant induction of total phenols, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in all the treatment as compare to control. Salicylic acid, *Pseudomonas fluorescens*, salicylic acid + *Pseudomonas fluorescens* were found most effective in induction of total phenols and peroxidase at 72 hrs after spray of elicitors. Polyphenol oxidase induction was found significantly high in oxalic acid, *Pseudomonas fluorescens* + *Trichoderma harzianum* and chitosan + *Pseudomonas fluorescens* at 72 hrs after spray of elicitors. Among all the treatments, maximum induction of Phenylalanine ammonia lyase activity was found in oxalic acid, *Trichoderma harzianum* + *Pseudomonas fluorescens* and isonicotinic acid + *Trichoderma harzianum* after 48hrs of spray of elicitors. Effect of different elicitors on percent disease index (PDI) 20 days after inoculation with uredospores of *U. viciae–fabae* showed least PDI in salicylic acid, *Trichoderma harzianum* + *Pseudomonas fluorescens* and chitosan + *Pseudomonas fluorescens* treated plants.

Several reviews have highlighted the potential of chemical treatments to activate and enhance natural plant disease resistance [17,18]. Dann and Deverall [27,28] stated that inoculation of unifoliate leaves of nine days old green bean (*Phaseolus vulgaris*) with spore suspension of *Colletotrichum lindemuthianum* (10^4 conidia/ml), causing local lesions, or spraying with 2-6-dichloroisonicotinic acid (20µg/ml) induces development of resistance in the upper leaves against challenge inoculation of *U. appendiculatus* afterwards. Rauscher et al. [29] reported the treatment of broad bean leaves with salicylic acid or 2, 6; dichloroisonicotinic acid induces resistance against the rust fungus *Uromyces viciae-fabae* resulting in reduced rust pustules density.

Pea (*Pisum sativum* L.) plants treated with different concentrations of salicylic acid and 4-aminobutyric acid increased activities of phenol metabolizing enzymes implicated in the defense of plants. The enzymes peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase and superoxide dismutase responded to treatment with variation in their activities. Phenolic content also varied following treatment with the inducers [30]. SA was the first synthetic compound shown to induce enhanced activation of a variety of defence responses against major pathogens on various crops [16,17] Peroxidase activity in cucumber (*Cucumis sativa* L.) and tobacco (*Nicotina tabaccum* L.) after treatment with SA have been reported [31]. Exogenous applications of salicylic acid (SA) and benzothiadiazole (BTH) solutions have been used in faba bean to induce systemic acquired resistance (SAR) to rust (*Uromyces viciae-fabae*), ascochyta blight (*Ascochyta fabae*) and broomrape (*Orobanche crenata*) by Sillero et al. [32]. Surekha et al. [33] conducted experiment to understand the role of *Trichoderma viride* in inducing defense enzymes (Peroxidase, Polyphenol Oxidase and Phenyl Alanine ammonia Lyase) and total phenolic content in black gram exposed to pathogens *Fusarium oxysporum* and *Alternaria alternata*. He found that the biocontrol agent, *T. viride* induce higher levels of defense enzymes in black gram during pathogenesis by *F. oxysporum* and *A. alteranata*. Nikoo et al. [34] also mentioned that plant-mediated systemic resistance against the *M. javanica* in tomato cv. CALJN3 was triggered using salicylic acid (SA) and *Pseudomonas fluorescens* CHAO as elicitors. Biochemical changes in *T. harzianum* treated plants, *M. phaseolina* inoculated plants and healthy plants were assayed at different stages of infection by Sreedevi et al. [35]. She found that treatment with *T. harzianum* and challenge inoculation of *M. phaseolina* enhanced induction of defense enzymes such as peroxidase (PO) and polyphenol oxidase (PPO) and defense compounds like total phenol and ortho-dihydric phenol. Numerous findings in other plant pathogen system such as *Puccinia helianthi*/sunflower [36,37], *Uromyces appendiculatus*/common bean [38], or *Uromyces pisi*/pea [39] based on reduction of infection frequency has also been reported. Systemic acquired resistance seems to be a mechanism different from the pre-existing resistance and is associated to the induction of pathogenesis related (PR) proteins [40]. Activation of SAR by exogenous application of elicitors can protect from a broad pathogens spectrum [29,38].

Therefore the above mentioned treatments might be an alternative for the conventional pesticides in pea crop protection, with the advantage of a low environmental impact.

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Mali et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00070.7

Vegetos- An International Journal of Plant Research

Research Article

A SCITECHNOL JOURNAL

Spatial Variability in Soil Properties of Mango Orchards in Eastern Plateau and Hill Region of India

Mali SS, Naik SK and Bhatt BP*

Abstract

Analysis and interpretation of spatial variability of soil chemical properties is important in framing site-specific fertilizer management practices for mango orchards. This paper aims to study the spatial structure of soil variables in the mango orchards in the eastern plateau region of India. Soil samples were collected from 90 points across the mango orchards from three soil horizons (0-30, 30-60 and 60-90cm). Eleven soil properties were analysed by classical statistical and geo-statistical methods. Soil pH exhibited the lowest statistical variation (CV<15%) while it was highest (CV 68.4 to 97.8%) for the phosphorous (P) content in all the soil layers. Spherical, Gaussian and Exponential semivariogram models were developed after accounting for necessary transformations. The findings of geostatistical analysis showed that spatial structures exist in the soil variables. In surface layers, soil pH, phosphorous (P), iron (Fe), calcium (Ca) and copper, (Cu) have the strong spatial dependence with nugget-sill ratios of less than 25%. Analysis suggested that both the internal and external factors are responsible for the spatial dependence of the soil properties. The magnitude and the pattern of spatial variability in soil chemical properties have implications for variable rate fertilizer application strategies in the mango orchards of the eastern region.

Keywords

Spatial variability; Geostatistics; Semivariogram; Soil fertility; Mango orchard

Introduction

Mango (*Mangifera Indica* L.) is one of the most important commercially grown fruit crop of India. It is cultivated over 2.5 Mha area producing about 18 Mt mangoes per year [1]. Many factors play a crucial role on the yield and quality of mango crop, the most important being the fertility of the soil. Soil physical and chemical properties vary in space and time due to the combined effect of physical, chemical and biological processes, which act simultaneously with different intensities at different spatiotemporal scales. Prior knowledge about the spatial variability of the soil fertility indicators over a field can be very useful in maintaining optimum nutrient status in soil and managing other important agronomical measures. Substantial spatial variability of soil nutrient levels at the macro-scale and micro-scale often results in over or under application of fertilizers [2]. Significant relation between leaf nutrient concentrations of the mango plants and the nutrient contents in the soil highlights the need for mapping spatial variability of soil nutrients in the mango orchards [3]. Growing body of literature suggests that spatial variability in soil properties should be considered for making recommendations on variable-rate fertilizer application in mango.

The variable rate fertilizers application and site specific nutrient management can be achieved on the basis of the precisely defined spatial variability of soil nutrients. Geostatistics is one of the most popular set of statistical tools for analysing spatial variability of geocoded parameters. Geostatistics is concerned with detecting, estimating and mapping the spatial variation trends of regional variables. It provides a set of statistical tools such as fitting of a semivariogram model for the description of spatial patterns of continuous and categorical soil properties [4] and it has become an important tool in characterizing the spatial variability of soil properties [5]. This method distinguishes variation in measurement separated by known distance. Semivariogram models provide the necessary information for Kriging, which is a method for interpolating data at unsampled points [6].

Geostatistical methods have been effectively used to assess the spatial variability in soil parameters and it has become an important tool in characterizing the spatial variability of soil properties [7,8]. Houlong et al., [9] used the geostatistical approach and kriging interpolation to map the spatial variability of soil properties in the Pengshui tobacco experiment station for better management of experimental treatments to achieve reliable experimental results. Liu [10] used geostatistical method to investigate the spatial variability of soil organic matter and nutrients in paddy fields in southeast China. Behera et al. [11] analysed the spatial variability in the soil properties of the oil palm plantations in the southern India and concluded that the soil properties were influenced by intrinsic, extrinsic and both intrinsic and extrinsic factors. Conventional and geostatistical methods can be used to understand the heterogeneity of soil chemical properties and to identify factors responsible for the spatial variation of soil properties [5].

Spatial variability of soil physical and chemical properties under different crops and management practices have been analysed by different researchers across the world. Although such studies provide information on the soil variability at the experimental sites, the variability at larger spatial scale, such as district, is not well characterised. Information on variation of soil properties under mango orchards is seriously lacking. Assessment of spatial variability of soil properties is important in fertility management of mango orchards in East India Plateau. The objective of this study was to evaluate the spatial variability of soil chemical attributes and principal soil fertility traits in the mango plantations using traditional statistics and geostatistics at district scale to provide information for better soil fertility management in mango orchards of the eastern plateau and hill region of India.

Materials and Methods

Study area

The study was carried out during 2012 and 2013 in the Gumla and Simdega districts located in the south-western part of the Jharkhand

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Received: June 18, 2016 **Accepted:** July 22, 2016 **Published:** July 29, 2016

state to assess the fertility status of the soils in the mango orchards. The study districts lies between 22^0 19' 40" N to 23^0 36' 43" N latitude and 84^0 00' 21" E to 85^0 05' 09" E longitude with average mean sea level of 300 and 424 m. The climate of the Gumla and Simdega districts is tropical monsoon type and receives an average annual rainfall of about 1100 and 1397 mm respectively. Soils of the region are characterised with red laterite, red and yellow with texture ranging from loamy to sandy loam having slightly acidic reaction.

Soil sampling and laboratory analysis

A field survey was undertaken to collect the soil samples from different mango orchards located in the study area. The age of the mango orchards varied from 6 to 18 years. A total of 90 soil samples were collected from the study orchards at various soil profile depths. Mango trees have very well spread, deep, and extensive root system and the widespreading feeder roots also extend many anchor roots to deeper depths. To represent the entire root zone more precisely, the soil samples were collected from0-30, 30-60 and 60-90 cm depth profiles. Each sample was formed from the four samples collected within 1.5 to 2 m radius of the four different trees and mixed well to form one representative sample of an orchard. The geographic coordinates (latitude, longitude and elevation) of every sampling point were recorded with a handheld global positioning system (GPS) (Oregon 550, Garmin Ltd, Kansas, USA).The soil samples were placed into plastic bags then air-dried, ground to pass through a 2-mm sieve and analysed for soil physicochemical properties.

The samples were analyzed for soil acidity (pH), organic carbon (OC), available nitrogen (N), available phosphorous (P), available potassium (K), exchangeable calcium (Ca) and magnesium (Mg), DTPA-extractable iron (Fe), DTPA-extractable manganese (Mn), DTPA-extractable copper, DTPA-extractable zinc. Determination of soil pH was done based on 1:2.5 soil water ratio (w/v) suspension using pH meter following half an hour equilibrium [12]. The soil organic carbon content was determined by Walkley and Black method [13]. The following methods were used to determine available nutrient contents: the method of Subbiah and Asija [14] for N, that of Bray and Curtz [15] for P, and the flame photometric method [12] for K. the exchangeable Ca and Mg was determined by Versenate method [16]. DTPA-extractable Fe, Mn, Cu and Zn were measured with an atomic absorption spectrophotometer by following the method of Lindsay and Norvel [17].

Descriptive statistics

Data were subjected to descriptive analysis. The minimum, maximum, mean, standard deviation (SD), coefficient of variation (CV), skewness and kurtosis for soil properties were computed. Skewness is the most common statistic parameter to identify a normal distribution that is confirmed with skewness values varying form – 1 to + 1. Criterion established by Warrick [18] was used to classify the parameter variability on the basis of variation coefficient values as low: <15%, moderate: from 15% to 50%, and high: >50%.

Geostatistical analysis

Exploratory Spatial Data Analysis (ESDA) was carried out to assess and correct the trend, periodicity and extreme values present in the datasets pertaining to soil properties. The ArcGIS10.0 was used for performing the ESDA.

Variance of the difference between two values is assumed to depend only on the distance h between the two points, and not on the location x. Spatial patterns were usually described using the experimental semivariogram $\gamma(h)$, which measures the average dissimilarity between data separated by distance h. The semivariance as a function of both the magnitude of the lag distance was computed using [6,19];

$$\gamma(h,\alpha)=\frac{1}{2N(h,\alpha)}\sum_{i=1}^{N(h)}[Z(x_i+h)-Z(x_i)]^2$$

Where, $\gamma(h,\alpha)$= semivariance as a function of both the magnitude of the lag distance or separation vector (h) and its direction (α); N(h,α) = number of observation pairs separated bydistance h and direction α used in each summation; $Z(x_i)$= random variable at location x_i.A semivariogram consists of three basic parameters that describe the spatial structure as: $\gamma(h) = C_0 + C$. $C0+ C$ is the sill (total variance), which is the lag distance between measurements at which one value for a variable does not influence neighbouring values. C_0 is the combination of random errors and sources of variation at distances smaller than the shortest sampling interval [20]. C is the structural variance, which is the constant semivariance value where the curve was stabilized. The range is the distance over which soil property is spatially related. The nugget ratio ($C0/(C0 + C)$; nugget-sill) represents the parameters that characterize the spatial structure of a property [21]. Several semivariogram functions (Spherical, Gaussian, Exponential, Linear, Linear to sill etc.) were evaluated to choose the best fit with the data. The best fit semivariogram model was selected on the basis of coefficient of determination (R^2) and the residual sum of squares (RSS). Semivariance calculation and semivariogram function model fitting were performed using the geostatistical software GS+ for Windows. Semivariogram, differences in nugget/sill ratio and range were examined for various soil properties.

Results and Discussion

Descriptive statistics

The data were analysed using classical statistical methods to understand the characteristics of the general soil properties prior to the investigation on the spatial structure (Table 1). The concentrations of soil properties (pH, OC, N, P, K, Ca, Mg, Fe, Mn, Zn, Cu) were described by minimum, maximum, mean, median, standard deviation (SD), coefficient of variation (CV), skewness and kurtosis of data distribution in the study area (Table 1). The summary statistics of soil properties suggested that all the soil properties exhibited considerable variability across the study region. The soil pH varied from 4.08 to 7.78 depending on the soil layer. The surface profile (0-30 cm) showed dominantly acidic reaction with pH varying from 4.08 to 6.61 with mean value of 5.15 ± 0.61. The pH also varied with depth with mean pH increasing from 5.15 in surface layer to 5.56 at subsurface layers. The values of CV for soil pH in all the soil layers revealed their moderate variability and these values were less compared to CV values of other measured soil properties. The CV values in the range of 10 to 100 are considered in the class of 'moderate variability' [22]. Low CV values for soil pH was due to transformed measurement of hydrogen ion concentration. Behera et al. [11] also reported the lower CV values of 17.1 and 19.5 for the pH of surface and subsurface soils of oil palm plantations in the southern plateau of India. Houlong et al. [9] observed lowest CV in case of soil pH as compared to other soil properties recorded in tobacco plantations of southern china.

The organic carbon content in the surface soil layers varied from 0.21 to 0.91% across the study region. The mean organic carbon content decreased with increasing soil depth. The mean value of

Table 1: Descriptive statistics* for selected soil properties of surface layers (n=90).

Soil Properties	Soil Layer (cm)	Min	Max	Mean	Med	SD	CV (%)	Skew.	Kurt.
pH	0-30	4.08	6.61	5.15	5.17	0.61	11.84	0.54	0.62
	30-60	4.37	7.78	5.46	5.47	0.73	13.47	1.49	1.34
	60-90	4.29	7.65	5.56	5.56	0.77	13.90	0.76	1.55
OC, %	0-30	0.26	0.91	0.51	0.49	0.20	38.87	0.47	-0.87
	30-60	0.13	0.84	0.40	0.36	0.19	46.00	0.49	-0.47
	60-90	0.02	0.72	0.31	0.28	0.14	45.47	0.73	1.81
N, kg/ha	0-30	87.8	175.6	126.4	125.4	19.96	15.80	0.30	0.75
	30-60	75.2	138.0	111.3	112.9	17.71	15.91	-0.65	-0.15
	60-90	37.6	138.0	101.3	100.4	26.02	25.69	-0.67	0.28
P, kg/ha	0-30	0.16	14.57	4.80	4.29	3.29	68.47	1.44	2.91
	30-60	0.16	16.66	3.83	2.92	3.29	85.99	2.52	8.44
	60-90	0.16	14.10	3.02	2.53	2.96	97.83	2.40	7.36
K, kg/ha	0-30	173.6	380.8	270.5	266.6	65.90	24.37	0.23	-1.22
	30-60	159.0	369.6	241.8	225.1	64.07	26.49	0.40	-1.10
	60-90	144.5	336.0	225.0	208.3	59.05	26.24	0.40	-1.14
Ca, g/kg	0-30	414.0	1320.0	725.8	620.0	272.1	37.49	0.56	-0.93
	30-60	600.0	1566.0	953.1	928.0	238.4	25.02	0.71	0.18
	60-90	488.0	1448.0	975.4	936.0	243.9	25.01	0.22	-0.60
Mg, g/kg	0-30	164.4	648.0	331.6	296.4	138.9	41.92	0.83	-0.31
	30-60	159.6	796.8	382.9	362.4	136.8	35.71	0.99	2.00
	60-90	220.8	654.0	395.1	387.6	102.5	25.95	0.61	0.52
Fe, g/kg	0-30	8.04	29.40	16.26	15.24	5.54	34.10	0.95	0.29
	30-60	5.21	22.76	10.59	9.30	5.25	49.61	1.37	0.95
	60-90	3.84	19.56	9.76	8.07	4.62	47.38	0.93	-0.05
Mn, g/kg	0-30	12.98	24.18	19.15	18.99	2.72	14.20	-0.10	0.84
	30-60	2.31	23.84	16.50	17.72	5.09	30.83	-1.30	1.72
	60-90	3.44	23.56	15.16	15.51	4.51	29.74	-0.78	1.17
Zn, g/kg	0-30	0.22	0.71	0.39	0.37	0.12	31.57	0.79	0.02
	30-60	0.22	0.43	0.31	0.29	0.05	17.59	0.35	-0.58
	60-90	0.15	0.57	0.30	0.30	0.10	31.32	0.81	0.91
Cu, g/kg	0-30	0.41	1.55	0.96	0.93	0.32	33.53	-0.03	-0.89
	30-60	0.36	1.51	0.82	0.75	0.31	38.19	0.64	-0.35
	60-90	0.05	1.38	0.77	0.71	0.30	38.45	0.31	0.71

*Min-minimum, Max-maximum, mean, SD-standard deviation, CV-coefficient of variation, Skew-skewness, Kurt- kurtosis.

the total N content is classed as 'low' and it further decreased with increasing depth. Coefficient of variation (CV) of available N at sub-surface layer is higher (25.7%) than at top-layer (15.8 %), meanwhile all the CV of available N in the soil could be classified as moderate variability. Available N content in the top layer was about 24.7% higher than the sub-surface layer (60-90 cm). This is probably due to the higher organic residues deposited on the soil surface than the sub surface soil. Highest spatial variation was observed in case of available P content of the soil. The variability of available P in deeper layer (CV = 97.8%) was higher than surface layer (CV=68.5 %). This condition is probably due to difference of soil pH and total organic carbon between the layers. Mean K content in the top layers was about 270.5 kg ha^{-1} which decreased to 225.0 kg ha^{-1}at the sub soil layer. The spatial variation in K content in all the studied soil profile was classed as 'moderate' (CV=24.4 to 26.5%). This variability is the result of the irregular cropping system and non-uniform management practices.

The secondary macronutrient (Ca and Mg) and micronutrient (Mn, Fe, Zn, Cu) content in all the soil layers was situated in the 'moderate variability' class except for the Mn content in the top layer. The CV of these parameters varied in a narrow range of 14.2 to 49.6 %. Among the secondary macronutrient and micronutrient, the Mg showed highest variation in the surface soil layer (CV=41.9%). Concentration of Ca was higher (>725 g kg^{-1}) in the soil layers. With the increasing depth, the concentration of Ca and Mg increased while that of Fe, Mn, Zn and Cu decreased.

The descriptive statistics of soil properties suggested that all variable distributions were only slightly skewed, and their medians values were close to their mean values, identifying a normal distribution of soil variables. The values for skewness and kurtosis between -2 and +2 are considered acceptable in order to prove normal univariate distribution [23]. Highly skewed properties indicated that these properties had a local distribution, the high values were recorded for these properties at some points, but most of the values of these properties were low [24]. The principal reason for some soil properties having non-normally distributions may be related with soil management practices [25]. The kurtosis values ranged from -1.22 to 8.44. The skewness and kurtosis values for available P data series was very high and also there was significant difference in the mean and medium values observed at all soil layers. The probability distributions of P concentration data at all the soil layers are positively skewed and have sharp peaks. The calculation of variation function should generally be in accordance with the normal distribution.

Therefore logarithmically transformed P concentration data were used in the geostatistical analysis of variation function.

Geostatistical Analysis

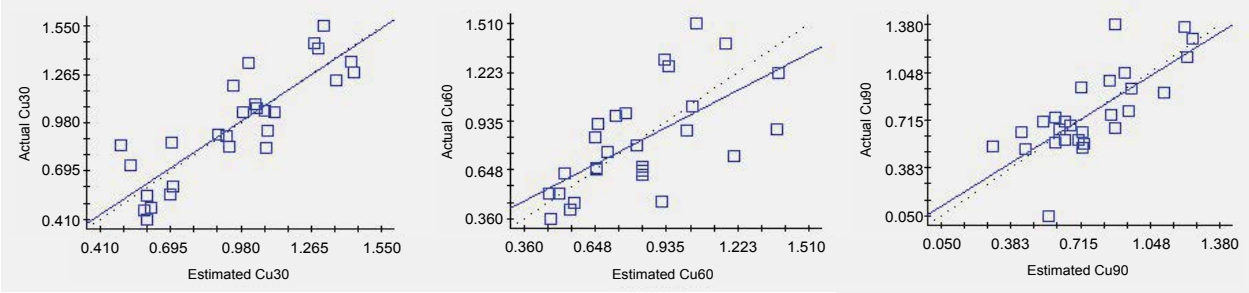
Knowledge about the spatial variability of soil properties is very useful in optimizing and determining the fertilizer application recommendations in mango orchards. Appropriate use of nutrients can contribute to enhance crop quantity and quality, while being environmentally sustainable [26]. The inherent limitation of predicting the soil properties at the un-sampled sites precludes the use of classical statistics in variability assessment of soil properties. Geostatistical analysis however permits examination and understanding of spatial dependency of a soil property [27].

The results of geostatistical analysis (Table 2) indicated different spatial distribution models for the soil properties. The geostatistical analysis indicated different spatial distribution models and spatial dependence levels for the soil properties. In most of the parameters, spherical variogram model was found to be ideal fitting model. Apart from spherical model, the Gaussian and Exponential models were also fitted well in case of some soil properties. In particular, the Zn had exponential best fit model at all soil layers. Among the major nutrient contents (N, P K) only N had the Gaussian best fit model for the surface layer (0-30) and K had an Exponential best fit model for the deeper soil layer. In selecting the best fit model, the prediction accuracy of the semivariogram model was also taken into consideration. The plots between observed and predicted values at the sampled location and the values of coefficient of determination (R^2) were considered in selecting the best fit model. The sample plot between observed and predicted values for Cu is shown in Figure 1. Several researchers [9,11,28] have reported spherical model as the best fit for soil parameters like N, P, K, OC, Fe, Cu, Mn and Zn.

The nugget to sill ratio is used to define spatial dependence of soil properties. If the ratio is <0.25, there is strong spatial dependence; if the ratio is 0.25 to 0.75, there is moderate spatially dependence; and if the ratio is >0.75, spatial dependence is weak. Strong spatial dependence of soil properties can be attributed to intrinsic factors such as soil properties and mineralogy, whereas, weak spatial dependence is due to extrinsic factors such as anthropogenic activities. Moderate spatial dependence is owing to both intrinsic and extrinsic factors [11]. As shown in Table 2, the ratio values indicated the presence of strong to weak spatial dependence for all soil parameters (values between

Table 2: Semivariogram parameters of soil properties at different layers.

Soil Property	Soil Layer (cm)	Parameters of Semi Variogram					
		Model	Nugget	Sill	Range (m)	Nugget/sill ratio	Spatial Class
pH	0-30	Spherical	0.04	0.40	1430	0.10	Strong
	30-60	Spherical	0.00	0.59	2530	0.00	Strong
	60-90	Spherical	0.01	0.66	2090	0.02	Strong
OC, g/kg	0-30	Spherical	0.03	0.06	21890	0.50	Moderate
	30-60	Spherical	0.03	0.06	21890	0.50	Moderate
	60-90	Spherical	0.02	0.03	21890	0.67	Moderate
N, kg/ha	0-30	Gaussian	302.4	604.9	6160	0.50	Moderate
	30-60	Spherical	285.8	295.8	6490	0.97	weak
	60-90	Spherical	61.00	689.0	1650	0.09	Strong
P, kg/ha	0-30	Spherical	1.84	7.65	4730	0.24	Strong
	30-60	Spherical	0.01	11.69	1210	0.00	Strong
	60-90	Spherical	0.01	11.44	1210	0.00	Strong
K, kg/ha	0-30	Spherical	3190	8063	16170	0.40	Moderate
	30-60	Spherical	2900	8095	19800	0.36	Moderate
	60-90	Exponential	2960	7292	14190	0.41	Moderate
Ca	0-30	Spherical	7200	85700	1210	0.08	Strong
	30-60	Spherical	18800	54000	1100	0.35	Moderate
	60-90	Exponential	47900	95810	19360	0.50	Moderate
Mg	0-30	Spherical	17827	15325	6490	1.16	weak
	30-60	Spherical	7950	19300	2750	0.41	Moderate
	60-90	Spherical	100.00	9440	660	0.01	Strong
Fe	0-30	Exponential	15.00	78.50	12870	0.19	Strong
	30-60	Spherical	19.96	39.90	11000	0.50	Moderate
	60-90	Exponential	0.01	24.41	660	0.00	Strong
Mn	0-30	Spherical	2.03	7.86	1430	0.26	Moderate
	30-60	Spherical	2.27	29.39	2750	0.08	Strong
	60-90	Spherical	0.01	23.87	2750	0.00	Strong
Zn	0-30	Exponential	0.01	0.03	21890	0.33	Moderate
	30-60	Exponential	0.00	0.01	14630	0.00	Strong
	60-90	Exponential	0.01	0.02	21890	0.50	Moderate
Cu	0-30	Spherical	0.00	0.12	1210	0.00	Strong
	30-60	Spherical	0.00	0.10	1210	0.00	Strong
	60-90	Spherical	0.00	0.10	1210	0.00	Strong



(a) (b) (c)

Figure 1: Observed and estimated copper (Cu) concentration at (a) 0-30 cm, (b) 30-60 cm and (c) 60-90 cm soil depths at sampled locations.

0 and 1.16). Stronger spatial dependence observed in case of pH, P and Cu (low nugget to sill ratio) and indicated that the soil properties might be affected by the internal factors. Total N content in the sub-surface layer and concentration of Mg in the surface layer showed weak spatial dependence. The rest of variables were in moderate spatial dependence with the nugget-sill, are between 0.25 and 0.75, illustrating that the soil variables might be affected by internal and external factors, such as cultivation and fertilization. Wang et al. [14] found that most of the soil properties in the studied area were classed as moderately spatially dependent. Liu et al. [10] reported the nugget-sill ratios of Zn and Cu were less than 0.50.

Range is the distance at which the semivariogram levels off and beyond which the semivariance is constant [29]. Knowledge of the range of influence for various soil properties allows for the construction of independent data sets that can be used for classical statistical analysis. A smaller range indicates that observed values of the soil variable are influenced by other values of this variable over lesser distances than soil variables which have larger ranges [30]. In the present mango orchards, the range for soil properties varied from 660 (deep layer Fe) to 21890 m (Surface layer Zn). In surface layer, the range for pH, P, Ca, Mn, Cu was about 1430, 4730, 1210, 1430 and 1210 m. Low range for these variables indicated that these values are influenced by the neighbouring values at lesser distance than other variables. The smaller range suggested that smaller sampling intervals are needed pH, P, Ca, Mn, Cu. In particular, OC, K and Zn showed consistently higher range values at all the sampling depths.

In present study, N exhibited moderate spatial dependence in surface soils (0-30 cm), weak spatial dependence in sub-surface layer (30-60 cm) and strong spatial dependence at deeper layers. Spatial dependence of K was classed as 'moderate' at all depths implying the impact of both the intrinsic and extrinsic factors on its spatial variability. This phenomenon might be explained by high mobility of K in sandy loam soils with low cation exchange capacity, which can accentuate leaching effects of strong rains characterising the study districts. Exchangeable K exhibited three spatial patterns: strong dependence at topsoil (0-0.05 m depth), moderate from 0.05 to 0.2 m depth, and no spatial correlation in the lower layer (0.2 –0.3 m). The Mg concentration in the surface horizon showed weak spatial dependence as the nugget-sill ratio was more than 0.75 indicating comparatively lesser influence of the Mg concentration at neighbouring points.

The presented results suggested that there is considerable spatial variability in the soil properties across the mango orchards of the eastern plateau region. While planning the field experiments in the farmers' field, it is necessary to obtain coincident soil conditions and avoid the test errors from the inconsistent soil properties [31]. Results obtained under this study can be used to facilitate the procedure of the preparation for field experiment in the mango plantation areas of the eastern plateau region.

Conclusion

The classic statistical analysis revealed a considerable statistical and spatial variability of pH, OC, N, P, K, Ca, Mg, Fe, Mn, Zn and Cu among soil horizons and across the mango orchards of the eastern plateau region. Then mean values of pH, Ca and Mg increased while that for OC, N, P, K, Fe, Mn, Zn and Cu decreased with increasing soil depth. The findings of geostatistical analysis showed that spatial structures exist in the soil variables. Apart from surface horizon (0-30 cm), a strong to moderate spatial dependence was observed for subsoil and deeper soil horizons. Higher range values for some of the soil parameters implied that the soil chemical properties had spatial dependence over larger distances. This study demonstrated that the variability of soil chemical properties was associated to the management practices (fertilizer, residue management etc.) and local conditions (topography, climate etc.). It is concluded that the orchard specific fertility management recommendations needs to be considered over the general fertilizer recommendations for entire region.

Acknowledgements

The authors gratefully acknowledge the help rendered by Mr. Ganga Ram, Technical officer, ICAR Research Complex for Eastern Region, Research Centre, Ranchi, India for collection of samples. The authors sincerely acknowledge the help rendered by PRADAN, Jharkhand, India for identification of mango orchards for the investigation. The authors wish to acknowledge Indian Council of Agricultural Research, New Delhi, India for providing facilities for carrying out this research.

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Kamran et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00071.9

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Genetic Evaluation of Crown Freezing Tolerance and Some Physiological Traits in Barley (*Hordeum vulgare* L.) Lines

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Abstract

In order to investigate cold tolerance in 20 barley genotypes based on crown survival percentage, a greenhouse experiment was conducted as split plots with three replicates, with temperatures (-8, -10, -12, -14 and -16°C) as main plots and barley genotypes constituting subplots. Randomized complete block design was performed to analyze physiological traits measured after acclimation and before applying chilling temperatures. Crown survival percentage was measured zero at -16°C. Error was not significant for main factor in split plots, therefore, data analysis for -8, -10, -12 and -14°C was executed as factorial. Results indicated that temperature, genotype and their interactions had significant influence on the crown survival percentage. Also, the genotypes were significantly different in terms of LT50, the glycine betaine content and leaf relative water content before and after adaptation to cold. Comparison of the means, based on LT50 and crown survival percentage, suggested the genotype number 15 (with K-096M3 pedigree) as the most tolerant to crown freezing, and genotypes 36 (Schulyer), 15 (K-096M3) and 14 (GK Omega) as possessing the most desirable physiological traits, with genotypes 15 and 36 possessed the lowest difference before and after leaf relative water content, and the maximum quantity of glycine betaine after adaptation to cold. Cluster analysis of the genotypes, based on the aforementioned traits, divided them into three distinct tolerant, semi- tolerant and sensitive groups.

Keywords

Barley; Crown survival percentage; Freezing tolerance; Glycine betaine; LT50; Relative water content

Introduction

Cold stress is an abiotic stress that limits the distribution, growth, and productivity of crop plants [1] in 42 percent of the surface of the earth [2] where lands experience temperatures below -20°C. Plants exhibit different degrees of cold tolerance, and some can increase their tolerance through a process known as cold acclimation, adaption to low temperatures. Cold acclimation involves a series of physical and biochemical mechanisms, which occur at low temperatures, or above the freezing point [3,4], including processes such as stability of cell permeability, change in the composition of membrane lipids and increase in antioxidants [5]. After cold adaptation, plant may withstand the following freezing conditions, in which case it is called cold tolerant [6].

In order to assess tolerance in cereals after acclimation, they are grown in natural environment or controlled conditions (growth chamber or fridge), which is known as direct evaluation. In indirect methods, on the other hand, molecular markers are employed to assess physiological and biochemical modifications during cold acclimation [7]. One direct method is fast, controllable and repeatable; uses controlled freezing tests to measure LT50 in cold acclimatized cultivars, and commonly used as a winter survival signifier of tolerant plants.LT50 is a temperature stage that causes 50 percent death to plants grown in a freezing chamber during monitored freezing tests [8,9] In some studies, there has been a significant linear correlation between LT50 and survival at -50°C [10]. In winter wheat, for example, physiological traits such as LT50, crown water content (CWC) and leaf relative water content (RWC) correlate with cold survival [11].

Most temperate plants, through evolution, have acquired varied abilities to develop cold tolerance in response to acclimating conditions. Accumulation of certain molecules with a cryoprotective role, for example, is a mechanism adapted by plants in response to low temperature conditions [12]. Glycine betaine (GB) is one such cold tolerance associated osmolyte [13], which plays many roles, including preserving the quaternary structure of enzymes and proteins [14], stabilizing membranes [15] and photosynthetic apparatus [16,17], under cold and freezing temperatures. It also reduces the peroxidation of membrane [18]. In some species, cold acclimation induces glycine betaine accumulation proportional to the degree of cold tolerance [19,20]. There is also evidence that GB concentrations in leaf correlates with leaf relative water content [21].

Losses of water and tissue water content are other attributes of cold tolerance. According to Fowler et al. [22], tissue water content measurement, as one important laboratory indicator, possesses in it all the desired characteristics of cold tolerance. It has also been established that leaf water content has had a major correlation with viability of plants) [11], and tissue water content declined in response to cold acclimation, which in turn leads to increase in cold tolerance [23].

Among autumn cereals, barley is the third sub tolerant to cold stress [24]. In terms of global production, it comes forth in the rank, after wheat, rice and corn. Barley autumn cultivars have higher yield than spring ones. Since they spend a part of their vegetative growth exposed to cold conditions, to avoid late heat and droughts, developing cold tolerate barley varieties is an important goal of breeding programs worldwide [25]. World have suffered significant economic losses due to injuries imposed by freezing temperatures to crop and horticultural industries [26]. In carrying out this study, the objectives have been to identify barley genotypes tolerant of freezing based on the crown freezing test, as well as determining the relationship between freezing tolerance and some physiological and biochemical characteristics.

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Received: March 11, 2016 **Accepted:** July 27, 2016 **Published:** August 03, 2016

Materials and Methods

Preparation of plant materials

In this experiment, plant materials, including 20 Barley (*Hordeum vulgare* L.) genotypes obtained from Seed and Plant Improvement Institute (SPII), Karaj, Iran (Table 1). Evaluation of the Barley genotypes was carried out using a split plot experiment with three replicates, within greenhouse and growth chamber in the Faculty of Agriculture of University of Tabriz (from November 2013 to the middle of February 2014).

Seeds, after have being sterilized in Mancozeb 2 ppt, were planted the rows in rectangular 50 × 40 cm plastic pots containing agronomy soil. Each pot involved four rows. 25 seeds were planted along each row, sown two cm down the soil. Irrigation performed when necessary. The greenhouse temperature was kept at 21°C and 18°C during the days and nights, respectively. After reaching three, four- leaf stage, the seedlings were transferred to a growth chamber for four weeks, with 4°C daytime and 2°C during nights, under a 12-h day length at 250 µmol m-2s-1 photo synthetically active radiations, in order to get acclimatized to low temperatures.

Relative water content (RWC) measurement

In order to execute RWC measurements prior and post acclimation, the third developed leaves were sampled from each genotype, fresh weights were determined immediately after wards. To determine saturated weight, leaf specimens were submerged in 100 ml water at room temperature for four h. The same samples were, then, wrapped in aluminum foil, put inside an Avon set at 75°C to dry out, and, finally, weighed to measure their dry mass. The RWC was determined using the following equation [27].

$$RWC = [(\text{fresh weight- dry weight})/(\text{saturated weight- dry weight})] \times 100.$$

Glycine betaine (GB) measurement

For glycine betaine measurement, sampling was carried out before and after cold acclimation from the third developed leaves. GB content was measured according to Grieve and Grattan [28]. After stirring leaf samples in distilled water for 48 h at 25°C and filtering, the solution was diluted using 2NH2SO4. Cold KI-I2 was added to the diluted liquid, and after centrifugation, the supernatant was mixed with 1, 2- dichloroethane. Absorption was recorded at 365 nm.

Crown survival percentage (CSP) assessment

CSP investigated after plants have been adapted to cold with the roots and leaves were cut two cm below and one cm above the crown respectively, so plants could recover by developing new roots and leaves. Ten crowns belonging to the same genotype were banded together. Samples were placed in aluminum cans filled with wet sand and transferred to a programmable freezer where they were, first, kept at -2°C. After 12 hours, the temperature plummeted gradually. From -8°C onwards, materials of the respective temperatures were taken out at two-hour intervals, and the crowns were put in a regular fridge to thaw at 4°C. The next day, the crown of any given temperature were planted in pots, then, grown in the greenhouse at 23°C for a 21-day period. The records of surviving and dead plants, as well as the CSP were calculated as followings [29].

$$CSP = (\text{the number of seedlings after freezing} / \text{the number of seedling before freezing}) \times 100.$$

LT50 measurements

LT50 in genotypes studied was calculated using data related to survival percentage for all temperatures and transformation of the probits [30]; variance analysis was conducted as randomized complete blocks. Comparison of the means was carried out with Duncan's test. Prior to analysis, data was suitably transformed in cases where some assumptions of the variance analysis were not true.

Statistical analysis

Before performing analyses, the assumption of variance homogeneity and error normality was examined. Most data relative to survival percentage scored zero at -16°C, and brought in homogeneity and abnormality to the variance, therefore, the pertaining data was excluded from the analysis. Since the amount of biochemical variables were measured prior and post acclimation, a complete randomized block design with three replicates was implemented to analysis the data. Data was analyzed in SPSS19 and MSTATC computer software.

Results

Data was, first, analyzed in the split plot, due to the nature of the experiments. However, as a result of main plot error being non-significant, a factorial design was used to analyze the variables.

The results of variance analysis pertaining to CSP of 20 barley genotypes at -8, -10, -12 and -14°C showed that the F for temperature, genotype and the interaction of temperature and genotype was significant at 1%. Interaction being significant indicates that genotypes

Table 1: Code/name and pedigree of barley genotypes us ed in evaluation of cold s tres s

Genotype No	Genotype Code/ Cultivar Name	Pedigree
1	EC79-10	Walfajre/Miraj 1
4	EC80-7	YEA389.3/ YEA475.4
5	EC80-11	ALGER/(CI10117/ CHOYO
9	EC82-5	Alger/(CI10117/ Choyo
11	EC82-11	Np106/Minn14133-Gva xduois //Gi10143
14	EC83-10	GkOmega
15	EC83-12	K-096M3
16	EC83-15	SCHUYLER//(M.RNB89.80/ NB1905//L.527)
18	A1C84-7	Star/Dundy
20	A1C84-12	Kozir/330
21	A1C84-14	As trix(C)/3/Mal/OWB753328-5H//Perga/ Boyer
22	A1C84-15	Monolit/Plais ant
28	A2C84-14	Cyclone/Arar
29	A2C84-18	Mal/OWB753328-5H//11840-76/3/ Radical
31	Makouee	Makouee
33	Rihane	Rihane
34	Kavir	Kavir
35	73M4-C	73M4-30
36	Schulyer	Schulyer
38	Aths	Aths

Table 2: Mean of s urvival percentage at -8, -10, -12, -14°C in barley genotypes

			-12°C		-14°C		The average mean temperatures
Genotype No.	**-8°C**	**-10°C**	**The original data**	**The converted data**	**The original data**	**The converted data**	
1	85.93	33.33	0	0.7080	0	0.0708	27.32
4	96.67	53.33	10	0.2902	0	0.0708	40
5	100	63.33	33.33	0.6151	0	0.0708	49.17
9	100	70	10	0.3300	3.33	0.1572	45.84
11	100	66.67	6.67	0.2436	3.33	0.1572	44.17
14	100	73.33	50	0.7904	6.67	0.2436	57.5
15	96.97	92.13	55	0.8424	10	0.3300	63.49
16	100	36.67	0	0.0708	0	0.0708	34.17
18	93.33	68.9	30	0.5851	0	0.0708	48.05
20	96.67	83.33	33.33	0.6151	0	0.0708	53.34
21	100	53.33	0	0.0708	0	0.0708	38.34
22	90	60	16.67	0.4233	3.33	0.1572	42.5
28	90	76.67	10	0.3300	3.33	0.1572	45
29	100	50	10	0.3300	3.33	0.1572	40.83
31	100	43.33	6.66	0.2038	0	0.0708	37.5
33	86.30	26.67	0	0.0708	0	0.0708	28.25
34	47.50	20	0	0.0708	0	0.0708	16.88
35	63.50	33.33	0	0.0708	0	0.0708	24.21
36	100	82.50	30	0.5816	10	0.3300	55.63
38	43.33	13.33	0	0.0708	0	0.0708	14.17
LSD5% LSD1%	0.1383 0.1852	0.1568 0.2100		0.1568 0.2100		0.0527 0.0700	

did not change equally at different temperatures. Therefore, analysis of variance and comparison of the means of genotypes for CSP for each individual temperature was conducted using a randomized complete block design, as genotypes performed differently at different temperatures, (Table 2).

There was a significant difference between genotypes for -8, -10, -12°C at level of 1% and for -14°C at level of 5%. At -8°C, all genotypes, except for 34, 35 and 38, scored above 80 percent survival, which also displayed a significant contrast to other genotypes at 1%. At -10°C, genotypes 15, 20 and 36 showed the maximum percentage of survival; the lowest percentage was obtained by genotype 38 as 13.33. As temperature declined to -14°C, some genotypes were killed; genotypes 15 and 14 obtained the maximum scores, respectively, with 55 and 50 percent survival. This was significant at 1%, compared to other genotypes.

At -14°C, most genotypes were destroyed. Genotypes 15 and 36 with 10 percent survival were significantly different from others at 1%, hence, designated as tolerant genotypes. Genotype 38 had the lowest average of survival across average temperatures; genotypes 15, 14, 36, 20 and 5 had survival percentage of at least 50 across mean temperatures (Table 2 and 3). The results of variance analysis for LT50 in genotypes studied revealed a significant difference at the level of 1% (Table 4). The coefficient of variation (C.V.) for this trait was 6.77, indicating a low experiment error. LT50 was higher in genotypes 15, 36, 14, 5, 20 and 9 and lower in genotypes 38, 34 and 35 than the rest. Genotypes with smaller LT50 had higher tolerance than those with bigger LT50 (Table 4).

Genotypes 15, 14, 36, 20 and 5 also scored a higher mark for CSP during the freezing test. The negative, significant correlation between LT50 and CSP indicated that the more the CSP, the less the number of dead plants.

Analysis of variance relative to the amount of GB and RWC were conducted as a factorial experiment involving temperature (in two levels) and genotype (in 20 levels) based on a complete randomized block design. Results showed that there was a significant difference between the two temperature conditions- prior and post acclimation to cold – in regard to aforementioned traits ($p \leq 0.01$). Likewise, a significant difference was observed between barley genotypes concerning RWC and GB, implying a variation in barley genotypes for these traits ($p \leq 0.01$). The interaction between genotype and temperature for GB showed significant difference at 1%, which, by comparing the means of this effect, made clear that the discrepancy was rooted from variation in GB content as developing adaptation to cold. In other words, there was no significant difference between genotypes before adaptation.

Investigating the means of GB and RWC between the genotypes under two temperature conditions showed that leaf RWC dropped significantly after adaptation to cold ($p \leq 0.01$) (Figure 1), and the amount of GB increased significantly post adaptation to cold ($p<0.01$) (Figure 2).

To better understand the contrasts between the genotypes, analysis of variance for two variables-GB content and RWC, before and after adaptation to cold and changes in the value of these traits-was conducted as a complete randomized block design in two conditions (Table 4). Analysis of variance showed that, in contrast to before adaptation to cold, which showed no significant difference between the genotypes, the amount of GB in genotypes had experienced a change from 1125 m mol per gram fresh weight in Sensitive genotypes to 2472 m mol per gram fresh weight in resistant ones, conferring a significant difference at 1%. Likewise, the change in the content of GB before and after adaptation brought about a significant difference among the genotypes at 1%, with the highest and lowest changes belonged to the genotypes 36 and 34, respectively (Figure 3). Accordingly, genotypes 38 and 34 are sensitive to cold and genotypes 5, 15 and cultivar 36 (Schulyer) are cold tolerant. Which means more GB carries with it more tolerance to cold. The same results have been reported on the accumulation of GB inducing tolerance in other plants undergoing drought and salinity stress [31].

Furthermore, there was no significant difference between the barley genotypes before cold acclimation for RWC, contrary to significant decline in RWC at 1% after the genotypes having been acclimatized. Variations of RWC before and after adaptation to cold were not significant between genotypes. However, these changes showed that, among all 20 genotypes, 38 and 34 possessed the maximum and 14, 15 and 36 had the least chaining of RWC in two conditions (Figure 4). Which means sensitive genotypes displayed a bigger fluctuation in RWC in response to cold adaptation. In other words, cold condition causes more loss of water in cold sensitive genotypes.

A negative, significant correlation existed between LT50 and GB content after acclimation to cold, and the difference between to temperature conditions. Which indicates that more tolerate genotypes has produced more GB. There was a significant, negative correlation between GB content after cold adaptation and RWC, before, after and the difference between the two cold treatments. Genotypes with lower RWC had greater GB content. Likewise, a significant, positive correlation was found between LT50 and RWC, before, after and the difference between the two cold treatments, indicating that sensitive genotypes possessed greater RWC in leaves (Table 5).

Table 3: Mean of LT50 in barley genotypes

Genotype NO	Genotype code or cultivar name	Mean
51		-12.39
63	EC83-12	-11.785
51	Schulyer	-11.274
1	EC83-10	-11.125
02	EC83-11	-11.068
9	A1C84-12	-11.048
51	EC82-5	-10.997
09	A1C84-7	-10.872
00	A2C84-18	-10.864
65	A1C84-15	-10.819
01	Makouee	-10.806
55	A2C84-14	-10.703
1	EC82-11	-10.547
05	EC80-7	-10.052
53	A1C84-14	-9.987
5	EC83-15	-9.52
66	EC79-10	-8.389
61	Rihane	-8.731
61	73M4-C Kavir Aths	-7.322
61		-6.7
1%LSD		0.7500
5%LSD		1.005

Table 4: Analys is of variance of GB and RWC content in barley genotypes leaves

M.S.							
G.B.					**R.W.C.**		
S.O.V	D.F.	Control	Acclimation	Changes between control and acclimation	Control	Acclimation	Changes between control and acclimation
	2	129.398ns	34243.438ns	335370.09ns	52.557ns	12.840ns	1114.439ns
Replication Genotype	19	6001.014ns	288364.093**	310132.916**	180.818**	69.680**	37.741ns
Error Non-additive	38	2556.659	30036.324	30746.398	23.617	11.688	47.746
Res idual	1	1.874**	2477714.5**	303005.948**	3.013ns	0.468*	1.160*
	37	2625.707	24151.590	23388.02	24.173	11.991	49.005
C.V		36.01	11.21	19.22	6.30	4.90	95.3

ns : not s ignificant, * s ignificant at 0.05% and ** s ignificant at 0.01%

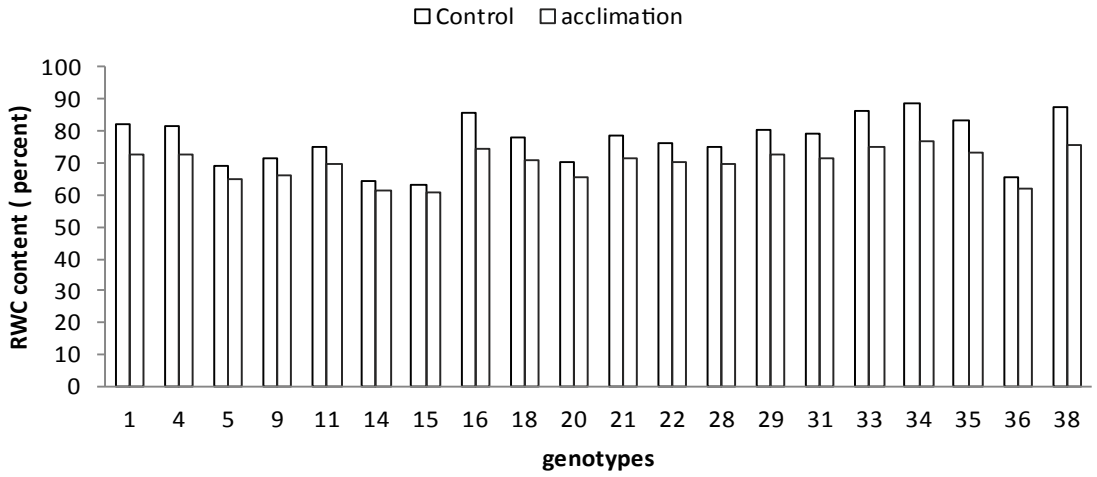

Figure 1: Content of RWC in the leaves of barley genotypes under control and cold conditions.

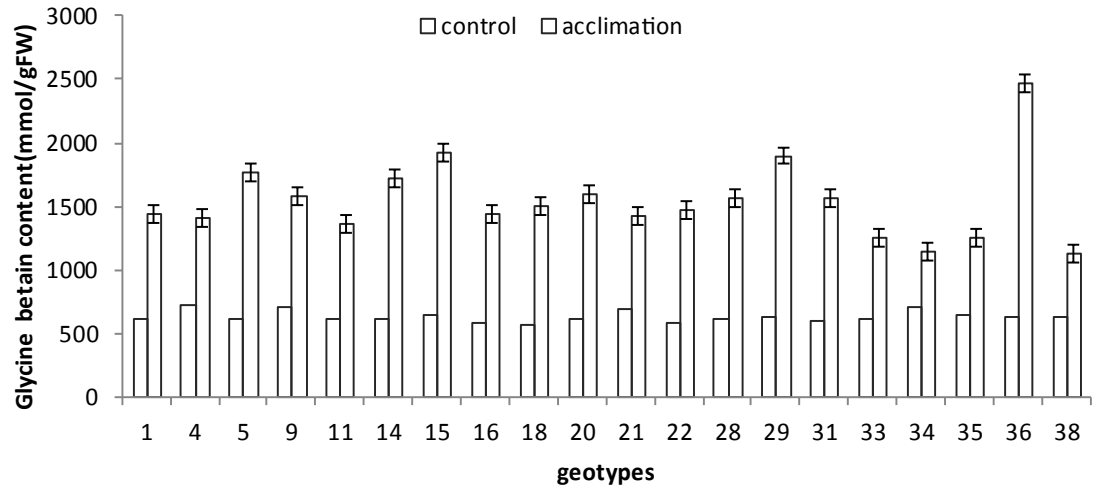

Figure 2: Changes in the content of GB in the leaves of barley genotypes under control and cold conditions.

Discussion

In this experiment, barley genotypes possessed different survival percentage and LT50 after cold adaptation at different temperatures. Autumn cultivars 36 and 15, due to being regularly cultivated in cold and moderate regions, displayed bigger survival percentage than spring's cultivar 38 did. Spring cultivars do not need adaptation to cold; they enter reproductive stage after a short time. Therefore, they are very sensitive to late spring as well as early fall cold weather. Transition from vegetative to reproductive stage is a vitally important phenomenon which keeps genes associated with cold tolerance under suppression, and raises the temperature in the crown [32]. In most areas, injury to the crown accounts for the main cause of death in plants. Therefore, soil temperature around the crown during the cold adaptation process is very important, and the crown will spoil if the soil temperature is lower than that of the crown [33]. In spring cultivars in which the temperature of the crown is warmer than the surrounding soil, this tissue spoils. Besides the plant's vegetative habit, genetics potential of the plants will also count for adaptation to cold [32] Autumn's genotypes varied in LT50 and survival percentage. And although the genetic potential varies in response to cold stress, the general pattern of response to cold weather during winter is the same for genotypes either inside or between cereal species. As a result, genetic variations related to cold tolerance can be determined using genetic coefficients of LT50 [34].

The results of this experiment and others [8,9] confirm the validation and reliability of LT50 as an indication of cold tolerance in barley. A high inheritability has been reported for LT50 [11] Here, the maximum LT50 scored by autumn genotype 15 as -12.39°C, and the minimum obtained by spring genotype 38 as -6.7°C. LT50 for wheat cultivar Nourstar has been determined in myriad of experiments around -23°C [35,36]. Therefore, it would be wise to use Nourstar as the landmark in identifying the precise LT50 for other genotypes, and setting them against Nourstars' LT50, as a means to reflex their potential capabilities [33]. Some management styles can additionally influence cold tolerance, which include cultivation date, plant age, depth of plantation and so forth [37]. In the current experiment, the effort was made to make sure every seed was planted in the same depth; the seedlings were acclimatized to cold stress in the same stage- a 3 to 4 leaf stage- to make sure the management errors have been averted, and LT50 was the only indicator of genetic potential.

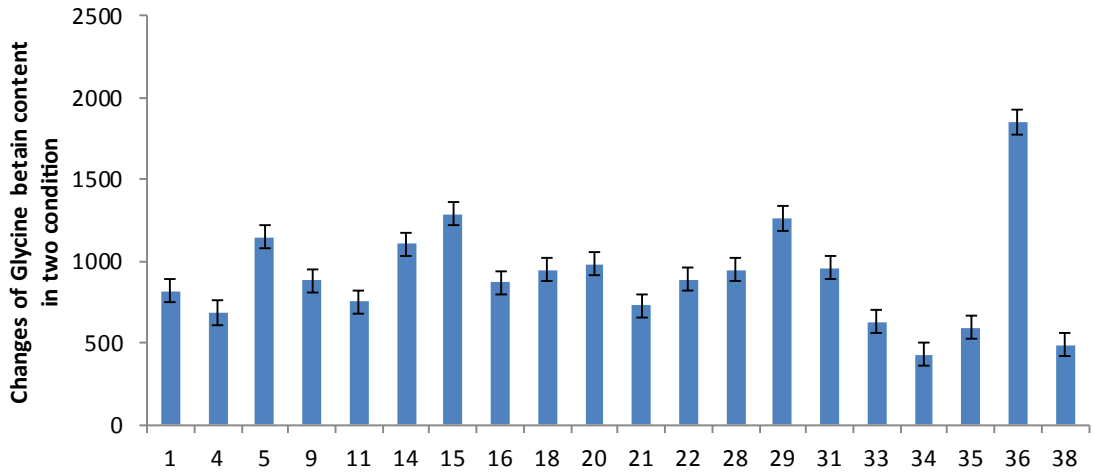

Figure 3:Changes of Glycine betaine content in the leaves of barley genotypes in two conditions.

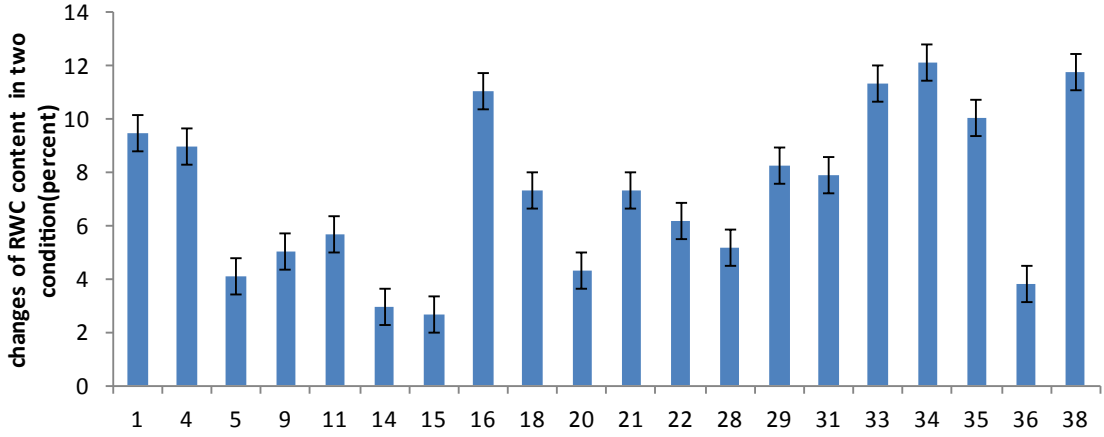

Figure 4. Changes of RWC content in the leaves of barley genotypes in two conditions.

Previous studies have confirmed a reduction in leaf RWC after acclimation to cold stress. Cold injury starts from the cell membrane, where low temperatures change the status of the membrane and leads to its damage [38]. The less cell membrane is damaged by freezing, the less amount of water is lost, and the greater the rate of survival will be [39].

Huner et al. [40] also reported that the leaves adapted to cold have 23% less water than plants without cold adaptation. Another research has also shown that plants tolerant to cold stress have higher competency to absorb and retain water during cold stress, hence experiencing fewer drops in RWC in leaves [41]. In the current study, the significant, positive correlation between leaf RWC and LT50, under normal conditions, signifies that tolerant genotypes with lower LT50 had a lower Leaf RWC than sensitive ones. Mirzaie-Asl et al. [42] also reported that tissues with less RWC in wheat were more tolerant to cold stress than those with bigger RWC. Ice formation is very damaging. Since ice crystals cannot exert a hydrophobic force necessary for preserving the bipolar status of lipids in cell membrane, they cause the disruption of cell membrane in contact [43]. Less cell membrane disruption in tolerate cultivars is due to less leaf RWC, less formation of ice inside the cell, and less production of H_2O_2.

In the current study, a significant, negative correlation existed between LT50 and GB content after cold adaptation as well as the difference between the two cold treatments, meaning GB content has increased with the reduction of LT50 (in more tolerate genotypes).

GB is one of the more common osmolytes, whose accumulation in surviving organisms is said to be used as a strategy for combating environmental stresses [44]. Although they are put in different groups, osmolytes assume similar functions in protecting plants against stress. However, the exact function of such solutes, including GB, under abiotic stresses, is not fully understood. There are two main functions attributed to these osmolytes: osmosis regulation and cell adaptation. Osmosis is regulated by the influence of forces related to concentration on osmosis pressure, which absorbs more water from the surrounding environment. In the cell adaptation process, these osmolytes substitute water in biochemical reactions, keeping the metabolism moving under stress conditions [45]. GB can substitute the lost water in tolerant genotypes, helping the plants survive by preserving the metabolism against cold stress. Contribution of GB accumulation to plants' tolerance to drought and salinity has been also reported in another study [31]. In the current study, GB content had increased in both tolerant and sensitive genotypes, but a bigger increase was seen in the former.

Table 5: Correlation between LT50, RWC, GB and Crown s urvival percent.

	LT50	Rwc before cold	Rwc after cold	Rwc difference between before and after cold	Gb before cold	Gb after cold	Gb difference between before and after cold	Crown s urvival percent at -8°C	Crown s urvival percent at -10°C	Crown s urvival percent at -12°C	Crown s urvival percent at -14°C
Rwc before cold	.819**	1									
Rwc after cold	.784**	.992**	1								
Rwc difference between before and after cold	.847**	.980**	.948**	1							
Gb before cold	.288	.154	.132	.185	1						
Gb after cold	-.759**	-.759**	-.775**	-.711**	-.190	1					
Gb difference between before and after cold	-.772**	-.754**	-.765**	-.711**	-.322	.991**	1				
Crown s urvival percent at -8°C	-.898**	-.597**	-.556*	-.643**	-.264	.598**	.613**	1			
Crown s urvival percent at -10°C	-.892**	-.912**	-.873**	-.944**	-.189	.704**	.705**	.688**	1		
Crown s urvival percent at -12°C	-.689**	-.866**	-.881**	-.814**	-.256	.643**	.655**	.412	.780**	1	
Crown s urvival percent at -14°C	-.591**	-.745**	-.770**	-.683**	-.073	.761**	.744**	.326	.661**	.649**	1

** Significant at 1%

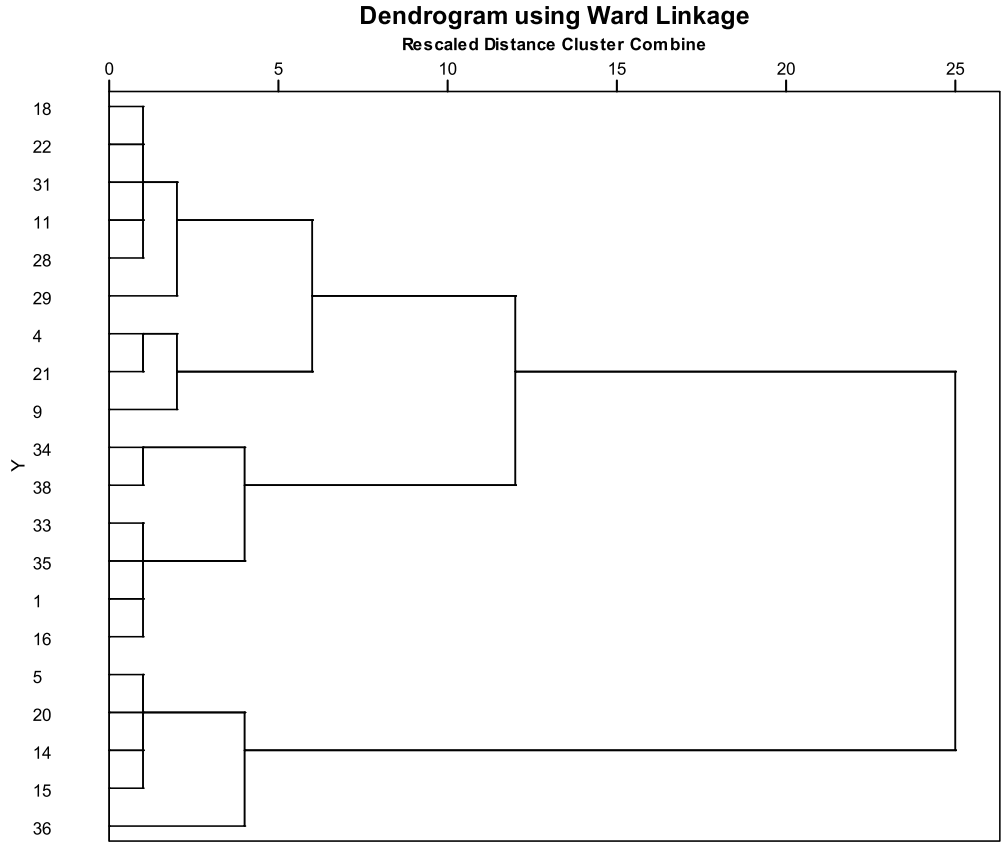

Figure 5: Cluster analysis for GB and RWC traits using the Ward method.

Cluster analysis

Cluster analysis was carried out using Ward method according to the squared Euclidean distance on standardized data (Figure 5). At a cut off 10 the dendrogram revealed three clusters. Group one includes 9 genotypes (18, 22, 31, 11, 28, 29, 4, 21,9) with negative deviation of mean (-16.38) for LT50, changing of GB and amount of RWC in two conditions, but deviation of mean percent is not a lot for GB and RWC. The group can considered as semi- tolerant genotypes based on the investigated characteristics under cold stress. Second group includes 34, 38, 33, 35, 1, 16 has positive deviation of mean (62.47) for LT50, negative deviation for changing of GB and RWC in two conditions. This group considered as sensitive to cold stress. Group three includes 5 genotypes (5, 20, 14, 15, 36) with highest negative deviation (-45.48) for LT50, positive changing of GB and RWC in two conditions, ranked as tolerant genotypes to cold stress. For LT50, more negative deviation from the mean and more positive deviation from the mean for GB and RWC, is a desirable features.

Conclusion

To sum up, LT50 is a suitable indication of tolerance to crown freezing but this method requires much time and cost and needs special systems for freezing test, thus by studying physiological traits and LT50 in seedling stage, a robust correlation can be made between these traits and tolerance to cold stress, hence differentiating tolerant genotypes from sensitive one at a lower cost and time. Glycine betaine and relative water content have a significant correlation with LT50, so that GB is more increased after cold in tolerant genotypes but RWC is more decrease in sensitive genotypes in response to stress condition. In conclusion, according to the results we suggested that in absence of freezing test systems, by measuring glycine betaine before and after cold stress, could be detected barley genotypes tolerance to cold conditions.

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Srivastava et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00072.0

Vegetos- An International Journal of Plant Research

Research Article

A SCITECHNOL JOURNAL

Evaluation of RIL Population Derived from Traditional and Modern Cultivar of Wheat (C 518/2* PBW 343) for Yield Potential under Drought Stress Conditions

Ashutosh Srivastava*, Puja Srivastava, Achla Sharma, Sarlach RS and Bains NS

Abstract

Present study was carried out to assess the yield potential of C518 and their potential utility in context of drought tolerance when introgressed in to a modern day wheat variety (PBW 343). These two cultivars (PBW 343 and C 518) belongs to distinct adaptation groups, offer several morpho-physiological and biochemical contrasts. C518 is tall and adapted to low input rainfed conditions whereas PBW 343 is semi-dwarf and input responsive. 175 recombinant inbred lines (C 518/2* PBW 343) along with parents and checks were evaluated for drought tolerance in account of yield potential under irrigated and rainfed environments during 2013 to 2014. Water stress was created by withholding irrigation. Different drought tolerance indices viz., stress susceptibility index, relative drought index, mean productivity, stress tolerance index, geometric mean productivity, yield stability index, drought resistance index were evaluated based on grain yield under irrigated and rainfed conditions. Out of 175 inbred lines, seven lines recorded higher grain yield under irrigated as well as rainfed environments. STI, DRI and MP showed highly significant positive correlation with yield in both stress and non-stress environments and with other drought tolerance indices. Thus application of these indices could be appropriate while screening the varieties for drought tolerance and on the basis of theses indices, the inbred lines 108, 84, 80 and 32 were found tolerance lines with high yield under both environments better than the parents.

Keywords

Drought indices; Principal component analysis; Recombinant inbred lines (RILs); Stress tolerance index; Wheat

Abbreviations

Ys: Yield Under Stress Condition; Yp: Yield Under Non-Stress Condition; STI: Stress Tolerance Index; SSI: Stress Susceptibility Index; TOL: Tolerance; MP: Mean Productivity ; GMP: Geometric Mean Productivity; YSI: Yield Stability Index; RDI: Relative Drought Index; DRI: Drought Resistance Index

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Received: July 05, 2016 **Accepted:** July 26, 2016 **Published:** July 30, 2016

Introduction

Wheat (*Triticum aestivum* L.) is an important food crop in the world and drought is one of the most common environmental stresses in agriculture [1,2]. In India, from 80% of the total cultivated wheat only one third is grown under full irrigation while the remaining receives only one to two irrigation in the crop season and hence water inadequacy is a critical factor for sustainable yield [3]. The unpredictability of duration and intensity of drought because of irregular rainfall patterns make the situation more intricate. Wheat crop can experience water deficit stress during growth and development in limited irrigation environments depending upon the water availability and in results of decline in yield productivity. In fact wheat crop often experiences drought in the post-anthesis and maturity period which most responsible plant behavior and development. Growing food demand and global warming would further drive wheat crop to heat and drought stress environments [4]. Therefore, in wheat breeding, drought tolerance has been a major objective in all breeding programs both nationally and internationally in order to improve crop productivity under water-limiting conditions [5]. There is a need for breeding approaches which couples higher yield and stress adaptation to combine higher yield potential and drought tolerance [5,6]. Fischer et al. [7] recommended relative drought index (RDI) as a positive indicator for stress tolerance. Rosielle and Hamblin [8] defined stress tolerance (TOL) as the differences in yield between water stressed and irrigated conditions and mean productivity (MP) as the average yield of genotypes under both these conditions. Since drought severity in field experiments vary over the years hence, some breeders use geometric mean productivity (GMP) to define the relative performance [9]. Fischer and Maurer [10] suggested the stress susceptibility index (SSI) for measurement of yield stability that perceives the changes in both potential and actual yields in under rainfed and irrigated environments. Clarke et al. [11] used SSI to evaluate drought tolerance in wheat genotypes and found year-to-year variation in SSI for genotypes. Guttieri et al. [12] evaluated yield potential by using SSI and suggested that an SSI >1 shows above-average susceptibility to drought stress. A yield stability index (YSI) was suggested by Bouslama and Schapaugh [13] in order to evaluate the stability of genotypes in the both stress (drought) and non-stress (irrigated) conditions. Stress tolerance index (STI) was defined as a useful tool for determining high yield and stress tolerance potential of genotypes under drought and irrigated conditions [9]. Prior to advent of present day semi-dwarf wheat in the 1960s, tall traditional cultivars were grown under rainfed conditions in the state. These cultivars (such as C306, C273, C518 and C591) were derived from landraces materials of this region. After the adaption of semi-dwarf, the tall traditional cutivars found very little use even as donors of traits in wheat breeding programme. Presently these materials have come under focus as a result of greater emphasis on breeding for abiotic stress tolerance with the aim of tall traditional cultivars C518 and their potential utility in context of drought tolerance when introgressed in to a modern day wheat variety (PBW 343). These two cultivars belonging to distinct adaptation groups, offer several morpho-physiological and biochemical contrasts. C518 is tall and adapted to low input rainfed conditions whereas PBW 343 is semi-dwarf and input responsive. The aim of the research reported in this

paper was to identify RILs combining drought tolerance with higher yield from the cross PBW 343 × C 518 based on tolerance indices to differentiate drought resistant inbred lines.

Materials and Methods

A Field experiment was carried out at experimental area of Department of Plant Breeding & Genetics, Punjab Agricultural University Ludhiana, Punjab, India (30^0-54' N and 75^0-48' E, 247 m altitude) during the 2012-13 and 2013-14 growing seasons. Plant material consisted of 175 recombinant inbred lines (C 518/2*PBW 343) along with 21 check cultivars viz. (PBW 343, PBW 621, C518, PBW 644, PBW 527, C306, C273, C591, C286, C281, C285, PBW 706, PBW 175, PBW 691, BWL 1856, HD 2967, Kirchauff, Babax, Excalibur, Gladius and Drysdale). The experimental design was 14 × 14 square lattice having 1m^2 plots with three replications. The drought environment was created by withholding irrigation and created temporary rain shelter over trails whenever required. Agrometerological data were recorded during crop season from Agrometerological station, PAU Ludhiana and is presented in Figure 1. Normal recommended agronomic practices for growing timely sown wheat crop was followed. Analysis of variance was computed by using SAS pro lattice (version 9.2). Correlation and Principal component analysis among different tolerance indices were analyzed by software JMP® SAS (version 12).

Calculation of drought tolerance indices

The grain yield/m^2 were recorded for each genotype under irrigated and rainfed environment and used to calculate the drought tolerance indices. The drought tolerance indices were calculated using the following formulas:

Stress Tolerance Index (STI): Drought tolerance indices were calculated based on grain yield over stress environment (drought) and non-stress environment (irrigated) by using the formula as below:

$$STI = \frac{Y_S * Y_p}{(\overline{Y_P})^2} \quad [9]$$

Stress susceptibility index (SSI) = $\frac{1-\left(Y_S/Y_P\right)}{1-\left(\overline{Y_S}/\overline{Y_P}\right)}$ [10]

Tolerance (TOL) = $Y_P - Y_S$ [8]

Mean productivity (MP) = $\frac{(Y_S + Y_P)}{2}$ [8]

Geometric mean productivity (GMP) = $\sqrt{(Y_S * Y_P)}$ [9]

Yield stability index (YSI) = $\frac{Y_S}{Y_P}$ [13]

Relative drought index (RDI) = $\frac{\left(Y_S/Y_P\right)}{\left(\overline{Y_S}/\overline{Y_P}\right)}$ [7]

Drought resistance index (DRI) = $\frac{Y_S\left(Y_S/Y_P\right)}{\left(\overline{Y_S}\right)}$ [14]

Where;

Y_P: yield of each genotype in non- stressed (Irrigated) environment

Y_S: yield of each genotype in stressed (drought) environment

$\overline{Y_P}$: mean of yield of all genotypes under non- stressed (irrigated) environment

Result and Discussion

The 175 inbred lines of wheat along with 21 check cultivars were evaluated for drought tolerance indices under normal and drought environments. Analysis of variance showed that genotypes were significantly differed for grain yield in both irrigated as well as rainfed conditions (Table 1). These results indicated high diversity among the genotypes that may enable breeder to select genotypes under stress as well as non-stress environments for grain yield potential. Stress-environment decreased grain yield by 12.19% as compared to non-stress environment. Several other researchers also reported the similar kind of observations under stress and non-stress conditions for grain yield [15]. Out of 175 lines tested for grain yield, seven

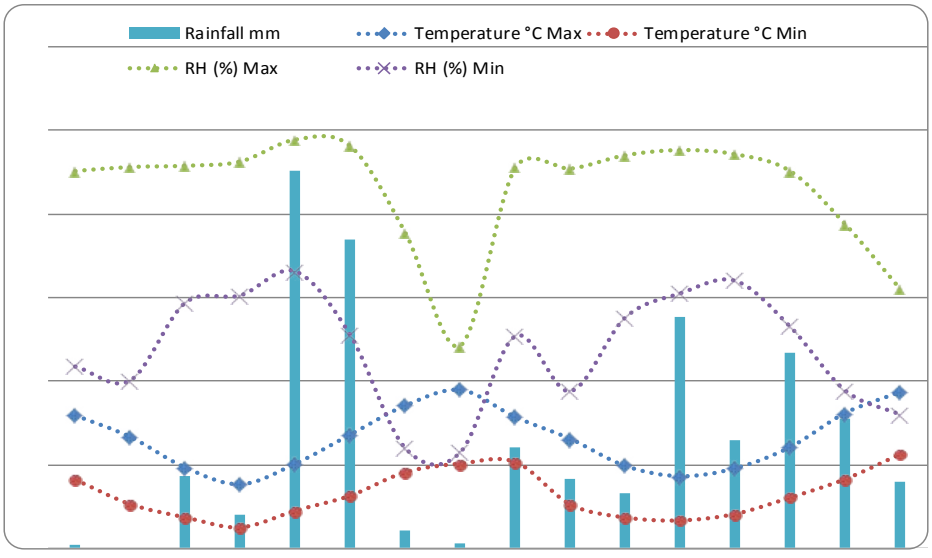

Figure 1: Agrometerological data during crop seasons 2012-13 and 2013-14.

Table 1: Analysis of variance for grain yield under irrigated and rainfed conditions.

		Mean square of characters	
Source of variation	**DF**	**Grain Yield (Irrigated)**	**Grain Yield (Rainfed)**
Replication	2	87.24	0.00952
Block within rep (adj)	39	1388.89	0.00669*
Treatment (unadj)	195	18100	0.01391
Treatment (adj)	195	16809.2**	0.0129**
Intra Block Error	351	919.64	0.00317
Randomized complete Block Error	390	966.56	0.00352
Total	587	6655.27	0.00699
Efficiency relative to RCBD		101.67	105.5
R^2		0.917	0.729
CV (%)		12.92	23.21

Table 2: Various drought indices of selected inbred lines along with check cultivars under irrigated and rainfed environments.

RILs	**Yp**	**Ys**	**STI**	**SSI**	**RDI**	**DRI**	**TOL**	**MP**	**GMP**	**YSI**
108	393.3	383.3	2.942	0.208	1.11	1.879	10	388.33	388.3	0.975
84	403.3	373.3	2.938	0.61	1.054	1.739	30	388.33	388.04	0.926
80	353.3	346.7	2.39	0.155	1.117	1.711	6.67	350	349.98	0.981
32	360	340	2.388	0.456	1.076	1.615	20	350	349.86	0.944
47	353.3	333.3	2.298	0.464	1.074	1.582	20	343.33	343.19	0.943
30	356.7	326.7	2.273	0.69	1.043	1.505	30	341.67	341.34	0.916
41	346.7	323.3	2.187	0.552	1.062	1.517	23.33	335	334.8	0.933
RILs Min	76.7	50	0.069	0.052	0.537	0.16	-33.33	63.33	61.91	0.467
RILs Max	403.3	383.3	2.729	4.096	1.307	1.828	153.33	388.33	388.3	1.137
RILs Mean	226.4	198.8	0.889	0.981	1.003	0.865	27.61	212.58	211.76	0.872
Checks	**Yp**	**Ys**	**STI**	**SSI**	**RDI**	**DRI**	**TOL**	**MP**	**GMP**	**YSI**
BWL 1856	520	460	4.33	0.886	1.017	1.991	60	490	489.08	0.885
HD 2967	493.3	433.3	3.869	0.934	1.01	1.862	60	463.3	462.33	0.878
PBW 644	440	400	3.186	0.698	1.045	1.779	40	420	419.52	0.909
PBW 621	426	383.3	2.956	0.77	1.034	1.687	42.7	404.65	404.09	0.9
PBW 706	423.5	381.7	2.926	0.758	1.036	1.683	41.8	402.6	402.06	0.901
GLADIUS	446.7	310	2.507	2.35	0.798	1.053	136.7	378.35	372.12	0.694
PBW 691	410	333.3	2.474	1.437	0.935	1.326	76.7	371.65	369.67	0.813
PBW 527	335.5	286.7	1.741	1.117	0.982	1.199	48.8	311.1	310.14	0.855
PBW 175	313.3	296.7	1.683	0.407	1.089	1.375	16.6	305	304.89	0.947
PBW 343	363.3	226.5	1.489	2.892	0.717	0.691	136.8	294.9	286.86	0.623
C 591	250	210	0.95	1.229	0.966	0.863	40	230	229.13	0.84
C 286	253	207	0.948	1.396	0.941	0.829	46	230	228.85	0.818
C 518	260	215.5	1.014	1.314	0.953	0.874	44.5	237.75	236.71	0.829
C 273	223.5	186.7	0.755	1.264	0.96	0.763	36.8	205.1	204.27	0.835
C 306	213.3	183	0.707	1.091	0.986	0.768	30.3	198.15	197.57	0.858
BABAX	206	130	0.485	2.833	0.726	0.401	76	168	163.65	0.631
EXCALIBUR	193.3	130	0.455	2.515	0.773	0.428	63.3	161.65	158.52	0.673
KIRCHAUFF	170	143.3	0.441	1.206	0.969	0.591	26.7	156.65	156.08	0.843
C 281	165.3	143.3	0.429	1.022	0.997	0.608	22	154.3	153.91	0.867
DRYSDALE	183.3	116.7	0.387	2.79	0.732	0.363	66.6	150	146.26	0.637
C 285	163	125	0.369	1.79	0.882	0.469	38	144	142.74	0.767

superior inbred lines were found higher grain yield over other lines under both stress and non- stress conditions. Drought indices which provide a measure of drought tolerance based on yield loss under drought conditions in comparison to normal conditions have been used for screening drought tolerant genotypes [16].

Evaluation of inbred lines on the basis of tolerance indices

Drought tolerance can only be evaluated, if drought stress causes significant reduction in yield [17]. Various drought tolerance indices were estimated for grain yield under rainfed with relation to performance under irrigated conditions and their values are presented in Table 2.

Stress Tolerance Index (STI) is a useful tool for determining stress tolerance potential and high yield of a genotype. Among the RILs the range of STI was 0.069 to 2.729 with an average of 0.889, whereas parents PBW 343 and C 518 had 1.489, 1.014 respectively. Line 108 showed higher STI value 2.94 followed by 84, 80, 32, 47, 30 and 41 (2.938, 2.39, 2.388, 2.298, 2.273 and 2.187 respectively).

RILs showed higher STI value either of parents indicating that inbred lines improved for drought resistance on at par or either of parents. Among the check cultivars higher STI value was recorded for BWL 1856 (4.33) followed by HD 2967, PBW 644, PBW 621, PBW 706, Gladius, PBW 527 and PBW 175 (3.86, 3.18, 2.95, 2.92, 2.50, 2.47,1.74 and 1.68 respectively). Among the RILs the range of SSI was 0.052 to 4.096 with an average of 0.981, whereas parents 343 and C 518 showed 2.892, 1.314 respectively. Line 80 recorded lowest SSI value (0.155) followed by 108, 32, 47, 41, 84 and 30 (0.208, 0.456, 0.464, 0.552, 0.610 and 0.690 respectively). Among the check cultivars, lowest SSI value was recorded for PBW 175 (0.407) followed by PBW 644, PBW 706, PBW 621, BWL 1856, HD 2967 (0.698, 0.758, 0.770, 0.886, 0.934 respectively). Thus, stress susceptibility index is independent of yield potential and drought intensity, and is potentially useful for comparisons of drought susceptibility of genotypes between drought and irrigated experiments, since larger values of SSI indicate greater drought susceptibility. On the basis of SSI, PBW 343 showed greater drought susceptibility followed by Babax (2.833), Drysdale (2.790), Excalibur (2.515) and Gladius (2.507). Similarly, Drought response indices (DRI) were calculated for stress condition. Genotypes with high DRI, low SSI and high grain yield performed consistently across the stress environments. Among the RILs the range of DRI was recorded 0.160 to 1.828 with an average of 0.865, whereas parents PBW 343 and C 518 showed 0.691, 0.874 respectively. Among the checks, highest RDI value was recorded for BWL 1856 (1.991) followed by HD 2967, PBW 644, PBW 621, PBW 706, PBW 175, PBW 691 and Gladius (1.862, 1.779, 1.687, 1.683, 1.375, 1.326 and 1.053 respectively). This indicates that greater DRI value have better yield under both the environments. Similarly for MP, GMP greater values considered for higher mean yield under both environments, based on these indices inbred lines 108, 84, 80, 32, 47 and 30 showed greater values as higher than either of parents PBW 343 and C 518 (Figure 2).

Correlation analysis among the tolerance indices

Correlation among various drought indices were observed and presented in Table 3. Under stress environment, grain yield (Ys) was found significant positive correlation with STI, RDI, DRI, MP, GMP, YSI (0.962, 0.440, 0.973, 0.985, 0.988, 0.440 respectively) whereas, it was significant negative correlated with SSI (-0.440). Grain yield in irrigated condition (Yp) showed significant positive correlation with STI, DRI, TOL, MP and GMP (0.962, 0.845, 0.311, 0.987, and o.983 respectively), Positive relationship between MP and STI with yield under both environments would be more effective criteria in identifying high yielding genotypes. Farshadfar et al. [18] also reported similar results for correlations of grain yield with MP and STI under both stress and non-stress environments. Stress tolerance index (STI) showed positive significant correlation with RDI, DRI, MP and YSI. Mean productivity (MP) was found significantly and positively correlated with STI, DRI, RDI and TOL, and negatively correlated with SSI. Relative drought index (RDI) had significant positive correlation with STI, YI, YSI, MP, GMP and DRI and strong negatively correlated with SSI (-1.00). Absolute correlations of RDI with SSI and YSI with SSI indicated mathematical similarity in their

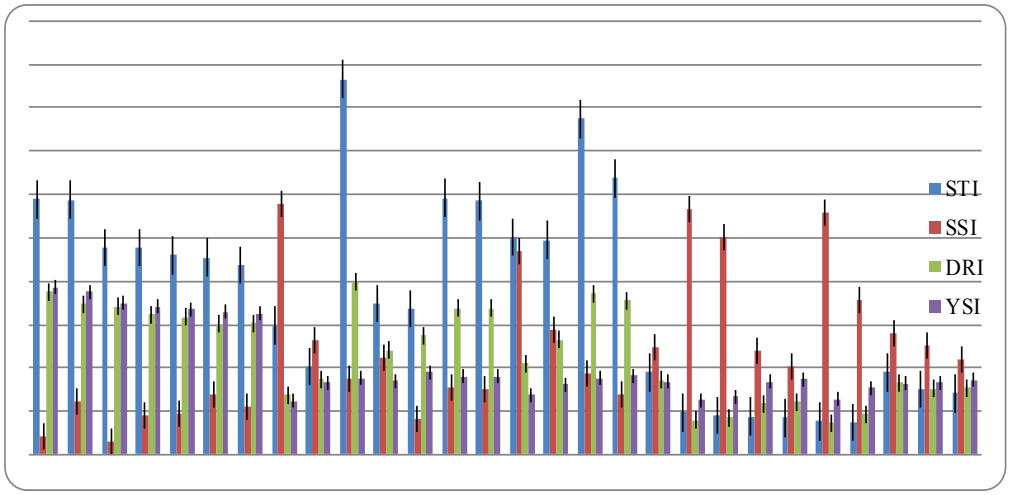

Figure 2: Different drought indices showed variability in inbred lines along with check cultivars.

Table 3: Correlation among different tolerance indices under both irrigated and rainfed environments.

	Ys	Yp	STI	SSI	RDI	DRI	TOL	MP	GMP	YSI
Ys	1	0.944**	0.962**	-0.440**	0.440**	0.973**	-0.02	0.985**	0.988**	0.440**
Yp	0.944**	1	0.962**	-0.137^{NS}	0.137^{NS}	0.845**	0.311**	0.987**	0.983**	0.137^{NS}
STI	0.962**	0.962**	1	-0.259**	0.259**	0.898**	0.145*	0.976**	0.976**	0.259**
SSI	-0.440**	-0.137^{NS}	-0.259**	1	-1.000**	-0.617**	0.853**	-0.288**	-0.306**	-1.000**
RDI	0.440**	0.137^{NS}	0.259**	-1.000**	1	0.617**	-0.853**	0.288**	0.305**	1.000**
DRI	0.973**	0.845**	0.898**	-0.617**	0.617**	1	-0.244**	0.920**	0.927**	0.617**
TOL	-0.020^{NS}	0.311**	0.145*	0.853**	-0.853**	-0.244**	1	0.151*	0.133^{NS}	-0.853**
MP	0.985**	0.987**	0.976**	-0.288**	0.288**	0.920**	0.151*	1	1.000**	0.288**
GMP	0.988**	0.983**	0.976**	-0.306**	0.305**	0.927**	0.133^{NS}	1.000**	1	0.305**
YSI	0.440**	0.137^{NS}	0.259**	-1.000**	1.000**	0.617**	-0.853**	0.288**	0.305**	1

Table 4: First two principal components for grain yields and stress indices under irrigated and rainfed conditions.

Variable	PC1	PC2	PC3
Yp	0.318	0.316	0.115
Ys	0.358	0.147	-0.094
STI	0.342	0.22	-0.317
SSI	-0.297	0.363	-0.318
RDI	0.297	-0.363	0.338
DRI	0.367	0.026	-0.266
TOL	-0.125	0.571	0.703
MP	0.342	0.234	0.011
GMP	0.341	0.224	0.003
YSI	0.298	-0.363	0.306
Eigenvalues	7.32	2.57	0.086
Cumulative %	73.23	99	99.8

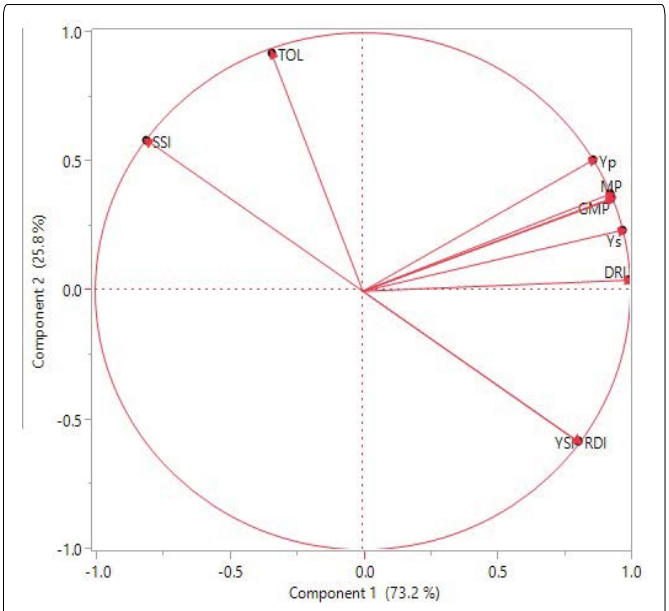

Figure 3: Biplot analysis for drought tolerance indices using two principal components.

formulae. So, these indices cannot be a proper index for selecting the genotypes which have a high yield in normal and drought stress environments [19].

Principal component analysis

The first two factors in the principal component analysis showed 99.0 % total variation (Table 4). The first PC explained 72.2% grain yield whereas the second contributed 25.8 %. The maximum contribution by individual for first factor was by DRI followed by STI and MP.

Therefore it reflects high grain yield as well as stress tolerance. The relationships among different indices are graphically displayed in a biplot of PCA1 and PCA2 (Figure 3). The angle direction between the attribute vectors illustrated the strength and the direction of correlation between any two attributes [9]. Significant positive correlation was observed between yield (Ys) with DRI, GMP, STI and MP and significant negatively correlated with SSI. These observations were conformity with correlation results indicating that DRI, STI, and MP could be reliable selection criteria for drought tolerance as reported by Abdi et al. and Mohammadi et al. [20,21].

It is concluded that, among the various tolerance indices, STI, DRI and MP showed strong correlation between them so on the basis of these, inbred lines 108, 84, 80 and 32 were found most tolerance lines as compared to other lines and better than parents as well as check cultivars. These RILs can be used as genetic material for further breeding programme and identify QTLs for drought tolerance traits.

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Gautam et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00073.2

Vegetos- An International Journal of Plant Research

Research Article

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Development and Evaluation of Inclined Plate Metering Mechanism for Onion Pelleted Seeds

Anand Gautam*, Rohinish khurana, Gursahib Singh Manes, Anoop Dixit and Aseem Verma

Abstract

The ability to place seeds at a given distance apart in a row is an important performance factor of a planter with single seed metering mechanism. Inclined plate seed metering device was developed and evaluated in laboratory for singulation and uniform placement of onion seeds with different pelleting ratio viz. 1:1, 1:2 and 1:3 pelleted. Metering device was tested at three inclinations of 40°, 45°and 50° using plates having three different groove number 18, 24 and 30 on cells. Average seed spacing obtained at in S_3 seed, θ_2 angle in forward speed 2.0 km/h, the average spacing was observed to be 5.90, 5.35 and 5.15 cm for 18, 24 and 30 groove plate respectively. Missing index at 2.0 km/h forward speed with 24 groove seed metering plate with 45° inclination angle was 5.0% and multiple index was 11.0%. The overall quality of feed index obtained with these parameters was 84.0% which is maximum when compared with other seed treatments, forward speeds and types of seed metering plate combinations with different angle of inclination of plates. The selection of plate inclination and type of metering cell for the planter was purely based on average spacing, missing index, multiple index and quality of feed index. With 24 groove seed metering plate with 45° inclination angle and forward speed of 2.0 km/h was selected for the field evaluation.

Keywords

Inclined plate; Miss index; Multiple index; Quality index; Cell; Pelleted seed

Introduction

Onion (Allium cepa L.) is one of the major vegetable crop grown throughout the country. Onion is one of those versatile crops that can be stored for a longer period under ambient conditions and safely withstands the hazards of rough handling and transportation. It is widely grown in different parts of the country mainly by small and marginal farmers. In Punjab onion is sown over an area in 2013-14 of about 8.3 thousand hectare having production of 185.4 thousand tonne [1]. Generally, the onion seeds are sown in nursery and transplanted with row to row spacing of 15 cm and plant to plant spacing of 7.5 cm to get optimum yield. During onion cultivation, transplanting of seedlings, weeding and harvesting are the most labour intensive operations that are presently done manually in India. The labour requirement in manual transplanting of onion seedlings is as high as 100-120 man- days/ha as 8.9 lakh seedlings per hectare are to be transplanted [2]. Because of high requirement and shortage of labour, the area under onion cultivation is low and can be increased by mechanization of this crop.

Performance of single seed planter mainly depends on its ability to place seeds at a given distance apart. Under field conditions, it is often impossible to directly measure seed placement. An alternative is to measure the spacing between plants after they emerge. When examining the spacing between the plants once they emerge, considerable variability often exists in the plant-to-plant distance. Much of the variability in spacing could be removed by evaluating planters under laboratory conditions. However, field trails are also needed to accurately evaluate how planters perform in field. The main aim is to quantify the observed variability in a way that will allow one to make meaningful comparisons between single seed metering devices. A number of factors affect the spacing of plants. The seed selection mechanism may fail to select or drop a seed resulting in large spacing between the seeds. The device may pick and drop multiple seeds resulting in small spacing between seeds. Seed tube design and soil conditions along with other factors determine the final placement of seed. As all seeds may not germinate, the distribution of plant spacing will differ from the intended seed spacing.

Kachman and Smith [3] tested and compared the most widely used measures; mean, standard deviation, quality of feed index, multiple index, miss index and precision. These measures were based on the theoretical spacing (X ref), specified in ISO 7256-1 standard [4], and gave a good indication of spacing distribution. Kachman and Smith [3] concluded that the mean and the standard deviation of seed spacing did not offer an appropriate evaluation of planter performance on seed distribution. The final selection of metering device also depends on multiple index and miss index. Shibata et al. [5] developed two devices for small seed metering based on the picking action with pincette type picking unit. One device was designed with a spring pick up unit and other device with electromagnetic pick-up type. Lower misses were observed for spring pickup type at low peripheral speed of 13.4 $cm.s^{-1}$ with 71% seeding efficiency. In case of electromagnetic pickup device, no misses were observed even at high peripheral speeds with 80% seeding efficiency. Zang and Guo [6] designed a special- shaped spiral groove precision seed metering device for small grain crops. The spiral groove sections with rectangular bottom, V-shaped bottom and U-shaped bottom were used for seed metering. V-shaped bottom was chosen, because of its stability for seeding. Development of manually operated electrostatic planter for small seed was reported by Ahmed and Gupta [7]. There was no damage to seeds passing through metering device, but number of seeds picked up by electrostatic charge varied from 2-6 seed per hill. Study on feasibility of precision planting by cell type metering device for radish seed was reported by Otsuka et al. [8]. Kowalczuk and Zarajczyk [9] examined the quality of carrot sowing with belt type seeder at 3 working speeds of 0.7, 1.0 and 1.4 $m.s^{-1}$, and found the best working speed as 0.7 $m.s^{-1}$. A large number of planter designs are available for bolder seeds, but very little information is available on small seed like onion, particularly under Indian situations. Hence,

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Received: July 11, 2016 **Accepted:** July 30, 2016 **Published:** July 30, 2016

the present study was conducted with the objective to design the metering mechanism for small sized seed like onion and evaluate it for uniformity of seed placement.

Materials and Methods

Metering system

Mechanical seed metering devices in planter usually have cells on a moving member to have positive seed metering. Commonly recommended metering systems on planters are horizontal plate, inclined plate, vertical rollers with cells, and cups over the periphery [4]. Since onion seeds are small in size and very susceptible to mechanical damages, metering with vertical and horizontal plate metering mechanism were not considered. Laboratory experiment was thus conducted with inclined plate cell type metering mechanism having different cell numbers (Figure 1). Details of the metering plates are shown in Figure 2. The types of onion seeds used for the study are shown in Figure 3. The average values of roundness value of onion seed were 0.75, 0.78 and 0.82 for 1:1, 1:2 and 1:3 pelleted seed respectively (Table 1). This gave clue to use slant shape of cells over plate periphery. The values of angle of repose for 1:1 pelleted (S_1), 1:2 pelleted (S_2) and 1:3 pelleted (S_3) seeds were 31.61°, 29.50°, 24.78°, and 23.70° respectively (Table 1).

The hopper slope was thus decided at 45° by the values of angle of repose of the seeds.

Laboratory test

The performance of cells of different shapes was evaluated using a sticky belt and by varying inclination of the metering device for both coated and uncoated seeds (Table 2). The sticky belt mechanism consisted of 4 m long endless canvass belt mounted on two endless rollers spaced 100 cm apart along with a seed hopper and power transmission unit of belt pulley system with reduction gear and driving roller driven by a 4 kW motor. Observations were taken on the spacing between two adjacent seeds over the greased belt. Based upon the in-between spacing of 50 seeds, five measures of performance parameters viz. average spacing, multiple index, miss index, quality of feed index and precision were determined [1].

Performance parameters

Multiple index: Multiple index (D) is an indicator of more than one seed dropped within a desired spacing. It is the percentage of spacing's that are less than or equal to half of the theoretical spacing:

$$D = n1/N \quad (1)$$

Where,

N = Total number of observations, and

n1 = Number of spacing's in the region less than or equal to 0.5 times of the theoretical spacing.

Quality of feed index: Quality of feed index (A) is the measure of how often the seed spacing's were close to the theoretical spacing [4]. It is the percentage of spacing's that are more than half, but not more than 1.5 times the theoretical spacing. The quality of feed index is mathematically expressed as follows:

$$A = n2/N \quad (2)$$

Where,

N = Total number of observations, and

n2 = Number of spacing's between 0.5 times the theoretical spacing and 1.5 times of the theoretical spacing.

Miss index: Miss index is an indicator of how often a seed skips the desired spacing. It is the percentage of spacing greater than 1.5 times the theoretical spacing, and expressed as:

$$M = n3/N \quad (3)$$

Where,

N = Total number of observations, and

n3 = Number of spacing's in the region > 1.5 times of the theoretical spacing.

Degree of variation: Degree of variation (c) is a measure of the variability in spacing after accounting for variability due to both multiples and skips. The degree of the variation is the coefficient of variation of the spacing that are classified as singles.

$$C = S / X_{ref} \quad (4)$$

Where,

S = Sample standard deviation of the n3 observation,

X ref = Theoretical spacing

Results and Discussion

Average spacing

The average spacing was significantly influenced by all combinations of design variables of the study at 5% level of significance. Inclination of metering device influenced the average spacing, followed by cell number as indicated by the F-values (Table 3).

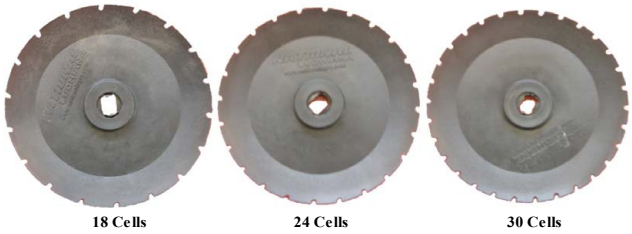

Figure 1: Metering plates used for metering of onion seed.

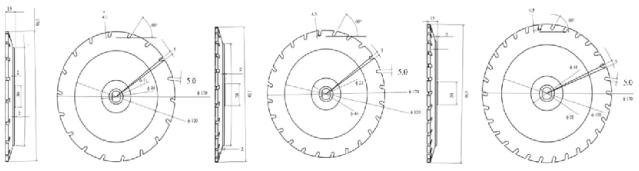

Figure 2: Details of metering plate used for onion seed metering.

Figure 3: Onion seeds used for evaluation of metering mechanism.

Table 1: Physical and engineering properties of onion seeds under different seed treatments.

Property	Treatment		
	S_1	S_2	S_3
Major Dimension	3.16	3.45	3.71
Intermediate Dimension	2.32	2.76	2.88
Minor Dimension	2.01	2.35	2.71
Spherecity value	0.77	0.81	0.82
Roundness value	0.75	0.78	0.82
Angle of repose (degree)	29.50	24.78	23.70

Table 2: Plan of experiment on metering device.

S. No.	System Variable	Level of Variable
1.	Seed Treatment (S)	S_1-1:1 Pelleted Seed
		S_2-1:2 Pelleted Seed
		S_3-1:3 Pelleted Seed
2.	Inclination of metering mechanism(θ)	θ_1- 40°
		θ_2- 45°
		θ_3- 50°
3.	Speed of operation (Sp)	Sp_1- 1 kmh^{-1}
		Sp_2- 2 kmh^{-1}
		Sp_3- 3 kmh^{-1}
4.	Cell (P)	P_1- 18 Number of grooves on Cell
		P_2- 24 Number of grooves on Cell
		P_3-30 Number of grooves on Cell

Table 3: F-values for performance parameters of seed metering mechanism.

Source	F-Value				
	Average Spacing	Multiple Index	Miss Index	Quality Feed Index	Degree of Variation
S	6668.486	811.685	212.839	239.651	60.089
θ	168.743	31.510	97.090	7.705	2.213
Sp	47.410	65.053	60.260	16.017	973.483
P	696.377	117.435	46.917	23.161	44.110
S * θ	22.272	5.848	12.759	.917	0.649
S * Sp	32.042	0.450	2.259	1.195	1.608
S * P	75.332	0.352	8.198	2.570	0.703
θ * Sp	2.782	2.253	19.345	7.566	3.927
θ * P	41.509	2.602	8.849	6.052	0.215
Sp * P	5.771	0.651	2.605	1.083	0.092
S * θ * Sp	7.155	0.808	1.540	1.756	1.418
S * θ * P	5.858	1.550	2.967	1.889	0.141
S * Sp * P	3.280	0.399	2.775	1.319	0.101
θ * Sp * P	1.441	1.354	5.579	3.223	0.208
S * θ * Sp * P	0.631	0.396	1.531	1.255	0.272

The data presented in Figures 4, 5 and 6 depicts that average spacing varied slightly with the change in type of treatments of seed, inclination angle of plate and type of seed metering plate. The average spacing increased with the increase in the ratio of seed treatment at forward speed of 1.0 km/h, whereas at forward speed of 2.0 km/h and 3.0 km/h and decreases with number of grooves the seed metering plate. For S_1 seed, θ_2 angle in forward speed 2.0 km/h, the average seed spacing was observed to be 3.460 cm, 3.400 cm and 3.290 cm for 18, 24 and 30 groove plate respectively. In S_3 seed, θ_2 angle in forward speed 2.0 km/h, the average spacing was observed to be 5.90, 5.35 and 5.15 cm for 18, 24 and 30 groove plate respectively.

Performance indices

The distance between plants within a row is influenced by a number of factors including multiple index, missing index, failure of a seed to emerge, and variability around the drop point. Missing, multiple and quality of feed index were highly influenced by all the three design variables at 5% level of significance.

Multiple index

Multiple index was influenced by inclination of the metering device, followed by cell shape and type of seed as indicated by the F-values (Table 3). The experimental multiple index for independent parameters are given in Figures 7, 8 and 9. It is apparent that the multiple index was affected by the parameters studied i.e. treatment of seed, inclination angle of planter, forward speed and types of seed metering plate. The average multiple index observed were at S_1 seed 30.0, 28.0, 27.0 for 18 groove plate, 32.0, 31.0, 29.0 for 24 groove plate and 35.0, 33.0, 32.0 for 30 groove plate at θ_2 angle and forward speed 1.0, 2.0, 3.0 km/h respectively. In S_3 seed, 19.0, 13.0, 12.0 for 18 groove plate, 20.0, 11.0, 10.0 for 24 groove plate and 24.0, 18.0, 17.0 for 30 groove plate respectively.

Missing index

Missing index was influenced most by plate angle, followed by cell shape, as indicated by the F-values (Table 3). Missing index increased with the increase in forward speed as shown in Figures 10, 11 and 12. The average miss index observed at S_1 seed were, 6.0, 6.0, 10.0 for 18 groove plate, 5.0, 5.0, 10.0 for 24 groove plate and 2.0, 3.0, 6.0 for 30 groove plate at θ_2 angle and forward speed 1.0, 2.0, 3.0 km/h respectively. For S_3 seed, 10.0, 12.0, 18.0 for 18 groove plate, 3.0, 5.0, 14.0 for 24 groove plate and 8.0, 11.0, 16.0 for 30 groove plate respectively.

Quality of feed index

Quality of feed index was highly influenced by angle of metering

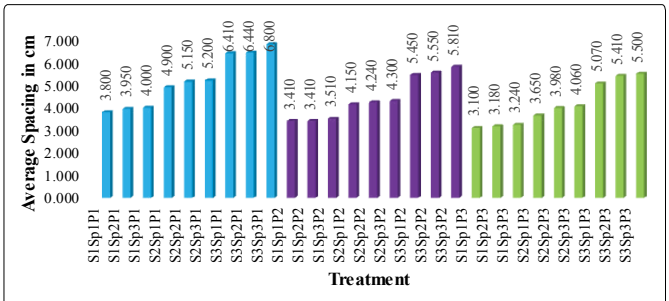

Figure 4: Effect of seed treatment, forward speed and type of seed metering plate on onion seeds average spacing at inclination angle of plate 50°.

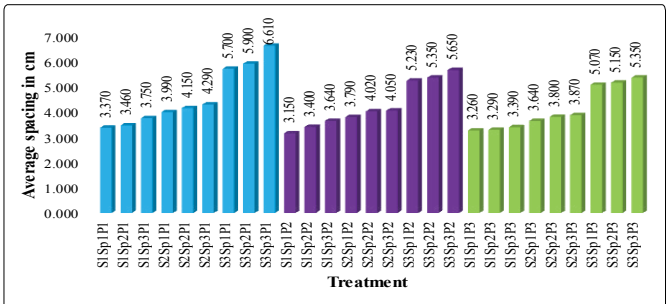

Figure 5: Effect of seed treatment, forward speed and type of seed metering plate on onion seeds average spacing at inclination angle of plate 45°.

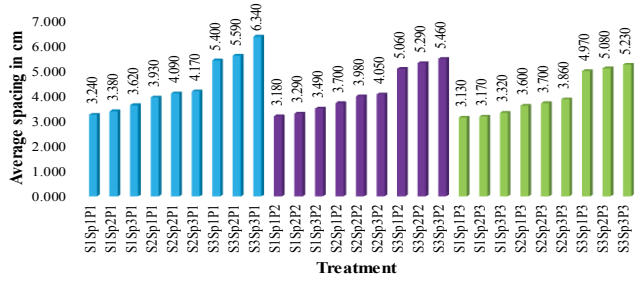

Figure 6: Effect of seed treatment, forward speed and type of seed metering plate on onion seeds average spacing at inclination angle of plate 40°

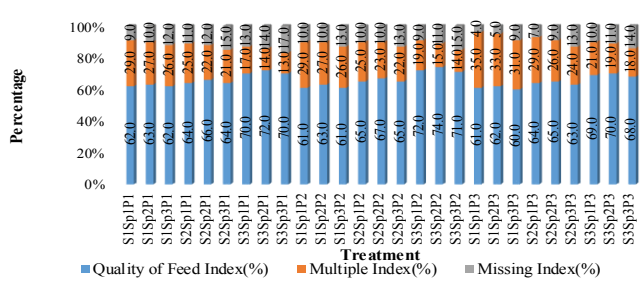

Figure 7: Effect of seed treatment, forward speed and type of seed metering plate on onion seeds performance indices at inclination angle of plate 50°.

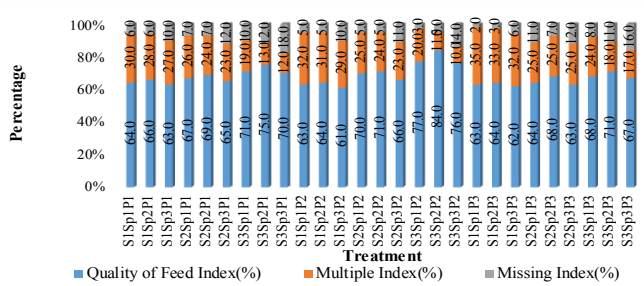

Figure 8: Effect of seed treatment, forward speed and type of seed metering plate on onion seeds performance indices at inclination angle of plate 45°.

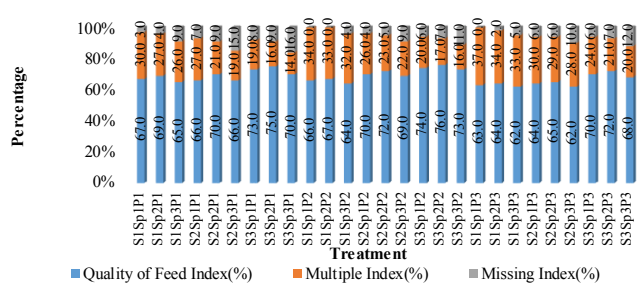

Figure 9: Effect of seed treatment, forward speed and type of seed metering plate on onion seeds performance indices at inclination angle of plate 40°.

plate, followed by seed and cell shape as indicated by F-values (Table 3). The data in depicts the variation of quality of feed index with respect to the forward speed. It can be seen that the quality of feed index initially increased when the forward speed was increased from 1.0 km/h to 2.0 km/h and when the forward speed was increased from 2.0 km/h to 3.0 km/h the quality of feed index also decreased.

Degree of variation

The coefficient of variation of spacing's is classified as singles. Lower the value of coefficient of variation in single's, better is the performance of a metering mechanism. It is evident from the degree of variation increases with the increase in speed for all types of seed metering plates. The average degree of variation observed were at S_1 seed, 17.0, 19.0, 26.0 for 18 groove plate, 16.0, 18.0, 25.0 for 24 groove plate and 15.0, 17.0, 24.0 for 30 groove plate at θ_2 angle and forward speed 1.0, 2.0, 3.0 km/h respectively. In S_3 seed, 19.0, 22.0, 30.0 for 18 groove plate, 18.0, 21.0, 29.0 for 24 groove plate and 17.0, 20.0, 28.0

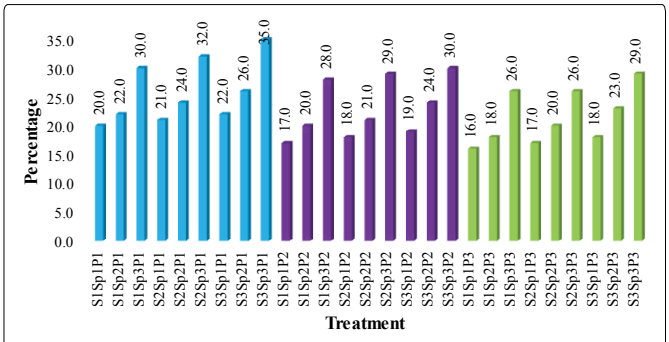

Figure 10: Effect of seed treatment, forward speed and type of seed metering plate on onion seeds degree of variation at inclination angle of plate 50°.

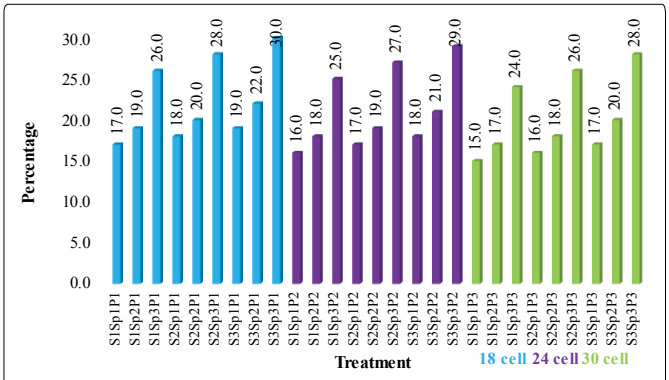

Figure 11: Effect of seed treatment, forward speed and type of seed metering plate on onion seeds degree of variation at inclination angle of plate 45°.

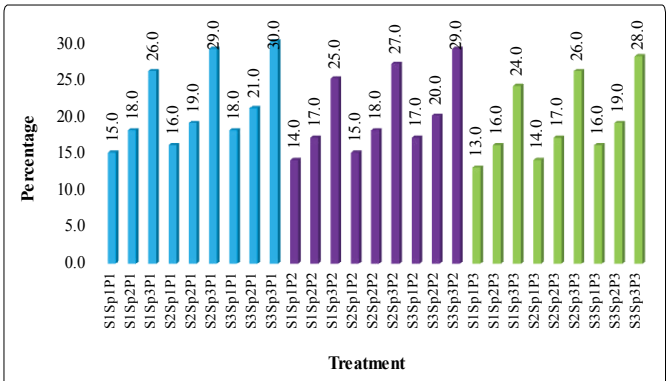

Figure 12: Effect of seed treatment, forward speed and type of seed metering plate on onion seeds degree of variation at inclination angle of plate 40°.

for 30 groove plate respectively.

Seed damage

The seed damage was estimated based on germination and visual damage and bruising. Bruising of pelleted seeds can be observed by visual observation of the samples used in the laboratory experiments on visual observation of the samples of S_1, S_2, and S_3 no prominent bruises were observed. There can be no damage to the seeds which are encapsulated within the outer coating in case of pelletized seeds.

Selection of metering mechanism

Final selection of metering system for onion planter based on the results of laboratory evaluation, with 24 groove seed metering plate with 45° inclination angle and a forward speed of 2.0 km/h was selected for the field evaluation. Missing index at 2.0 km/h forward speed with 24 groove seed metering plate with 45° inclination angle was 5.0% and multiple index was 11.0%. The overall quality of feed index obtained with these parameters was 84.0% which is maximum when compared with other seed treatments, forward speeds and types of seed metering plate combinations with different angle of inclination of plates.

Conclusions

The inclination of metering device statistically influenced the average seed spacing and performance indices, followed by cell number, inclination angle, speed of operation and seed treatment. Highest feed index of 84.0% was obtained at plate inclination of 45°, with 2 km/h speed of operation and 24 groove cell number when tested for S_3 seed. Metering plate with 24 groove cells at 45° inclination at 2 km/h speed of metering device gave best performance with S_3 seed, and thus recommended.

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Sreejith et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00074.4

Vegetos- An International Journal of Plant Research

Research Article

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Microhabitat Diversity in a Lateritic Hillock of Northern Kerala, India

Sreejith KA*, Prashob P, Sreekumar VB, Manjunatha HP and Prejith MP

Abstract

Lateritic hillocks of Kerala which are often considered as 'wastelands' in remote sensing images due to devoid of vegetation were analyzed for its floral wealth and microhabitat diversity. The study identified 9 microhabitats in the study area which supports 263 angiosperms in which majority are distributed in specific microhabitats. Floral inventory and comparison of microhabitats using Sorensen's Similarity Index indicates that each microhabitat was characterized by a specific group of species. The result showed the Shannon diversity indices ranged from 2.11 to 3.64, the Simpson index ranged from 0.0324 to 0.1515 and the Species richness index ranged from 0.0736 to 0.3369 in different microhabitats. Plants on the plateaus are adapted to various microhabitats and each of these microhabitats is unique in its edaphic properties, water availability and species composition. A detailed long-term quantitative study on the vegetation dynamics of each microhabitat is essential to understand the structure, composition and function of each microhabitat and the landscape as a whole. The baseline information generated on the vegetation and microhabitats of lateritic hills of Northern Kerala can be used to develop conservation and restoration activities on these hills which are highly threatened due to habitat degradation, fragmentation, laterite mining and change in land-use pattern.

Keywords

Lateritic hillocks; Lateritic flora; Microhabitat diversity; Kavvayi river basin; Northern Kerala

Introduction

The environment of Northern Kerala is governed by its peculiar geographic features. A unique feature of this region is the presence of lateritic hillocks which give fascinating undulations for the midland terrain in particular. Such hillocks are present up to Malappuram district towards south and they are spread to South Karnataka towards North. The lateritic hills are the most imposing but extremely threatened topographical floristic and faunistic feature of Northern Kerala. The alteration of very wet and dry condition creates an unusual ecological situation and varying microhabitats that supports unique biota. Plants on the plateaus are adapted to various microhabitats and each of these microhabitats is unique in its edaphic properties, water availability and species composition. Owing to the scarcity of woody species or forest cover the plateaus appear devoid of vegetation in remote sensing images and often considered as 'waste lands' but in reality they are landscape units having high biodiversity value and ecological significance. In monsoon, the impermeable nature of the hard rock surfaces leads to water logging and creation of ephemeral wetlands [1]. Plants species commonly seen in these areas are showing high specificity towards their microhabitat. Some species are capable of growing in very closely related microhabitats were as some are seen in two or more different microhabitats. But the species that grow in many different microhabitats having less dominance when comparing with the microhabitat specific plants. Most of the species are able to grow across a wide range of soil depths and slopes, although their dominance varies with difference in microhabitats. Regional floristic studies have reported the occurrence of many narrow-niched endemic and habitat specialist angiosperms from other lateritic plateaus [2-4] throughout the Western Ghats. The lateritic hills of Northern Kerala is also not an exception as indicated by 7 new angiosperm species reported in past couple of years by Ansari and Balakrishnan [5], Swapna et al. [6], Sunil et al. [7], Narayanan et al. [8], Sunil et al. [9] and Pradeep et al. [10]. Still, the 'wasteland' status supports the over exploitation or conversion of these important habitats for mining, monoculture plantation, industries and infrastructure development. This is happening throughout the study area and only a small portion of the lateritic hills remain un-disturbed in the study area. The management of pressures due to human impact is often misguided due to poor understanding about the special ecological features of the habitat [1]. The lateritic hillocks in the Northern Kerala are highly neglected areas from conservation point of view since they remain apparently barren for at least 7 months (November-May). Secondly, majority of the flora (90%) comprises herbaceous annuals and hence they are not considered important when compared to the woody green cover as in forest ecosystems. It has been noted that these hillocks differ completely from their surrounding vegetation in both physiognomy and floristic aspects. In spite of harsh environment, these hillocks are unique in having various micro habitat conditions and species which are habitat specific. Hence baseline scientific information regarding ecological significance of lateritic hills is needed to evolve strategies for conservation and management of this landscape especially when habitat destruction at its peak. This study is a part of collating baseline information to highlight the ecological significance of the lateritic hills of Northern Kerala, Western Ghats.

Materials and Methods

Study area

The present study was carried out in the lateritic hill of Madayipara (Figure 1) in Northern Kerala located between 12º2' North latitude and 75º16' East longitude respectively to document floral diversity and to classify and compare different microhabitats present in the hillock.

Floral inventory

A check-list of angiosperms in the study site has been prepared by visiting sites along with locality details including microhabitat. At least two field visits were made during every month for a period of one year from December 2012-November 2013. Each location was marked with GPS. Plant specimens were collected and identified

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Received: March 03, 2016 **Accepted:** July 30, 2016 **Published:** August 05, 2016

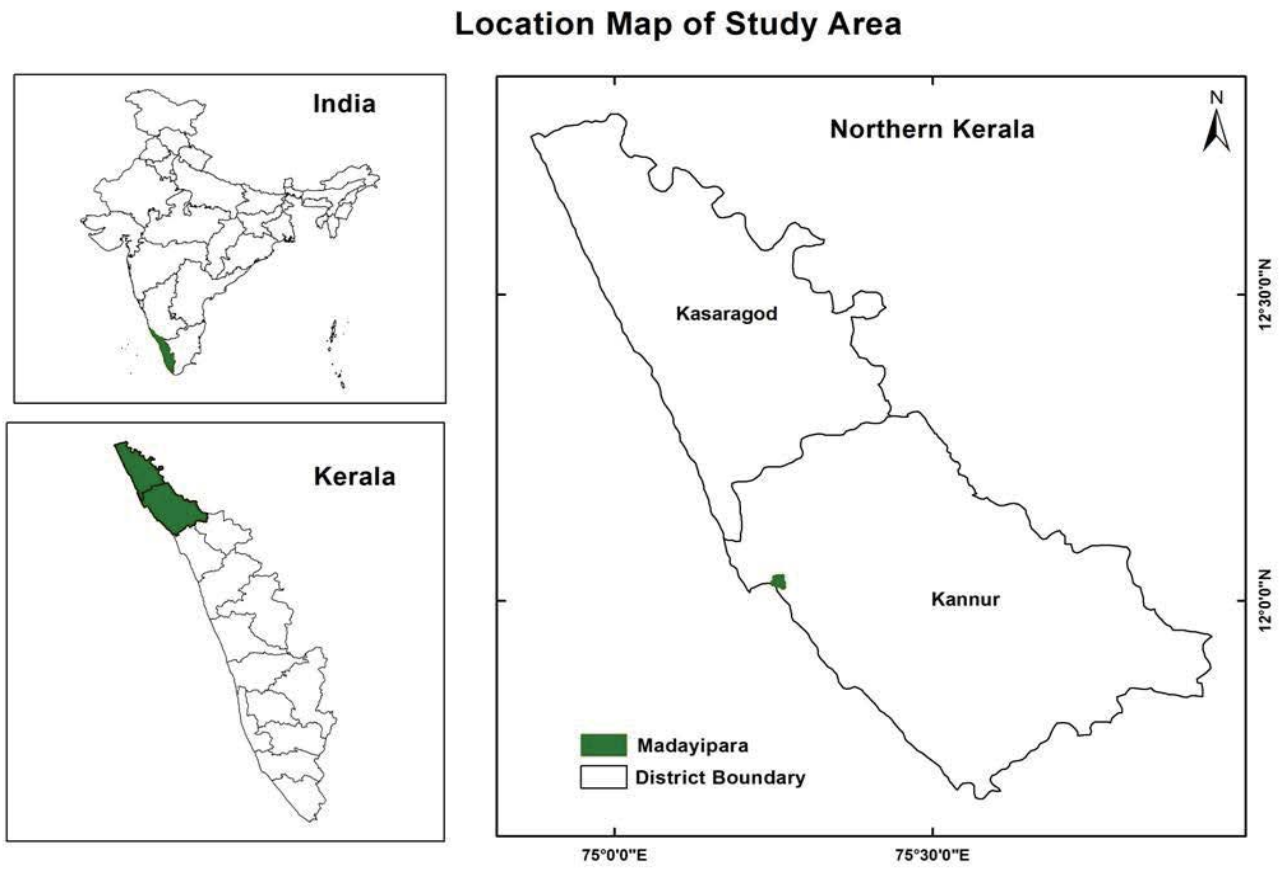


Figure 1: Study area.

using local and regional floras and deposited in KFRI herbarium.

Classification of microhabitats

The microhabitats were classified based on edaphic properties, water availability and species composition. All existing classifications attempted in other rocky plateaus of Western Ghats and other regions were referred while classifying microhabitats [11-15]. But the present classification is more site specific and does not suits exactly to any existing systems since there are factors especially species composition which are peculiar to these habitats. But the current and all the above microhabitat classifications on the rocky plateaus/hillocks are limited by the fact that there is no clear physical demarcation between the microhabitats. The microhabitats classified were further analyzed for their similarity in species composition by Sorensen's Similarity index.

In order to compare two communities we calculated the Sorensen's Similarity index is suggested by Sorensen [16].

Sorensen's Similarity index S=2C/(A+B)

Where S= Similarity index; A= number of species in sample; B= number of species in sample; C=number of species in sample common to A and B both.

Diversity index were calculated for diversity and species richness was determined by using Shannon-Wiener diversity index [17] and Menhinick's [18] species richness index as:

Species diversity index (H)=-Σ [(ni/N) log2 (ni/N)]

Where, H is Shannon –Weiner index, calculated to the base 2 of species diversity; n_i number of individual of a species i; N= total number of individual of all species in the community.

Species richness index (R)=S/ √N

Where, R= Menhinick's index of species richness; S= Number of a species in a collection; N= Number of individuals collected.

The index of dominance of the community was calculated by Simpson's index (Simpson [19])

Index of dominance (C)=Σ [ni/N] ^{2}

Where, C= Simpson's index of dominance; ni=number of individuals of species i; N= total number of individuals of all species in the community.

Results and Discussions

Floral inventory

A total of 263 angiosperm species were recorded from the study area in which 62 are endemic to Western Ghats. It is interesting and noteworthy that these species were distributed in 9 microhabitats and majority of them shows very narrow ecological niche (Table 1). Four species (*Eriocaulon heterolepis, Utricularia cecilii, Eriocaulon cuspidatum and Impatiens minor*) found to be distributed in more than 5 microhabitats with a wide ecological niche as indicated by its presence in different microhabitats (Table 1).

Table 1: Species distribution pattern in different microhabitats of Lateritic hills of Northern Kerala.

No	Species Name	Microhabitats									Presence of Species*
		B	CEC	EFV	ERS	RCF	SEP	SFD	SRA	TCTA	
1	*Abrus precatorius L.*									*	1
2	*Abutilon indicum (L.) Sweet.*									*	1
3	*Acacia caesia (L.) Willd.*									*	1
4	*Acacia pennata (L.) Willd.*									*	1
5	*Acalypha indica L.*									*	1
6	*Acalypha paniculata Miq.*									*	1
7	*Acampae praemorsa (Roxb.) Blatt. & McCann.*									*	1
8	*Acanthospermum hispidum DC.*									*	1
9	*Achyranthes aspera L.*									*	1
10	*Achyranthes bidentata Blume.*									*	1
11	*Aegle marmelos (L.) Correa.*									*	1
12	*Aeschynomene americana L.*									*	1
13	*Aeschynomene aspera L.*									*	1
14	*Aganosma cymosa (Roxb.) G. Don*									*	1
15	*Ageratum conyzoides (L.) L.*									*	1
16	*Aglaia elaeagnoidea (A.Juss.) Benth.*									*	1
17	*Alangium alpinum (C.B.Clarke) W.W.Sm. & Cave.*									*	1
18	*Allamanda cathartica L.*									*	1
19	*Alloteropsis cimicina (L.) Stapf .*			*							1
20	*Alloteropsis semialata (R.Br.) Hitchc.*			*							1
21	*Alseodaphne semecarpifolia Nees.*									*	1
22	*Alstonia scholaris (L.) R. Br.*									*	1
23	*Alternanthera bettzickiana (Regel) G.Nicholson.*									*	1
24	*Alternanthera brasiliana (L.) Kuntze.*									*	1
25	*Alysicarpus bupleurifolius (L.) DC.*									*	1
26	*Amaranthus spinosus L.*									*	1
27	*Amaranthus viridis L.*									*	1
28	*Amorphophallus paeoniifolius (Dennst.) Nicolson.*									*	1
29	*Ampelocissus latifolia (Roxb.) Planch.*									*	1
30	*Anamirta cocculus (L.) Wight & Arn.*									*	1
31	*Ancistrocladus heyneanus Wall. ex J.Graham.*									*	1
32	*Anisomeles indica (L.) Kuntze.*									*	1
33	*Antidesma acidum Retz.*									*	1
34	*Antidesma bunius (L.) Spreng.*									*	1
35	*Apluda mutica L.*									*	1
36	*Apocopis mangalorensis (Hochst. ex Steud.) Henrard.*									*	1
37	*Ariopsis peltata Nimmo.*	*				*					2
38	*Aristolochia indica L.*									*	1
39	*Asystasia dalzelliana Santapau*									*	1
40	*Asystasia gangetica (L.) T.Anderson*									*	1
41	*Atlantia wightii Tanaka.*									*	1
42	*Axonopus compressus (Sw.) P.Beauv.*									*	1
43	*Azadirachta indica A.Juss.*									*	1
44	*Barleria cristata L.*									*	1
45	*Barleria prionitis L.*									*	1
46	*Begonia crenata Dryand*	*	*			*					3
47	*Begonia integrifolia Dalzell*	*									1
48	*Begonia malabarica Lam.*	*									1
49	*Begonia trichocarpa Dalzell.*	*									1
50	*Benkara malabarica (Lam.) Tirveng.*									*	1
51	*Bhidea fischeri Sreek. & B.V. Shetty.*									*	1

52	*Bidnes pilosa (Blume) Sherff.*									*	1
53	*Biophytum reinwardtii (Zucc.) Klotzsch.*			*							1
54	*Blumea membranacea DC.*									*	1
55	*Blumea oxyodonta DC.*									*	1
56	*Blyxa octandra (Roxb.) Planch. ex Thwaites.*									*	1
57	*Boerhavia diffusa L.*								*		1
58	*Bombax ceiba L.*									*	1
59	*Brachiaria reptans (L.) C.A.Gardner & C.E.Hubb.*									*	1
60	*Breynia retusa (Dennst.) Alston.*								*		1
61	*Breynia vitis-idaea (Burm.f.) C.E.C.Fisch.*									*	1
62	*Bridelia retusa (L.) A.Juss.*									*	1
63	*Bridelia stipularis (L.) Blume.*									*	1
64	*Bulbophyllum neilgherrense Wight.*									*	1
65	*Burmannia coelestis D.Don.*									*	1
66	*Butea monosperma (Lam.) Taub.*									*	1
67	*Cajanus scarabaeoides (L.) Thouars.*									*	1
68	*Callicarpa tomentosa (L.) L.*									*	1
69	*Calopogonium mucunoides Desv.*									*	1
70	*Calotropis gigantea (L.) Dryand.*									*	1
71	*Calycopteris floribunda (Roxb.) Lam. ex Poir.*									*	1
72	*Canavalia gladiata (Jacq.) DC.*									*	1
73	*Canscora diffusa (Vahl) R.Br. ex Roem. & Schult.*									*	1
74	*Canscora pauciflora Dalzell.*									*	1
75	*Cansjera rheedei J.F.Gmel.*									*	1
76	*Canthium coromandelicum (Burm.f.) Alston.*									*	1
77	*Capparis rheedei DC.*									*	1
78	*Capparis sepiaria L.*									*	1
79	*Capparis zeylanica L.*									*	1
80	*Capsicum annuum L.*									*	1
81	*Capsicum frutescens L.*									*	1
82	*Caralia brachiatia (Lour.) Merr.*									*	1
83	*Cardiospermum halicacabum L.*								*		1
84	*Careya arborea Roxb.*									*	1
85	*Cassia fistula L.*									*	1
86	*Catunaregam spinosa (Thunb.) Tirveng.*									*	1
87	*Celosia argentea L.*									*	1
88	*Cinnamomum malabatrum (Burm.f.) J.Presl.*									*	1
89	*Cissus latifolia Lam.*									*	1
90	*Cleome rutidosperma DC.*									*	1
91	*Cleome viscosa L.*									*	1
92	*Clerodendrum infortunatum L.*								*	*	2
93	*Clitoria ternatea L.*								*	*	2
94	*Commelina benghalensis L.*								*		1
95	*Commelina diffusa Burm.f.*			*	*			*	*		4
96	*Commelina erecta L.*			*	*			*	*	*	5
97	*Connarus wightii Hook.f.*								*	*	2
98	*Cosmos caudatus Kunth*								*	*	2
99	*Croton roxburghii Wall.*								*	*	2
100	*Curculigo orchioides Gaert.*								*		1
101	*Cyclea peltata (Lam.) Hook.f. & Thomson.*								*	*	2
102	*Cymbopogon citratus (DC.) Stapf.*								*	*	2
103	*Cynodon dactylon (L.) Pers.*								*	*	2
104	*Dalbergia horrida (Dennst.) Mabb.*								*	*	2
105	*Delonix regia (Hook.) Raf.*									*	1
106	*Derris scandens (Roxb.) Benth.*									*	1
107	*Desmodium gangeticum (L.) DC.*									*	1
108	*Desmodium heterophyllum (Willd.) DC.*			*							1

109	*Desmodium triflorum (L.) DC.*			*							1
110	*Dopatrium junceum (Roxb.) Buch.-Ham. ex Benth.*						*				1
111	*Drosera indica L.*			*							1
112	*Eragrostis nigra Nees ex Steud.*			*					*		2
113	*Eragrostis unioloides (Retz.) Nees ex Steud.*			*					*		2
114	*Eriocaulon cuspidatum Dalzell.*			*	*	*	*	*	*		6
115	*Eriocaulon heterolepis Steud.*		*	*	*	*	*	*	*		7
116	*Eriocaulon lanceolatum Miq. ex Körn*							*			1
117	*Eriocaulon lanceolatum Miq. ex Körn.*				*						1
118	*Eriocaulon madayiparense.*		*	*			*				3
119	*Eriocaulon parviflorum (Fyson) R.Ansari & N.P.Balakr.*				*			*			2
120	*Eriocaulon quinquangulare L.*		*	*							2
121	*Eriocaulon robustobrownianum Ruhland.*		*	*			*	*			4
122	*Eriocaulon sexangulare L.*			*			*	*	*		4
123	*Eriocaulon stellulatum Körn.*			*			*	*	*		4
124	*Eriocaulon truncatum Buch.-Ham. ex Mart.*			*			*	*			3
125	*Eriocaulon xeranthemum Mart.*			*			*	*	*		4
126	*Erycibe paniculata Roxb.*									*	1
127	*Erythrina variegata L.*									*	1
128	*Evolvulus alsinoides (L.) L.*								*		1
129	*Ficus arnottiana (Miq.) Miq.*									*	1
130	*Ficus benghalensis L.*									*	1
131	*Ficus callosa Willd.*									*	1
132	*Ficus exasperata Vahl .*									*	1
133	*Ficus religiosa L.*									*	1
134	*Flacourtia indica (Burm. f.) Merr.*								*		1
135	*Flueggea virosa (Roxb. ex Willd.) Royle .*									*	1
136	*Gloriosa superba L.*									*	1
137	*Glycosmis mauritiana (Lam.) Tanaka*									*	1
138	*Glycosmis pentaphylla (Retz.) DC.*									*	1
139	*Grewia nervosa (Lour.) Panigrahi .*									*	1
140	*Gymnema sylvestre (Retz.) Schult.*								*	*	2
141	*Habenaria longicorniculata J.Graham .*							*	*		2
142	*Habenaria periyarensis Sasidh., K.P.Rajesh & Augustine.*							*	*		2
143	*Haplanthodes nilgherrensis (Wight) R.B.Majumdar.*									*	1
144	*Heliotropium indicum L.*									*	1
145	*Heliotropium keralense Sivarajan & Manilal.*									*	1
146	*Heliotropium marifolium J.König ex Retz.*			*		*		*	*		4
147	*Hemidesmus indicus (L.) R. Br. ex Schult.*								*	*	2
148	*Hippocratea arnottiana Wight.*									*	1
149	*Holarrhena pubescens Wall.*								*		1
150	*Holigarna arnottiana Hook.f.*									*	1
151	*Hopea fastigiata (Griseb.) C.B.Clarke*									*	1
152	*Hopea ponga (Dennst.) Mabb.*									*	1
153	*Hugonia mystax Cav.*									*	1
154	*Hydnocarpus pentandrus (Buch.-Ham.) Oken.*									*	1
155	*Hyptis suaveolens (L.) Poit.*								*	*	2
156	*Ichnocarpus frutescens (L.) W.T.Aiton*								*		1
157	*Impatiens dasysperma Wight.*			*				*	*		3
158	*Impatiens diversifolia B.Heyne.*	*	*						*		3
159	*Impatiens minor (DC.) S.M. Almeida.*	*	*	*		*		*	*		6
160	*Impatiens scapiflora B.Heyne.*	*									1
161	*Ipomoea cairica (L.) Sweet.*									*	1
162	*Ipomoea hederifolia L.*									*	1

163	*Ixora brachiata Roxb.*									*	1
164	*Ixora coccinea L.*									*	1
165	*Jasminum azoricum L.*									*	1
166	*Jasminum malabaricum Wight*									*	1
167	*Jatropha curcas L.*									*	1
168	*Justicia adhatoda L.*									*	1
169	*Justicia ekakusuma Pradeep & Sivar.*									*	1
170	*Justicia japonica Thunb.*			*							1
171	*Kyllinga nemoralis (J.R.Forst. & G.Forst.) Dandy ex Hutch. & Dalziel.*					*					1
172	*Lannea coromandelica (Houtt.) Merr.*									*	1
173	*Lantana camara L.*								*	*	2
174	*Leea indica (Burm. f.) Merr.*									*	1
175	*Leea macrophylla Roxb. ex Hornem.*									*	1
176	*Lepidagathis keralensis Madhus. & N.P.Singh .*			*	*	*		*		*	5
177	*Leucas aspera (Willd.) Link .*								*	*	2
178	*Leucas biflora (Vahl) R.Br. ex Sm.*									*	1
179	*Lindernia hyssopoides (L.) Haines.*									*	1
180	*Mallotus philippensis (Lam.) Müll.Arg.*									*	1
181	*Mallotus repandus (Willd.) Müll.Arg.*									*	1
182	*Mangifera indica L.*									*	1
183	*Melastoma malabathricum L.*									*	1
184	*Memecylon randerianum SM & MR Almeida*									*	1
185	*Memecylon umbellatum Burm. f.*								*	*	2
186	*Merremia tridentata (L.) Hallier f.*									*	1
187	*Mimusops elengi L.*									*	1
188	*Mitracarpus hirtus (L.) DC.*									*	1
189	*Mollugo pentaphylla L.*									*	1
190	*Mukia maderaspatana (L.) M.Roem.*									*	1
191	*Murdannia crocea (Griff.) Faden .*				*			*			2
192	*Murdannia nudiflora (L.) Brenan.*			*							1
193	*Murdannia semiteres (Dalzell) Santapau.*			*							1
194	*Naregamia alata Wight & Arn.*							*	*		2
195	*Neanotis hohenackeri P.Daniel & Vajr.*			*	*			*	*		4
196	*Neanotis rheedei (Wight & Arn.) W.H.Lewis.*				*	*		*			3
197	*Neanotis tubulosa (G.Don) Mabb.*					*					1
198	*Neuropeltis malabarica Ooststr.*									*	1
199	*Nymphoides indica (L.) Kuntze.*						*				1
200	*Nymphoides krishnakesara K.T.Joseph & Sivar.*						*				1
201	*Oldenlandia corymbosa L.*									*	1
202	*Oldenlandia corymbosa L.*					*					1
203	*Olea dioica Roxb.*									*	1
204	*Parasopubia delphiniifolia (L.) H.-P.Hofm. & Eb.Fisch.*			*				*	*		3
205	*Paspalum conjugatum P.J.Bergius.*			*				*	*		3
206	*Paspalum scrobiculatum L.*			*				*	*		3
207	*Phyla nodiflora (L.) Greene.*									*	1
208	*Piper nigrum L.*									*	1
209	*Plumbago zeylanica L.*									*	1
210	*Pogostemon deccanensis (Panigrahi) Press.*							*	*	*	3
211	*Pogostemon quadrifolius (Benth.) F.Muell.*									*	1
212	*Polyalthia korintii (Dunal) Benth. & Hook.f. ex Hook.f. & Thoms.*								*	*	2
213	*Polycarpaea corymbosa (L.) Lam.*			*	*	*		*			4
214	*Polygala chinensis L.*			*							1
215	*Polygala elongata Klein ex Willd.*			*						*	2
216	*Pongamia pinnata (L.) Pierre.*								*	*	2
217	*Pseuderanthemum malabaricum Gamble.*								*	*	2
218	*Pterospermum diversifolium Blume, Bijdr.*								*	*	2

219	*Rotala malabarica Pradeep, K.T.Joseph & Sivar.*						*				1
220	*Rotala malampuzhensis R.V. Nair ex Cook.*						*	*	*		3
221	*Rotala rosea (Poir.) C.D.K. Cook ex H. Hara .*						*				1
222	*Samanea saman (Jacq.) Merr.*								*	*	2
223	*Santalum album L.*								*	*	2
224	*Sapindus trifoliatus L.*								*	*	2
225	*Schleichera oleosa (Lour.) Merr.*								*	*	2
226	*Scoparia dulcis L.*								*	*	2
227	*Sesamum indicum L.*								*	*	2
228	*Sesamum radiatum Schumach. & Thonn.*								*	*	2
229	*Sida acuta Burm.f.*								*	*	2
230	*Sida cordifolia L.*								*	*	2
231	*Sida rhombifolia L.*								*	*	2
232	*Smilax zeylanica L.*								*	*	2
233	*Solanum torvum Sw.*								*	*	2
234	*Stachytarpheta jamaicensis (L.) Vahl.*								*	*	2
235	*Striga angustifolia (D. Don) C.J. Saldanha.*			*				*			2
236	*Striga asiatica (L.) Kuntze*			*				*			2
237	*Striga gesnerioides (Willd.) Vatke*			*				*	*		3
238	*Strychnos nux-vomica L.*									*	1
239	*Symphorema involucratum Roxb.*									*	1
240	*Synedrella nodiflora (L.) Gaertn.*									*	1
241	*Syzygium caryophyllatum (L.) Alston.*									*	1
242	*Syzygium cumini (L.) Skeels.*									*	1
243	*Tabernaemontana alternifolia L.*									*	1
244	*Tamarindus indica L.*									*	1
245	*Tectona grandis L.f.*									*	1
246	*Terminalia bellirica (Gaertn.) Roxb.*									*	1
247	*Tinospora cordifolia (Willd.) Miers .*									*	1
248	*Trema orientalis (L.) Blume.*									*	1
249	*Trianthema portulacastrum L.*									*	1
250	*Tridax procumbens (L.) L.*									*	1
251	*Tylophora indica (Burm. f.) Merr.*									*	1
252	*Utricularia aurea Lour.*			*				*	*		3
253	*Utricularia caerulea L.*		*	*				*	*		4
254	*Utricularia cecilii P.Taylor.*	*	*	*	*		*	*	*		7
255	*Utricularia graminifolia Vahl*		*	*			*	*	*		5
256	*Utricularia reticulata Sm.*			*				*			2
257	*Utricularia striatula Sm.*			*							1
258	*Utricularia uliginosa Vahl.*			*							1
259	*Uvaria narum (Dunal) Wall. ex Hook.f. & Thoms.*									*	1
260	*Vernonia cinerea (L.) Less.*									*	1
261	*Vitex negundo L.*									*	1
262	*Wrightia tinctoria R.Br.*									*	1
263	*Ziziphus oenopolia (L.) Mill.*								*	*	2

Note: *indicates the total number of microhabitats where particular species recorded

EFV: Ephemeral flush Vegetation, ERS=Exposed rock Surfaces, RCF=Rock crevices/fissures, SEP=Small ephemeral pools, SFD=Soil-filled depressions, TCTA=Tree cover and tree associated, B=Boulders, CEC=Crust edges or cliffs, SRA=Soil-rich Areas.

Classification of microhabitats

Plants on the plateaus are adapted to various microhabitats and each of these microhabitats is unique in its edaphic properties, water availability and species composition. The most common Microhabitats types on the lateritic hillock plateaus have been described along with characteristic species assemblages (Figure 2, Table 1).

Ephemeral flush vegetation: It occurs on rocks where water seeps continuously through the rainy season and soil deposition is negligible (Figure 2a). It occupies a large area on plateaus, colonized predominantly by *Desmodium heterophyllum, Desmodium triflorum, Eriocaulon cuspidatum, Drosera indica, Utricularia graminifolia* etc. We could record 45 species in this particular microhabitat, in which 14 species specifically confined to this microhabitat (Table 1).

Exposed rock surfaces: They are flat or uneven rock surfaces, exposed to direct sunlight. They may gradually get covered by grasses during monsoon (Figure 2b). Some common plants of the habitat are. *Polycarpaea corymbosa, Lepidagathis keralensis, Eriocaulon cuspidatum, Neanotis rheedei* etc. We could record 13 species in this

(a): Laterite hill at Madayipara

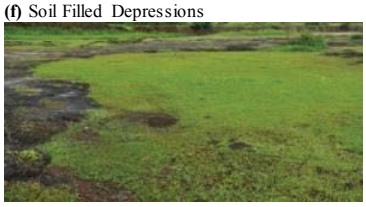
(b) Ephemeral Flush Vegetation

(c) Exposed Rock Surfaces

(d) Rock Crevices or Fissures

(e) Small Ephemeral Pools

(f) Soil Filled Depressions



(g) Soil Rich Areas

(h) Tree Cover and Tree Associates

(i) Boulders

(j) Crust Edges or Cliffs

Figure 2: The most common Microhabitats types on the lateritic hillock plateaus.

particular microhabitat in which 2 species specifically confined to this microhabitat (Table 1).

Rock crevices/fissures: They are frequently found on lateritic plateaus by providing a unique ecological niche specific to small number of species (Figure 2c). Many species such as *Begonia crenata, Heliotropium marifolium, Polycarpaea corymbosa*, *Impatiens minor*, etc. could list from this microhabitat. We could record 12 species in this particular microhabitat, in which 3 species specifically confined to this microhabitat (Table 1).

Small Ephemeral Pools (SEP): They are shallow depressions which remain filled with water during monsoon (Figure 2d). Since there is hardly any soil deposition plateau crust can be seen easily. The dominant species in this microhabitat includes *Dopatrium junceum, Rotala malabarica* and *Nymphoides krishnakesara*. We could record 16 species in this particular microhabitat in which 5 species specifically confined to this microhabitat (Table 1).

Soil-Filled Depressions (SFD): A specific microhabitat which are depressions that accumulate soil and water (Figure 2e). *Eriocaulon cuspidatum, Curculigo orchioides, Utricularia graminifolia*, etc. are the most common species found here. We could record 35 species in this particular microhabitat in which 1 species specifically confined to this microhabitat (Table 1).

Soil-Rich Areas (SRA): These are soil-rich microhabitats with more than 20 cm soil-thickness (Figure 2f). The gaps left between mats are mainly occupied by species *Commelina diffusa, Commelina erecta, Heliotropium marifolium,* etc. We could record 69 species in this particular microhabitat in which 9 species specifically confined to this microhabitat (Table 1).

Tree Cover and Tree Associated (TCTA): This microhabitat is entirely different from the rest simply because the dominance of tree species (Figure 2g). These are soil-rich areas of plateaus where tree could grow and survive. The covered shady areas provide a habitat which is entirely different from the harsh extreme environmental conditions prevailing on the exposed surfaces of plateaus. *Ficus arnottiana, Holigarna arnottiana, Hugonia mystax, Bridelia retusa, Benkara malabarica,* etc. are commonly found in this area. We could record 193 species in this particular microhabitat in which 156 species specifically confined to this microhabitat (Table 1).

Boulders (B): They are large rocks either isolated or in groups (Figure 2h). They are usually covered by lichens and bryophytes. Some typical angiosperm species also found to be associated with the systems which include *Begonia crenata, Begonia malabarica, Utricularia cecilii, Ariopsis peltata,* etc. We could record 9 species in this particular microhabitat in which 4 species specifically confined to this microhabitat (Table 1).

Crust Edges or Cliffs (CEC): They are edges of the plateaus mainly inhabited species such as *Eriocaulon cuspidatum, Begonia crenata, Ariopsis peltata,* etc. (Figure 2i). We could record 10 species in this particular microhabitat (Table 1).

When we analyzed these communities for their similarity in species composition, the index varied from 0-0.71. A total of 36 combinations were possible when 9 communities were compared between each other for their similarity in species composition, but interestingly only 2 found to have more than 50% similarity (Table 2) which clearly indicates the diversity of habitats within the lateritic hillock. On the other hand, 2 microhabitats found 100% dissimilar (Table 2). This diversity in habitats and seasonality supports unique plant community in each habitat which further supports associated faunal elements and responsible for the high species diversity of the lateritic hills. Diversity is further supported by change in species composition and community within microhabitat due to seasonal fluctuations. Barren Rock (B) and Tree cover and Tree Associates (TCTA) were found to be the two communities which show more dissimilarity with remaining seven communities. This also indicates the succession pattern in the laterite hillock in which Barren Rock (B) represents the pioneer community and Tree cover and Tree Associates (TCTA) represent the climax community. A detailed study supported by quantitative information on each community may reveal the role and level of remaining communities in the process of succession and their role in the landscape.

The Shannon diversity index (H) is ranged from 2.11 to 3.64 (Table 2). The highest Shannon diversity index was recorded in Soil Rich Areas (3.64) followed by Ephemeral Flush Vegetation (3.51), Soil Filled Depressions (3.29), Small Ephemeral Pools (2.56), Exposed Rock Surfaces (2.25), Crust Edges (2.19), and Rock Crevices (2.15) and Boulders (2.11). The Simpson index of dominance ranged from 0.0324 to 0.1515 (Table 2). The highest Simpson index was recorded in Rock Crevices (0.1515) followed by Boulders (0.1304), Exposed Rock Surfaces (0.1296), Crust Edges (0.1288), Small Ephemeral Pools (0.0951), Soil Filled Depressions (0.0489), Ephemeral Flush Vegetation (0.0380) and Soil Rich Areas (0.0324). The species richness index in different microhabitat of study area ranged from 0.0736 to 0.3369 (Table 3). The highest species richness index (R) was recorded in Soil Rich Areas (0.3369) followed by Soil Filled Depressions (0.1486), Ephemeral Flush Vegetation (0.1447), Small Ephemeral Pools (0.1016), Crust Edges (0.0862), Rock Crevices (0.0809), Boulders (0.0752) and Exposed Rock Surfaces (0.0736).

Discussion

The lateritic hillocks in Northern Kerala lack proper substrate (soil) and exhibit extreme climatic conditions. This type of environment usually share a series of stressful characteristics, such as high UV exposure, daily thermal variation, constant winds, high evapotranspiration, low water retention and impermeable soils [12]. Plant species commonly seen in the microhabitats have been described by Watve [14] and Lekhak ans Yadav [15]. The general vegetation is similar to ephemeral communities from granitic rock outcrops-Inselbergs and iron rich plateaus-in East and West Africa and Brazil [13,20,21]. Presence of *Cyanotis, Neanotis, Murdannia, Drosera, Utricularia, Lindernia, Burmannia, Fimbristylis, Rhamphicarpa* matches with that on paleotropical inselbergs described by Porembski and Brown [22] and Dörrstock et al. [23].

Table 2: Similarity index value between microhabitats in a lateritic hillock, of Northern Kerala.

	EFV	ERS	RCF	SEP	SFD	TCTA	B	CEC	SRA
EFV		0.26	0.13	0.36	0.71[a]	0.071	0.1	0.31	0.37
ERS			0.24	0.19	0.54[a]	0.073	0.09	0.17	0.14
RCF				0.12	0.39	0.055	0.26	0.25	0.07
SEP					0.043	0.045	0.074	0.42	0.25
SFD						0.025	0.011	0.26	0.47
TCTA							0[b]	0[b]	0.26
B								0.42	0.125
CEC									0.14
SRA									

Note: [a] microhabitats which having more than 50% similarity; [b] microhabitat which are 100% dissimilar

Table 3: Species Number, Shannon Diversity Index (H), Simpson Index (C) and Species Richness Index (R) of different microhabitats in a Lateritic Hillock, Northern Kerala.

Microhabitat	No. of Species	Shannon Index	Simpson Index	Species Richness Index
EFV	45	3.51	0.0380	0.1447
ERS	13	2.25	0.1296	0.0736
RCF	12	2.15	0.1515	0.0809
SEP	16	2.56	0.0951	0.1016
SFD	35	3.29	0.0489	0.1486
SRA	69	3.64	0.0324	0.3369
B	9	2.11	0.1304	0.0752
CEC	10	2.19	0.1288	0.0862

The dominance of therophytes is possibly due to their greater ability to survive under harsh environmental conditions. Due to their short life-cycle and high reproduction rate, they are well-adapted to extreme environments and high levels of disturbances. Therophytes may face high risk of mortality if rain fails or if rain is followed by a drought as these species are highly susceptible to drought without specific adaptations. Early phase of monsoon (July) was period of vegetative growth of most species and very few species started flowering in this period. Late phase of monsoon (August end and September) was a period of characteristic mass blooming mainly of non-grass families (Fabaceae, Eriocaulaceae, Rubiaceae) observed on rocky plateaus. Species richness and species diversity peaked at this period as most species of forbs and grasses completed their growth during this period. The presence of 263 species of angiosperms clearly indicates the ecological significance of lateritic hills and associated microhabitats. The conservation of these microhabitats is essential for survival and protection of these species which having specific ecological niche associated with the microhabitats of this lateritic hills. Due to its wide ecological niche, they are comparatively at lower level of risk since it could survive in varying microhabitats successfully. Further species specific quantitative studies are required to understand the population, regeneration and threat status of species especially those who are in narrow ecological niche. Seasonal fluctuations influence major changes in community structure and composition in majority of microhabitats, hence a quantitative study on the vegetation dynamics of each microhabitat along with microclimatic data is essential to understand the structure, composition, function and dynamics of the system and the landscape as a whole. Plant communities on the hillocks are continuously changing with respect to changing regimes of the climate. The growing season starts with the dominance of ephemerals and this was later replaced by perennials. Both the number of species and the number of individuals declined after a peak at the beginning of the growing season. Similar pattern is observed on the plateaus in India. The season starts with annuals which are mainly grasses and ends with perennials. Harsh environmental conditions on the lateritic hillocks have given rise to plants with certain traits that allow them to overcome environmental adversities. These traits help the plants to overcome major environmental stresses such as drought, high temperature and light intensities and nutrient deficiency.

Conclusion

Current study proved that the lateritic hills are characteristic with their diverse microhabitat and associated species diversity. The baseline information on microhabitats and floristic composition of a lateritic hill in Northern Kerala generated through this study revealed the biological and ecological significance of lateritic hills. Currently these systems are undergoing severe degradation, destruction, alteration through various activities such as mining, shifting to mono culture plantation etc. and there is no effective conservation and management activities are exists. Hence, the baseline information provided here on the biological and ecological significance of lateritic hills may further helps to develop strategies for conservation and management of this ecologically fragile system found in a human dominated landscape. Further detailed ecological and species inventory studies are required to understand the ecological process within the microhabitat and between microhabitats of lateritic hills of Northern Kerala.

Acknowledgement

We are thankful to the Kerala State Council for Science, Technology & Environment, Government of Kerala for providing financial support. We acknowledge the service of Mr. Alex CJ, Research Fellow, KFRI for preparing study area map. We also acknowledge Dr. Sankaran KV, Former Director and Director, Kerala Forest Research Institute for their support, encouragement and facilities provided.

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Fard et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00075.6

Vegetos- An International Journal of Plant Research

Research Article

A SCITECHNOL JOURNAL

Response of Six Turf Species to Cadmium Stress: Displaying the Most Tolerant Cultivar

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Abstract

This study was accomplished to monitor the interspecific differences in response to cadmium (Cd) stress among six turfgrass species through investigating the seed germination, seedling growth and some biochemical characteristics. After an environmentally controlled experiment under different Cd treatments (0, 100, 200 and 300 µM), *Agropyron elongatum* showed the highest germination percentage (80.33%) under 300 µM Cd, followed by *Lollium perenne* with 72.59%. The lowest germination parameters including germination vigor, index and percentage were found in *Festuca ovina,* which referred as the more sensitive species to Cd than other studied turfgrasses. The highest shoot growth followed by the lowest chlorophyll degradation was observed in *Agropyron elongatum*, *Festuca rubra* and *Lolium perenne* respectively. Furthermore, the low chlorophyll degradation and the high seedling growth in turf species were correlated with increasing in some antioxidant enzymes activities. It might display the crucial indirect effect of Cd, that is, oxidative stress. Overall, *Agropyron elongatum* is introduced as the most tolerant cool-season turfgrass among studied species, which could be considered for engaging in advanced studies of heavy metal stresses.

Keywords

Agropyron; Antioxidant enzymes; *Festuca*, *Lollium*; *Poa*

Introduction

Contamination of soil and water with toxic metals is severe environmental problem [1]. Heavy metals are mainly considered as both natural and anthropogenic-induced stress, caused by weathering of rocks, industrial effluents and agricultural runoffs [2]. Cadmium (Cd) is one of the most phytotoxic heavy metals to plants because it is highly soluble in water and promptly taken up by plants, and can interfere with numerous biochemical and physiological processes including photosynthesis, respiration, nitrogen and protein metabolism, and nutrient uptake [3]. Since it is active at concentrations much lower than those of other toxic elements, Cd could greatly influence the plants germination and growth, such as decreasing the amount of chlorophyll, depressing photosynthesis, disturbing the uptake and translocation of mineral nutrients, dwarfing plants, inhibiting the growth of roots, decreasing biomass, and even leading to death in some severe cases [4,5].

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Received: June 10, 2016 **Accepted:** July 30, 2016 **Published:** August 08, 2016

It has been reported that seed germination and early seedling growth are crucial in plant life and are extremely responsive to the environmental alterations [6]. Delay in germination has been often observed after exposure to heavy metals [7]. This can be associated with disorders in germinating metabolism, which is a highly complex multistage process regulated at the physiological, biochemical and molecular programs [8]. Moreover, Cd as a non-redox metal is unable to participate in Fenton-type reactions, but it causes oxidative stress by generating reactive oxygen species [9]. It has also been reported that great variation exists in heavy metal accumulation and tolerance among plant species [10]. Therefore, selection or development of Cd tolerant genotypes through screening in germination as well as biochemical and physiological variations in plants under different concentrations of Cd would be promising approaches to decrease the flow of Cd from contaminated soils and water into the food chain and to ameliorate Cd-induced stress.

Turf, a plant with high irrigation requirement, is commonly used in landscapes at widespread scales throughout the world [11]. Several factors such as water deficiency, water pollution, and heavy metal-contaminated soils have strongly restricted the plant germination and even subsequent growth and development. Bidar et al. [12] reported that *L. perenne* was more sensitive than *Trifolium repens* to heavy metal toxicity. González [13] showed that some grass species such as *Festuca arundinacea*, *F. rubra genuina*, *F. rubra fallax* and *Lolium perenne* were able to hyper-accumulate Cu, Ni, Zn, Pb and Cd in their tissues, but the more tolerant species among them were remained unknown.

The cessation of seed germination and the growth retardation have been illustrated as a response to elevated concentrations of Cd in *Triticum aestivum* [14], *Poa pratensis L.* [15], *Oryza sativa L.* [16], *Sorghum bicolor L.* [17], *Peganum harmala* and *Halogeton glomeratus* [18], soybean [19], *Elymus dahuricus* [20], *F. arundinacea* and *P. pratensis* [21]. However, most studies about the effects of Cd on seed germination and growth of turfgrass species have been relied only on morphological parameters not biochemical ones.

Moreover, the role of antioxidant enzymes as determining factors in response of cool- season turfgrasses to various concentrations of Cd has not been well understood. Bidar et al. [12] evaluated only superoxide dismutase (SOD) activity in *L. perenne* as affected by Cd, Zn, and Pb. Xu and Wang [21] also reported only growth parameters in *P. pratensis* and *F. arundinacea* under Cd exposure.

So far, the analogy of tolerance/sensitivity of six commonly used cool-season turfgrasses, perennial ryegrass, tall fescue, blue sheep fescue, red fescue, kentucky bluegrass and agropyron, to Cd-induced stress, has not been reported. Therefore, the present study was accomplished to identify the most sustainable ground cover under toxic levels of Cd for use not only in heavy metal-contaminated soils, but also in cultivations which are irrigated using waste water.

Materials and Methods

Plant material

In the present study, the seeds of six turf species; perennial ryegrass (*Lollium perenne*), tall fescue (*Festuca aurandinaceae*), blue

sheep fescue (*Festuca ovina*), red fescue (*Festuca rubra*), kentucky bluegrass (*Poa pratensis*) and agropyron (*Agropyron elongatum*) were purchased from Pakan Bazr Company, Esfahan, Iran and took to the Horticultural laboratory of University of Guilan, Iran.

Germination experiments

The first seeds were surface-sterilized using 5% (v/v) sodium hypochlorite for 10 min., followed by thoroughly rinsing with sterile deionized water and then allowed to imbibe for 3 h. After water imbibition, the seeds were placed into Petri dishes (90 mm diameter) containing sterile filter sheets (Whatman No. 42) moistened with either 6 mL of distilled water as a control or Cd solutions (100, 200, 300 µM). The cadmium solutions were freshly prepared by dissolving $CdCl_2$ in deionized water and their pH was adjusted to 7.0–7.2. There were four replicates per each treatment. Petri dishes were kept in an incubator at 25 ± 2.5 ˚C and 70% RH under a light irradiance of 30 mmol m^{-2} s^{-1} (12/12 h light/dark conditions), and the various parameters of germination and growth indices were recorded after two weeks from culturing.

Germination and seedling growth indices

The number of seeds germinated was determined in each treatment every 24 h intervals for 14 days using radicle protrusion (i.e., appearance of a radicle ≥ 2 mm in length) as a criterion [22], and the seed germination percent (GP) was calculated as follows: (number of seeds geminated/total number of seeds) × 100. Day 14^{th} of the experiment was considered as the final day, because no more germination was observed and roots and shoots length were measurable for all dishes. Germination rate (GR) was determined daily until the seedling stage [23]. Germination vigor (GV) and germination index (GI) were measured using following formulae: GV=seedling length (cm) × GP, and GI=(GP/DT), where GP is the germination percentage on the T days, and DT is the day of germination. The length of shoots and roots were also recorded after two weeks from culturing.

Biochemical analysis

Chlorophyll content: Weighed 0.5 g of leaf samples from controls and the different Cd treated seedlings were homogenized in 20 mL 80% acetone. The absorbance of the supernatant measured at 663 and 645 nm using a Spectrophotometer (Itd T80 + UV/VIS; PG Instruments, Leicestershire, UK). It was finally calculated according to Wellburn and Lichtenthaler [24] and expressed as mg g^{-1} fresh weight (Fw).

Preparations and assays of antioxidant enzymes: The leaves of the turfgrasses (200 mg) were homogenized in 1 mL ice-cold extraction buffer containing 50 mM phosphate buffer (pH 7), 1 mM EDTA and 1.5% (w/v) of PVP. The homogenate was centrifuged at 9000 g for 15 min. The supernatant was used as the crude extract for determination of enzyme activities [25]. The SOD activity was measured spectrophotometrically as described by Beyer and Fridovich [26]. The reaction solution (1 mL) contained 50 mM phosphate buffer (pH=7), 12 mM riboflavin, 13 mM methionine, 0.1mM EDTA , 7 mM nitro blue tetrazolium (NBT) and 10 µL of extracted enzyme solution. A solution with no enzyme was used as the control. Test tubes were irradiated under fluorescent lights at 100 mmol m^{-2} s^{-1} for 20 min. The absorbance of each solution was measured at 560 nm using a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme that would inhibit 50 percent of NBT photo reduction.

The catalase (CAT) activity was assayed as reported by Brouwer and Brouwer [27], The reaction solution (0.5 mL) contained 25 mM phosphate buffer (pH=7), 10 mM H_2O_2 and 10 µL of extracted enzyme solution. The reaction was initiated by adding the enzyme solution. Changes in absorbance at 240 nm were read every 10 s for 60 s using a spectrophotometer. One unit of CAT activity was defined as the absorbance change of 0.01 units per minute. The peroxidase (POD) activity was determined by the method of Chance and Maehly [28]. POD activity in leaves was assayed by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance was recorded at 470 nm. The reaction mixture contained 100 µL of crude enzyme extract, 500 µL of 5 mM H_2O_2, 500 µL of 28 mM guaiacol, and 1,900 µL of 50 mM potassium phosphate buffer (pH 7.0). POD activity of the extract was expressed as activity U/g FW min.

Statistical analysis

Statistical analysis was performed using SAS software (Version 9.0). The significance of the differences among treatments was tested by one-way ANOVA at $p < 0.01$. Values reported here are means of four replicates.

Results

Seeds germination characteristics

The germination indices (germination index, percentage, vigor and rate) of different turfgrass seeds treated with $CdCl_2$ are summarized in Table 1. Germination index (GI) of all studied turfgrasses was significantly affected by Cd concentrations. With increasing Cd levels in the solution GI decreased in the all studied species, except for *A. elongatum*. The maximum and minimum GI was observed in *A. elongatum* and *F. ovina*, as compared to their controls, with 6.01% and 47.24% reduction, respectively.

As the Table 1 shows, the germination percentage (GP) of seeds declined, when Cd level in the solution increased from 100 to 300 µM. However no significant difference was found for GP in *L. perenne* and *F. aurandinaceae* when exposed to Cd from 0 to 200 µM. Furthermore, no significant difference was found between Cd levels in *A. elongatum*. The lowest GP was observed in *F. ovina*, when exposed to 300 µM Cd (Table 1).

The germination vigor (GV) of all studied turf species was significantly decreased when Cd concentration increased in the solution (Table 1). The GV of the seeds of *F. aurandinaceae* was considerably affected by Cd treatments, so as it reduced from 13084 in control to 5280 in plants treated by 300 µM Cd. In other words, declined 59.64% compared to control. But *L. perenne* had the maximum GV about 35% compared to control when exposed to 300 µM Cd.

Effects of Cd on germination rate (GR) are shown in Table 1. Except for *F. rubra*, no significant difference was found among control, 100 and 200 µM Cd treated turf seeds in GR. But, with increasing Cd level from 200 to 300 µM, GR was significantly declined in all turfgrass species except for *A. elongatum*. Therefore, this species could be the less sensitive to Cd heavy metal.

Seedlings shoot and root growth

Shoot and root length of all studied species were decreased when exposed to Cd treatment as compared to control (Figure 1A and 1B). The highest dose of Cd (300 µM) markedly hindered root length. The highest suppressing effect of Cd contamination on root elongation was found in *F. aurandinaceae* (about 91% of control) and the lowest

Table 1: Germination index, percentage, vigor and rate of six turfgrass species in response to different concentrations of Cd.

				Turfgrass species			
Indices	**$CdCl_2$ concentration (µM)**	***Agropyron elongatum***	***Festuca rubra***	***Festuca aurandiance***	***Festuca ovina***	***Lolium perenne***	***Poa pratensis***
	0	35.1aA	24.76cA	22.7cA	27.26bA	32.86abA	28.06cA
Germination	**100**	34.51aA	23bcAB	20.04cA	23.58bcA	31.94aA	25.49bAB
Index	**200**	33.7aA	20.6bcBC	19.13bcA	18.75cB	30.61aAB	22.92bB
	300	32.99aA	18.7cC	15.68dB	14.38dC	27.93bB	19.89cB
	0	88.33aA	84.11aA	87.13aA	93.36aA	91.1aA	88.23aA
Germination	**100**	86.71aA	76.18bB	75.19bB	77.16bB	87.61aAB	77.93bB
percentage	**200**	83.56aAB	66.98bC	71.43bB	56.49cC	81.51aB	66.81bC
	300	80.33aB	58.4cD	56.09cC	37.18dD	72.59bC	53.36cD
	0	26111aA	10176cA	13084bA	10753cA	14069bA	10473cA
Germination	**100**	18969aB	8362.1bcAB	10966bA	7684.2cA	10871bB	8053bcAB
vigor	**200**	16487aB	7168bBC	5528.1cB	6395cB	9362bB	6925cB
	300	14297aB	5841.6cC	5280cB	4911.6cB	9078.3bB	5221.7cB
	0	9.2aA	4.93cA	4.46cA	5.26bcA	6.86bA	4.06cA
Germination	**100**	9.51aA	4.38cAB	4.31cA	5.18bcA	6.5bA	4cA
rate	**200**	9.66aA	3.7cB	4.06cA	4.59cAB	6.1bA	3.85cAB
	300	9.73aA	3.54cB	3.48cB	4.07bcB	5.3bB	3.01cB

Means in each row and column followed by different letters were significantly different (P< 0.01 level). Capital and small letters show statistical differences for data in columns and rows, respectively.

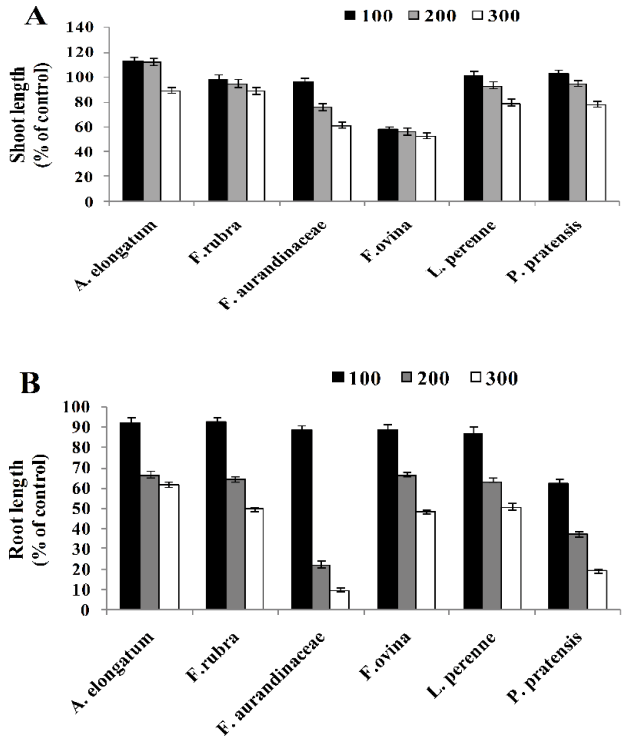

Figure 1: Percentage decrease in shoot length (**A**) and root length (**B**) in *Agropyron elongatum, Festuca rubra, Festuca aurandiance, Festuca ovina, Lolium perenne and Poa pratensis* seedlings seedlings at different concentrations of $CdCl_2$ (100, 200 and 300 µM). Bars indicated mean ± SE (n=3).

one was in *A. elongatum* roots with 70.87 mm about 38% of control in length (Figure 1A).

Shoot growth was also decreased with increasing Cd concentrations in Petri dish solution. Unlike roots, the seedling shoots were less sensitive to Cd (Figure 1B). No significant difference was found between 100 µM Cd treated seedlings and control for shoot growth except for *F. ovina*. Thereafter, with increasing Cd level from 100 to 200 µM, shoot length was significantly declined in *F. aurandinaceae*, *F. ovina* and *L. perenne*. Except for *F. rubra*, all turfgrass species showed a significant reduction in their shoot length when exposed to 300 µM Cd. The highest shoot growth was observed in *F. rubra and A. elongatum* with 89.09% and 87.58% of control, respectively, and the minimum was found in *F. ovina* with 53.22% of control.

Chlorophyll content

Leaf chlorophyll contents of the six turfgrass species was generally declined with increasing Cd concentrations (Table 2). However, no significant difference was found between control and 100 µM Cd in some turfgrass species. The lowest chlorophyll content was found in *F. ovina,* when treated with 300 µM Cd. It was declined as 45.96% as control in *F. ovina*, therefore, this species could be the most sensitive turf to Cd contamination for chlorophyll content.

Antioxidant enzymes activities

Effects of Cd stress on SOD activity are shown in Figure 2A. As the results showed, SOD activity gradually increased when Cd concentration increase from 100 to 300 µM and the maximum activity was assayed at 300 µM Cd, although there were no statistical difference. The maximum SOD activity was observed in *L. perenne* and *A. elongatum* (234 and 217 U g^{-1} Fw, respectively) after exposure to 300 µM Cd, whereas the minimum activity was shown in *F. ovina* (125 U g^{-1} Fw).

CAT activity was also increased significantly in the most of turfgrass species, when exposed to 100 and 200 µM Cd (Figure 2B), but it significantly decreased at 300 µM. CAT activity was continuously suppressed in *F. ovina*, and this inhibition was strengthened as Cd concentrations were increased. Also in *F. aurandinaceae* seedlings, CAT activity was significantly decreased at 300 µM. The highest increase in CAT activity was observed in *A. elongatum*, as compared to its control.

Table 2: Shoot chlorophyll content of six turfgrass species (mg/g fresh weight) when exposed to different Cd concentrations.

			Turfgrass species			
$CdCl_2$ concentration (µM)	*Agropyron elongatum*	*Festuca rubra*	*Festuca aurandiance*	*Festuca ovina*	*Lolium perenne*	*Poa pratensis*
0	4.25abA	4.38abA	4.92aA	3.72bA	3.91bA	3.95bA
100	4.18abAB	4.2abAB	4.79aAB	3.49bA	3.82bA	3.85bA
200	3.85abAB	3.69bB	4.25aB	2.69cB	3.51bAB	2.81cB
300	3.51aB	3.46bC	3.48aC	2.01cC	3.09bB	2.076cC

Means in each row and column followed by different letters were significantly different (P< 0.01 level). Capital and small letters show statistical differences for data in columns and rows, respectively.

POD activity tended to increase when Cd concentration increased from 100 to 200 µM, but suppressed at 300 µM Cd in the all turf species except for *F. aurandinaceae* (Figure 2C). The highest and lowest increase in POD activity at 300 µM Cd were observed in *A. elongatum* and *F. ovina,* about 33% and 13%, respectively with respect to their controls. POD activity in *F. aurandinaceae* significantly increased and reached its peak value at 300 µM.

Discussion

Seed germination and early growth performance of plants are usually considered in evaluating the toxicity of heavy metals [29]. In the present study, seed germination response of different turf species to Cd treatment was completely species dependent. Only *A. elongatum* and *L. perenne* have been shown to germinate even under the highest level (300 µM) of Cd. All seed germination parameters, except for GR, were significantly affected by high concentrations of Cd. Previous study also showed the toxic effects of the heavy metal on the seed germination and seedling growth Sengar et al. [30] have reported that inhibition of seed germination may result from the interference of Cd with germination-involved enzymes. Decrease in seed germination of plant can be attributed to the accelerated breakdown of stored nutrients in seed and alterations of selection permeability properties of cell membrane, due to negative effects of heavy metals [31]. The seed germination of *F. ovina* was inhibited more than that of other studied species at higher Cd levels; however, its root growth was not as reduced as that of *P. pratensis* and *F. aurandinaceae*. Gąbka and Wolski [32] showed that *F. ovina* "Noni" was able to accumulate heavy metals in its tissues, but its general aspect (aesthetic value) became worse than that of non-heavy metal accumulated plants.

In general, the terrestrial parts of all Cd-treated turfgrasses were more strictly affected than the aerial parts. These results have been certified by other researchers. It has been well established that root growth is more sensitive than seed germination to metal toxicity, because they are the primary targets of metal ions, and hence root testing is widely used for evaluating the toxicity levels of heavy metals [33]. In this study, the roots of six turfgrass species were affected when high levels of Cd were applied. Shafiq et al. [31] have been explained that the reduction in root length of *Leucaena leucocephala* under Cd treatments could be due to the reduced mitotic cells in meristematic zone of root. Lerda (1992) had similar observations in roots of onion (*Allium cepa*). The exact reason for different responses of root growth to heavy metals is not known but might be due to either the rapid accumulation of heavy metals in root than shoot or the faster rate of detoxification in shoot than root [31].

F. rubra and *A. elongatum* had an appropriate growth even under high Cd concentrations which may be resulted from less chlorophyll degradation. In contrast, the other species such as *F. ovina* and *P. pratensis* showed enhanced chlorophyll decomposition at elevated levels of Cd, so as their shoots were eventually died. Reduction in chlorophyll contents by excess Cd has been reported in *Bacopa monnieri* and hybrid Bermudagrass, which might be due to the interaction of Cd to –SH group of enzymes of chlorophyll biosynthesis.

It has been well established that Cd toxicity results in enhanced reactive oxygen species (ROS) generation [3]. ROS are dangerous primarily due to reaction with lipids, proteins, and nucleic acids [34]. In this study, POD, CAT and SOD activities were further in more tolerant species such as *A. elongatum* and *F. rubra* than sensitive ones such as *F. ovina* and *F. aurandinaceae*. It could indicate that the antioxidant enzymes activities manner was in accordance with the growth of turfgrasses exposed to exogenous Cd toxicity. In addition, an increase in antioxidant enzymes of *A. elongatum* and *F. rubra* as well as a decrease in those of *F. ovina* observed after exposure to 300 µM Cd were in accordance with their tolerance and sensitivity, respectively, might indicate a strong relationship between Cd tolerance and antioxidant system in these grasses. Zhang et al. [3] also reported that *Vicia sativa* was more tolerant to Cd than *Phaseolus aureus* because of higher leaf symplastic SOD and APX activities. Therefore, the more tolerance to Cd levels in *A. elongatum* and *F. rubra* could be related to the higher efficiency of antioxidant enzymes. Zhang et al. [35] have reported that Cd stress induces intracellular oxidizing conditions leading to the production of ROS, as shown by the increased lipid peroxidation in *Achnatherum inebrians* exposed to the heavy metal. According to our findings, CAT and POD activities were more strongly related to the Cd tolerance capabilities than SOD. The increased antioxidant activities particularly in *A. elongatum* and *F. rubra* after high concentration Cd exposure may indicated that the turfgrasses cells presented endogenous protective effects and the antioxidant enzymes were induced to prevent against Cd-induced ROS. However, our results showed that the tolerance induction to Cd stress through antioxidant enzymes activities was species-dependent. Moreover, It was in parallel to that of kenaf (*Hibiscus cannabinus*) seedlings reported by Li et al. [36]. They have reported that appropriate growth of kenaf seedlings when exposed to Cd toxicity can be due to the ROS scavenging by highly activated antioxidant enzymes.

According to these results *A. elongatum* seems to have a strong defense system during both seed germination and subsequent seedling growth. It might be due to the perturbation in Cd ion entrance into the tissues resulted from its thicker seed coat in comparison with that of any other studied turfgrasses. Kranner and Colville [37] have found that the influences of metals on seed germination in different plants depend mainly on interspecies differences in seed structure, particularly seed coat anatomy. Jian-Ling and Li-Qiang [38] have also reported that seed coat plays a crucial role in protecting seed for having the unique ability to obstruction and retention of the heavy metal. It is consistent with our seed germination results indicating that seeds of *A. elongatum*, apart from thick coat, may have a substantially higher threshold for toxicity than other studied species. Overall, *A. elongatum* and *F. rubra* not only had the more seed germination under high Cd concentrations, but also showed

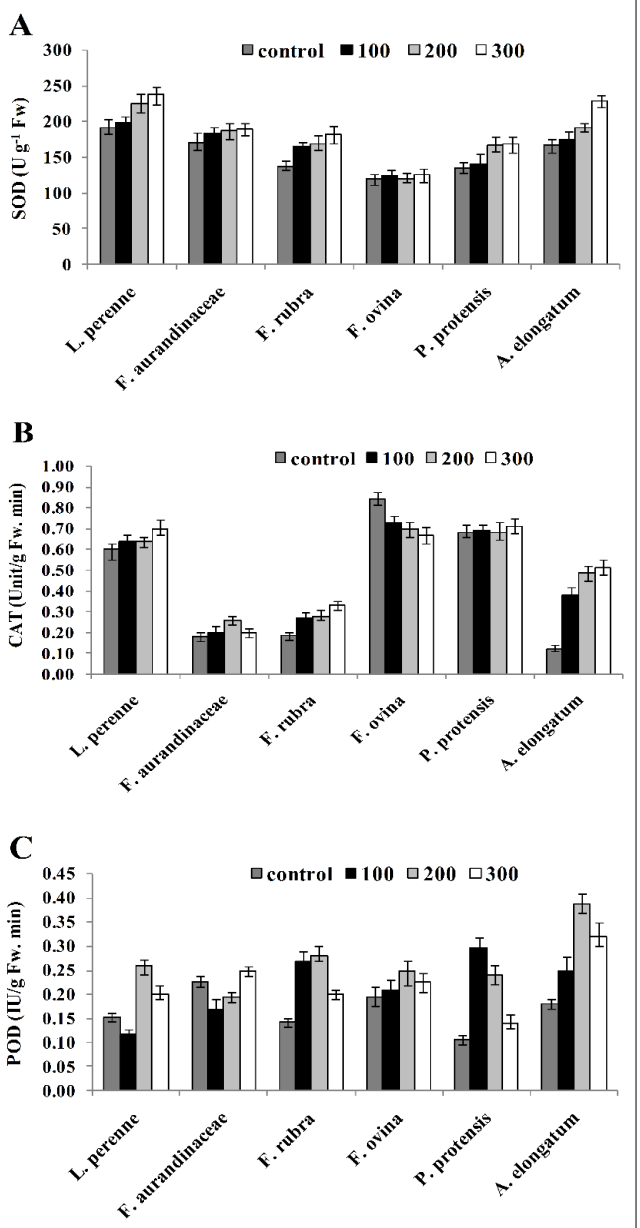

Figure 2: The activities of SOD (A), CAT (B) and POD (C) in *Agropyron elongatum, Festuca rubra, Festuca aurandiance, Festuca ovina, Lolium perenne and Poa pratensis* seedlings treated with 0, 100, 200 and 300 µM $CdCl_2$. Data are expressed as mean ± SE (n=4).

the better growth during subsequent stages. It may be hypothesized that there was a relationship between seed germination sensitivity/tolerance and subsequent plant growth to Cd stress.

Conclusion

In conclusion, the response of cool-season turfgrasses to Cd stress was species dependent. Cd treatment, particularly at high concentrations influenced seed germination and growth rate of the studied turfgrasses. Our results demonstrated that the higher seed germination was correlated with the more tolerance to the contamination during early seedling growth. In all studied species, shoot growth was not influenced as much as root growth to Cd treatment. The higher antioxidant enzymes (CAT, SOD, and POD) activities could relatively mitigate the adverse effects of Cd, and keep on their appropriate growth. It might disclose the important role of the side-effect of Cd, that is, oxidative stress. In general, based on the different assessments from seedling growth to chlorophyll content, the tolerance of the six studied turfgrasses was ordered as follows; *A. elongatum* > *F. rubra* > *L. perenne*> *P. pratensis* > *F. aurandinacea*> *F. ovina*. Overall, *A. elongatum*, from a morpho-physiologically and biochemically point of view, could be introduced as the most tolerant turfgrass, which could be used not only for cultivation in Cd-polluted sites, but also for more researches on the deciphering of the exact mechanisms of the heavy metal tolerance.

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Prajapati et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00076.8

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Studies on Effect of *A. niger* on Physiological Weight Loss and Biochemical Changes in Black Mould Rot Diseased Onion

Prajapati BK*, Patil RK and Patel NJ

Abstract

Black mould rot of onion is an important post-harvest diseases of onion which effects on the quality deterioration of onion bulbs by utilizing its nutrient contents. Here, present research was carried out to study the losses of different nutrients from onion bulbs after the infection of *A. niger* in three different varieties. Onion bulbs inoculated with *A. niger* showed loss in weight as compared to uninoculated bulbs at 30, 60 and 90 days after inoculation. Highest per cent physiological weight loss was recorded in Gujarat Anand white onion-3 variety (10.48 g and 16.70%) followed by Nasik yellow (11.54 g and 14.45%) and Nasik red (11.73 g and 10.59%) after 90 DAI. Total soluble sugar content in onion bulbs inoculated with *A. niger* progressively decreased as the incubation period was increased over control in all three varieties. Least TSS (10.90, 6.39 and 6.09%) and reducing sugar content (1.81, 1.13 and 1.09%) was recorded in onion bulbs after 90 DAI over control (18.21, 17.25 and 14.11%); (6.91, 5.48 and 5.23%) in Nasik red, yellow and Gujarat Anand white onion-3 varieties, respectively. There was appreciable decrease in phenol content in onion bulbs inoculated with *A. niger* up to 60 DAI due to utilization by fungi and then there was increase in total phenol content in onion bulbs up to 90 DAI when inoculated with *A. niger*. Total phenol content of healthy Nasik red, yellow and Gujarat Anand white onion-3 varieties were 51.23, 46.07 and 38.78 per cent, which showed maximum reduction after 60 DAI (30.69, 27.36 and 20.24%) and found increased after 90 DAI (33.72, 29.60 and 26.22%). Significantly lowest amount of ascorbic acid content was recorded at 90 DAI (0.03, 0.014 and 0.014 mg/100g) over control (0.14, 0.14 and 0.13 mg/100g), least pyruvic acid content was found at 90 DAI (257.82, 217.11 and 158.65 mg/100 g) over control (755.48, 589.77 and 512.78 mg/100 g), least acidity content was found at 90 DAI (2.05, 2.81 and 1.28%) over control (6.40, 5.76 and 3.84%) in Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties, respectively.

Keywords

Onion; *Aspergillus niger*; Total sugar; Reducing sugar; Phenol; Ascorbic acid; Pyruvic acid; Acidity content

Highlight

Onion bulbs inoculated with *A. niger* showed loss in weight, loss in total sugar content, reducing sugar content, ascorbic acid content, pyruvic acid content and acidity content as compared to uninoculated bulbs at 30, 60 and 90 days after inoculation. While, phenol content in onion bulbs inoculated with *A. niger* showed decrease up to 60 days after inoculation and after that it showed increased content up to 90 days.

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Received: June 18, 2016 **Accepted:** August 02, 2016 **Published:** August 07, 2016

Introduction

Onion (*Allium cepa* L.) is an important vegetable crop widely cultivated and used in the world. It is pungent when chopped and contains sulfenic acid which irritates the eyes with tears [1]. Onion contain phenolics and flavonoides which has potential anti-inflammatory, anti-cholesterol, anticancer and antioxidant properties. Most onion cultivars contains about 89% water, 4% sugar, 1% protein, 2% fiber and 0.1% fat, vitamin C, vitamin B_6, folic acid and numerous other nutrients in small quantity. They are low in fats and sodium. They can contribute their flavor to savory dishes without increasing caloric content [1]. After harvest of the bulbs when they are preserved for longer period, many fungal and bacterial pathogens cause deterioration of bulbs. Under storage conditions these fungal rotting cause heavy bulb losses. Black mould rot is the most destructive disease of onion in storage as well as under field conditions [2]. Raju and Naik [3] reported 16.52 and 14.76 per cent disease incidence of black mould rot of onion in Gulbarga and Bellary vegetable market, respectively. Nanda et al., [4] estimated 7.51 per cent overall post-harvest losses in *kharif* onion. Storage losses of *Rabi* harvested onions ranged from 10-15 per cent due to microbial decay or rotting due to fungal diseases [5]. The present research work was undertaken to study the effect of *Aspergillus niger* infection on physiological weight loss and biochemical changes in onion bulbs.

Materials and Methods

Collection and identification of the pathogen

Fresh naturally infected diseased onion bulbs showing typical characteristic symptoms of black mould rot were collected from the Sardar Patel vegetable market, Anand and brought to the laboratory for isolation of the pathogen. Pathogenicity was proved by following Koch's postulates. The pure culture obtained was sent for identification to Indian Type Culture Collection (I.T.C.C.), Division of Plant Pathology, I.A.R.I., New Delhi and was identified as *A. niger* (ID. No. 9610.14).

Physiological weight loss

Mature, healthy uniform size onion bulbs of three varieties *i.e.*, Nasik Red, Nasik Yellow and Gujarat Anand White Onion-3; were washed with tap water and then surface sterilized with 1 per cent NaOCl and finally washed thoroughly with distilled sterile water and separately inoculated with *A. niger* (10^6 spores/ml) by random pin-pricking method. The inoculated bulbs were incubated at ambient temperature. Physiological weight loss of infected bulbs was assessed after 30th, 60th and 90th days and losses in weight were calculated by the following formula:

$$= \frac{W_1 - W_2}{W_1} * 100$$

Where,

W_1 = The weight of bulb recorded at the time of inoculation

W_2= The weight of bulb recorded after 30th, 60th and 90th days after inoculation

Total soluble sugar content: Total soluble sugar content of inoculated and un-inoculated mature onion bulbs was determined by phenol sulphuric acid method as described by Dubois [6].

Reducing sugar content: Reducing sugar content of inoculated and un-inoculated mature onion bulbs was determined by the method of Nelson's modification of Somogyi's method [7,8].

Total phenol content: Total phenol content of inoculated and un-inoculated mature onion bulbs both was estimated by Folin ciocalteau method as described by Bhatnagar et al. [9].

Ascorbic acid content: The titramatric method described by Ranganna [10] was adopted to estimate the ascorbic acid content.

Pyruvic acid content: The DNPH (dinitrophenylhydrazine) method described by Anthon and Barrett [11] was employed to estimate the pyruvic acid content.

Acidity content: The method described by Ranganna [10] was employed to estimate the acidity.

Results and Discussion

Physiological weight loss

The results presented in Table 1 revealed that onion bulbs inoculated with *Aspergillus niger* showed loss in weight as compared to uninoculated bulbs at 30, 60 and 90 days after inoculation. Highest per cent physiological weight loss was recorded in Gujarat Anand white onion-3 variety (16.70%) followed by Nasik yellow (14.45%) and red (10.59%), after 90 days of inoculation (DAI). It was observed that physiological weight of onion bulbs decreased quickly when inoculated with *A. niger* as compared to control bulbs. The results of present investigation corroborate with the results obtained by Kapadiya et al., [12]. They reported maximum weight loss (5.44%) in black mould rot infected onion bulbs as compare to healthy bulbs (2.66%) through fresh neck cutting injury method. Damaram [13] reported that when tomato fruits are inoculated with *F. pallidoroseum* showed drastic loss in physiological weight compared to uninoculated healthy fruits. Highest physiological weight loss was recorded after 8th day of inoculation.

Total soluble sugar content

The results of total sugar content in onion bulbs inoculated with *Aspergillus niger* and control (without pathogen) at different incubation periods are given in Table 2. The results revealed that TSS in inoculated onion bulbs with *A. niger* progressively decreased as the incubation period was increased over control in all three (Nasik red, yellow and Gujarat Anand white onion-3 varieties). Least TSS content was found after 90 DAI (10.90, 6.39 and 6.09%) followed by 60 DAI (12.69, 10.76 and 10.33%) and 30 DAI (16.14, 15.51 and 13.20%) over control (18.21, 17.25 and 14.11%) in Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties. The results of present investigation are in consonance with the results obtained by Ghangaonkar [14]. He reported that *A. niger* significantly utilized total sugar contents from red and white onion varieties. Further Singh and Sinha [15] found that *A. flavus* and *A. parsiticus* resulted in depletion of total sugars from guava fruit. Rathod and Chavan [16] reported maximum reduction in total sugar content of papaya fruits due to *A. niger*. Bilgrami et al., [17] revealed that there was sharp decline in the level of total sugars of dry fruit during infection by *A. flavus*.

Reducing sugar content

The results of reducing sugar content in onion bulbs inoculated with *A. niger* and control (without pathogen) after different periods of incubation are given in Table 2. The results revealed that reducing sugar content in inoculated onion bulbs with *A. niger* progressively decreased as the incubation period was increased over control in all the three varieties. Least reducing sugar content was recorded after 90 DAI (1.81, 1.13 and 1.09%) followed by 60 (2.37. 2.31 and 2.71%) and 30 DAI (4.31, 4.07 and 4.10%) in Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties over control (6.91, 5.48 and 5.23%), respectively. It was observed that reducing sugar content of onion bulb decreased when inoculated with *A. niger* as compared to control bulbs. Results of present investigation corroborate with the results obtained by Singh and Sinha [15]. They reported that *A. flavus* and *A.*

Table 1: Physiological weight loss in different onion varieties inoculated with *A. niger.*

Sr. No.	Treatments	Initial bulb wt. (g) at the period			Bulb wt. (g) after inoculation at different periods			Physiological loss in weight			Per cent physiological loss in weight		
		30 days	60 days	90 days	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI
1.	Nasik red	106.27	109.2	110.71	104.29	103.48	98.98	1.98	5.72	11.73	1.86	5.24	10.59
2.	Nasik yellow	64.7	63.48	79.85	62.74	58.10	68.31	1.96	5.38	11.54	3.03	8.47	14.45
3.	Gujarat Anand White onion-3	55.26	59.72	62.74	52.77	52.87	52.26	2.49	6.85	10.48	4.51	11.47	16.70

***DAI= Days after inoculation**

Table 2: Effect of *A. niger* infection on total sugar, reducing sugar and phenol contents of different onion varieties

Sr. No.	Treatments	Total sugar (%)			Reducing sugar (%)			Total phenol (mg/100g)		
		Nasik Red	Nasik Yellow	Gujarat Anand White onion-3	Nasik Red	Nasik Yellow	Gujarat Anand White onion-3	Nasik Red	Nasik Yellow	Gujarat Anand White onion-3
1.	Control	18.21	17.25	14.11	6.91	5.48	5.23	51.23	46.07	38.78
2.	30 DAI	16.14	15.51	13.20	4.31	4.07	4.10	43.41	37.53	30.95
3.	60 DAI	12.69	10.76	10.33	2.37	2.31	2.71	30.69	27.36	20.24
4.	90 DAI	10.90	06.39	06.09	1.81	1.13	1.09	33.72	29.60	26.22
SEm±		0.031	0.088	0.045	0.011	0.013	0.015	0.312	0.602	0.387
C.D. (5 %)		0.092	0.264	0.135	0.033	0.038	0.046	0.935	1.804	1.161
C.V. %		0.472	1.579	0.922	0.632	0.868	1.042	1.753	3.839	2.980

parsiticus resulted in depletion of reducing and non-reducing sugars from guava fruit. Rajmane and Korekar [18] reported maximum decrease in reducing sugar in local varieties of mango (Nanded, Aurangabad) when infected by *A. niger*. Rathod and Chavan [16] reported maximum reduction in reducing and non-reducing sugar content in papaya fruits due to infection by *A. niger*. Bilgrami et al., [17] revealed that there was sharp decline in the level of reducing and non-reducing sugars of dry fruit during *A. flavus* infection.

Total phenol content

The results revealed that there was appreciable decrease in phenol content up to 60 DAI due to utilization by fungi and then there was increase in total phenol content in onion bulbs up to 90 DAI when inoculated with *A. niger*. Total phenol content of healthy Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties was 51.23, 46.07 and 38.78 per cent, which showed maximum decrease after 60 DAI (30.69, 27.36 and 20.24%) followed by 30 DAI (43.41, 37.53 and 30.95%) and found increased after 90 DAI (33.72, 29.60 and 26.22%). Phenol accumulated in the bulbs infected by pathogen which might be inactivate the enzymes of pathogens by forming poly phenol oxydase which in turn prevented further advancement of pathogen by limiting its source of nutrients [18]. The significance of phenols in onion bulb is to trigger the resistance. Results similar to the present investigation were obtained by Ghangaonkar [14]. He reported that *A. niger* significantly utilized phenol content from red and white onion varieties. Especially phenols which are responsible for inhibiting the growth of fungi were utilized by the pathogen. The white onion variety seems to be more susceptible to rot than red. Clark and Lorbeer [19] reported that catachol (phenol) which is responsible for inhibiting the growth of fungi was absent in white onions, while it is very common in red and yellow varieties. Wagh and Bhale [20] found that phenol content was decreased significantly due to five isolates of *A. niger* in sapota.

Ascorbic acid content

The results presented in Table 3 revealed that there was appreciable decrease in ascorbic acid content as per incubation period increased in onion bulbs when inoculated with *A. niger*. Significantly lowest amount of ascorbic acid content was recorded at 90 DAI (0.03, 0.014 and 0.014 mg/100g) followed by 60 DAI (0.07, 0.07 and 0.05 mg/100g) over control (0.14, 0.14 and 0.13 mg/100g) in Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties. Thus, it was observed that ascorbic acid content was progressively decreased as the inoculation period is increased in inoculated onion bulbs as compared to control. The probable reason for drastic reduction in ascorbic acid content of onion bulb infected with *A. niger* could be due to changes in biochemical content of bulbs which revealed that the fungus (*A. niger*) might have utilized it as a substrate. Similar trend of the results was reported by Singh et al. [21]. They reported that ascorbic acid content of papaya fruits inoculated with *A. flavus* and *F. moniliforme* showed 71.10 and 62.10 per cent decrease in ascorbic acid content, respectively as compared to control (76.00%). Ghosh et al. [22] noted post-infectional change in ascorbic acid content of mango and papaya fruits when inoculated with *A. niger*. As the incubation progressed, there was a decrease in ascorbic acid content in both healthy as well as infected fruits. After four days of incubation the quantity of ascorbic acid content was reduced to nearly half the original amount.

Ogaraku et al. [23] carried out studies on storage decay of tomato fruits and vitamin C content in fruits inoculated with *A. niger, A. flavus, Alternaria alternata, A. solani* and *Fusarium oxysporium*. The results revealed that the infected fruit contained 2.20 mg/100 g vitamin C, while the healthy tomato fruits contained 2.51 mg/100 g vitamin C.

Pyruvic acid content

The results of pyruvic acid content presented in Table 3 revealed that pyruvic acid content in inoculated onion bulbs with *A. niger* progressively decreased as the incubation period was increased over control bulbs in all three varieties. Least pyruvic acid content was found after 90 DAI (257.82, 217.11 and 158.65 mg/100 g) followed by 60 (460.60, 349.43 and 216.43 mg/100 g) and 30 DAI (604.38, 461.37 and 361.95 mg/100 g) in Nasik red, Nasik yellow and Gujarat Anand white onion-3 bulbs, respectively. It was observed that pyruvic acid content of onion bulb decreased when inoculated with *A. niger* as compared to control bulbs (755.48, 589.77 and 512.78 mg/100 g).

Acidity content

The results of acidity content presented in Table 3 revealed that acidity content in inoculated onion bulbs with *A. niger* progressively decreased as the incubation period was increased over control in all three varieties. Least acidity content was found after 90 DAI (2.05, 2.81 and 1.28%) followed by 60 (3.07, 4.09 and 2.56%) and 30 DAI (5.12, 4.61 and 3.33%) in Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties. It was observed that acidity content of onion bulb decreased when inoculated with *A. niger* as compared to control (6.40, 5.76 and 3.84%). Results of present investigation corroborate with the results obtained by Sharma et al., [24]. They reported the post-infectional changes pertaining to physical, biochemical and nutritional aspects caused due to major post-harvest microbial rot pathogens (*Alternaria alternata, Botryodiplodia theobromae, G. candidum, Penicillium digitatum* and *P. italicum*) in kinnow fruits. The results revealed significant decrease in fruit acidity and ascorbic

Table 3: Effect of *A. niger* infection on ascorbic, pyruvic acid and acidity contents of different onion varieties.

Sr. No.	Treatments	Ascorbic acid (mg/100g)			Pyruvic acid (mg/100g)			Acidity (%)		
		Nasik Red	Nasik Yellow	Gujarat Anand White onion-3	Nasik Red	Nasik Yellow	Gujarat Anand White onion-3	Nasik Red	Nasik Yellow	Gujarat Anand White onion-3
1.	Control	0.14	0.14	0.13	755.48	589.77	512.78	6.40	5.76	3.84
2.	30 DAI	0.11	0.09	0.08	604.38	461.37	361.95	5.12	4.61	3.33
3.	60 DAI	0.07	0.07	0.05	460.60	349.43	216.33	3.07	4.09	2.56
4.	90 DAI	0.03	0.014	0.014	257.82	217.11	158.65	2.05	2.81	1.28
SEm±		0.003	0.002	0.002	3.242	2.507	3.767	0.222	0.239	0.157
C.D. (5 %)		0.009	0.006	0.006	9.721	7.518	11.294	0.665	0.718	0.470
C.V. %		7.744	5.590	6.681	1.395	1.386	2.696	11.917	12.395	12.738

acid content as compare to uninoculated healthy fruits. Damaram [7] observed that acidity content of inoculated tomato fruits with *F. pallidoroseum* progressively decreased (0.78, 0.66, 0.55 and 0.48%) as the incubation period is increased (2nd, 4th, 6th and 8th day), while in control fruit acidity was 0.87 percent.

Conclusion

Onion bulbs inoculated with *A. niger* showed loss in weight as compared to uninoculated bulbs at 30, 60 and 90 days after inoculation in all three varieties. Total soluble sugar and reducing sugar content in onion bulbs inoculated with *A. niger* progressively decreased as the incubation period was increased over control in all three varieties. There was appreciable decrease in phenol content in onion bulbs inoculated with *A. niger* up to 60 DAI due to utilization by fungi and then there was increase in total phenol content in onion bulbs up to 90 DAI when inoculated with *A. niger*. This may be due to resistance mechanism of phenol. Similarly ascorbic acid, pyruvic acid and acidity content of onion bulbs infected with *A. niger* were found decreasing as the period of storage increased as compared to control in Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties, respectively.

Acknowledgements

The authors are grateful to the Professor and Head, Department of Biochemistry and Dean, Faculty of Agriculture, B. A. College of Agriculture, Anand Agricultural University, Anand for providing the necessary facilities during the course of investigation.

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Ranganathswamy et al., Vegetos 2016, 29:4
10.5958/2229-4473.2016.00077.X

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Molecular Characterization and Management of *Aspergillus flavus* Link Ex Fries in Groundnut

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Abstract

Thirty one isolates collected from different parts of major groundnut growing areas of Karnataka and one from ICRISAT were identified as *Aspergillus flavus* by molecular technique using species specific primers. Later a field experiment was conducted at two locations under artificial inoculation and natural infection conditions for the pre-harvest management of *A. flavus* incidence and thereby aflatoxin. The results indicated that all the treatments are effective in reducing the *A. flavus* incidence and there by aflatoxin level in the produce. Among all the treatments, T-7 was most effective under both the situations. Under artificial inoculation conditions T-7 recorded minimum incidence of *A. flavus* (1.00 per cent) and aflatoxin content (2.40 µg/kg) with higher benefit to cost ratio (2.86) as compared to control (25.90%, 19.90 µg/kg). In natural conditions also T-7 recorded lowest *A. flavus* incidence (1.40 per cent) and aflatoxin content (0.0 µg/kg) with highest benefit to cost ratio (2.77). Among all the treatments, T-5 (foliar spray with neem oil @ 5 ml/l) was the least effective under both the conditions. The integrated approach was found best under both the situations.

Keywords

A. flavus; Aflatoxin; Characterization; Integrated management

Introduction

Groundnut (*Arachis hypogea* L.) is one of the premier oilseed crops of the world. It is cultivated in the tropical and subtropical regions (40° N to 40° S) of the world. It is the thirteenth most important food crop of the world and third most important oil seed crop used for vegetable oil production. Groundnut suffers from many major diseases *viz.,* leaf spots, rust, stem rot, root rot, collar rot, bud necrosis and many others. Of late aflatoxin contamination caused by *Aspergillus flavus* Link Ex Fries and *Aspergillus parasiticus* Speare has become a serious problem in groundnut since it affects the quality of produce [1]. Of the different types of aflatoxin analogues aflatoxin B_1 is the most toxic leading to teratogenicity and liver cancer in human beings. Due to food safety, problems posed by aflatoxins, importing countries have prescribed the standards for groundnut. Maximum of 20 ppb of aflatoxin in exporting material is permissible in international trade (WTO). Groundnut producers in both developing and developed countries with advanced agriculture have found it almost impossible to meet above regulations as *Aspergillus* spp. can infect and produce aflatoxin at various stages of the cropping period including pre-harvest, post-harvest, and storage [2]. Hence, it necessitates taking the precautionary measures before sowing until it reaches the end users. Aid of molecular technique for accurate identification and exploring the integrated approach will be a best solution to address this problem. In this context, species specific primers used for accurate identification and the different components of integrated management (chemicals, botanicals and biocontrol agents) were screened under *in vitro* against *A. flavus* and most effective ones were further tested in the field by applying each component as individually as well as integrated manner. The present paper will through the light on the molecular characterization and management of *Aspergillus flavus* producing aflatoxin in groundnut.

Materials and Methods

Molecular identification of isolates of *Aspergillus* sp.

DNA extraction: Potato Dextrose Broth (PDB) was used for mycelial growth of fungus from which DNA was extracted. One hundred fifty ml of broth was dispensed in 250 ml conical flasks and sterilized at 121.6 °C at 1.1 kg/cm^2 pressure for 15 min. Each flask containing PDB was inoculated with spore suspension (100 µl) of different isolates. The inoculated flasks were incubated for 48 hrs at 27 ± 1°C. After incubation, the mycelial mats were harvested by filtering through sterilized Whatman No.1 filter paper. The harvested mycelial mats were freeze-dried and DNA extraction was performed using CTAB (Cetyl Trimethyl Ammonium Bromide) method [3]. The DNA pellet was rehydrated in 100 µl TE buffer and allowed to re-suspend at 4°C overnight.

Polymerase chain reaction (PCR)

The Fungal DNA (rDNA) was amplified with the specific primers ASPITSF2 (5' –GCCCGCCATTCATGG-3') and ASPITSR3 (5'-CCTACAGAGCGGGTGACAAA-3') [4]. Primers for amplification were custom synthesized at Bangalore Genie Pvt Ltd, Bangalore and supplied as lyophilised products of desalted oligos. Amplification reaction mixture was prepared in 0.2 ml thin walled PCR tubes containing the 1.0 µl Template DNA (25 ng/µl), 1.0 µl of each Primer (5PM/µl), 1.0 µl dNTPs mix (2.5 mM each), 2.0 µl of 10 x assay buffer with 15 mM $MgCl_2$ and 0.5 µl *Taq* DNA polymerase (6.0U µl^{-1}). Except template the master mix was distributed to PCR tubes (19 µl/tube) and later 1 µl of template DNA from the respective isolates was added making the final volume of 20 µl. The PCR was carried out in thermo cycler as follows: Initial denaturation 94 for 5 min, 35 cycles of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and extension at 72°C for 2 min and a final extension at 72°C for 10 min.

Separation of amplified products by agarose gel electrophoresis

The PCR products were resolved using 1.2 per cent agarose in 1X TBE (Tris Borate EDTA) buffer, 0.5 mg ml^{-1} of Ethidium Bromide and loading buffer (0.25% Bromophenol Blue in 40% sucrose).

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Received: July 27, 2016 **Accepted:** September 08, 2016 **Published:** September 09, 2016

Electrophoresis was carried at 70 V for 1.0 hr. The gel was observed under UV light and documented using gel documentation unit.

Pre-harvest management of *A. flavus* incidence

A field experiment was conducted during *kharif,* 2013 on the pre-harvest management of *A. flavus* incidence and there by aflatoxin in Groundnut at Main Agricultural Research Station (MARS), Dharwad, Karnataka and in the farmer's fields located at Murgod, Soundatti taluk, Belagavi district of Karnataka. The highly susceptible variety GPBD-4 was taken for the experiment (Tables 1 and 2).

Mass multiplication of pathogen and inoculation

A. flavus culture (AF-11-4 from ICRISAT) was inoculated to 1000 ml conical flask containing 500 ml Potato Dextrose Broth and incubated at 27 ± 1^0 C for 10 days. The culture was filtered through Whatman No. 40 filter paper and conidia were collected by washing the mycelial mat with water. The concentration of conidia was adjusted (1x10^6 conidia/ml) and spore suspension was applied through spraying at flowering and pod development stage.

Observations

***Aspergillus flavus* incidence:** The harvested kernels were analysed for *A. flavus* colonization by following rolled towel method and disease incidence in the field was assessed with the formula [5].

$$Percent\ A.flavus\ incidence = \frac{No\ of\ seeds\ colonized\ with\ A.flavus}{Total\ no.of\ seeds\ incubated} * 100$$

Aflatoxin quantification: The efficacy of different treatments were analysed by estimating the aflatoxin in the harvested crop by following indirect competitive ELISA [6].

Results and Discussion

Molecular characterization of *Aspergillus* sp.

All the 32 isolates of *Aspergillus* sp. were amplified by using *A. flavus* specific primers ASPITSF2 and ASPITSR3 and expected PCR product size of 397 bp was obtained in all the 32 isolates of *Aspergillus* sp. including standard isolate from ICRISAT (*AF-11-4*). Primer ASPITSF2 targeting within the ITS 1 region is specific at species level (*A. flavus*) and primer ASPITSR3 targeting within the ITS 2 region is specific at genus level. Midorikawa et al. [4] designed the *Aspergillus flavus*-specific PCR primers, ASPITSF2 and ASPITSR3 from ribosomal DNA internal transcribed spacers (ITS 1 and 2) and identified that all the strains isolated from Brazil nut and cashew were *Aspergillus flavus.* The present results are in line with the Midorikawa et al. [4] report (Plate 1).

Pre-harvest management of *A. flavus* incidence

Under artificial inoculation at MARS, Dharwad

***A. flavus* incidence:** Field experiment conducted during kharif, 2013 for pre-harvest management of *A. flavus* incidence at MARS, Dharwad indicated that incidence of *A. flavus* was lower in all the treatments compared to control. Among the different treatments, T-7 (Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/ kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2 % at pegging stage) recorded minimum *A. flavus* incidence (1.00 %) as compared to control (25.93 %) followed by T-6. Among the various treatments foliar spray with neem oil @ 5 ml /l (T-5) was least effective (19.44 % incidence) (Table 3). Kumar et al. [7] evaluated an integrated package at ICRISAT, Patancheru, Andhra Pradesh, India

Table 1: Details of Experiment.

Year: *kharif,* 2013	Location: MARS, Dharwad
Variety: GPBD-4	Design: RCBD
No. of treatments : 12	Replications: 3
Plot size: 2 X 2.7 m (9 lines)	Spacing: 30 X 10 cm
Soil type: Black clay	Observations: *Aspergillus flavus* incidence, Aflatoxin quantification

Table 2: Treatment details.

Year: *kharif,* 2013	Location: Farmer's field (Village: Murgod)
Variety: GPBD-4	Design: RCBD
No. of treatments : 12	Replications: 3
Plot size: 2 X 2.7 m (9 lines)	Spacing: 30 X 10 cm
Soil type: Black clay	Observations: *Aspergillus flavus* incidence and aflatoxin quantification

during the rainy season in 2001 to demonstrate the effectiveness of improved package vis-a-vis farmers' practice. In Integrated package the seed infection by *A. flavus* was very less (2.0 %) compared to farmers' practice (10.0 %). The results are in comparison with Kumar et al. [7], as integrated management practice was more promisable than individual approach.

Aflatoxin contamination: Aflatoxin contamination in the kernels obtained from various treatments ranged from 2.40 to 19.90 µg/kg. Among the different treatments, T-7 (Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2 % at pegging stage) and T-6 (seed treatment with tebuconazole @1 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + foliar spray with tebuconazole @0.1 % at pegging stage) recorded the least aflatoxin contamination (2.40 µg/kg) and they differed significantly from other treatments. The highest aflatoxin was recorded in control (19.90 µg/kg) (Table 3).

Yield and benefit: Cost ratio (B:C): The result revealed that, all the treatments recorded higher yields compared to control. Among different treatments, highest yield was recorded in T-7 (27.20 q/ha) with a benefit cost ratio of 2.86 followed by T-10 (25.20 q/ha) with B:C. ratio of 2.69 while lowest was recorded in T-5 (17.70 q/ha) with a B:C ratio of 1.97 (Table 3a)

Under natural conditions in farmer's field

***A. flavus* incidence:** Field experiment conducted during *kharif* 2013 for Pre- harvest management of *A. flavus* incidence in the farmer's field revealed that the *A. flavus* incidence ranged from 15.74 per cent (Control T-12) to 1.4 per cent (T-7). All the treatments were effective in reducing the *A. flavus* incidence. Among all the treatments, T-7 was found most effective with lower *A. flavus* incidence (1.40 per cent) while T-5 was least effective (Table 4).

Aflatoxin contamination: Aflatoxin contamination in the kernels obtained from various treatments ranged from 0.0 to 11.10 µg/kg. All the treatments were effective in reducing the aflatoxin content compared to control. Among the different treatments, T-7, T-8, T-9, T-10 and T-11 showed nil aflatoxin contamination The highest aflatoxin was recorded in control (Table 4).

Yield and benefit: cost ratio (B:C): The result showed that highest yield (26.30 q/ha) was obtained in T-7 (carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/50 kg of FYM + foliar spray with carbendazim 12%

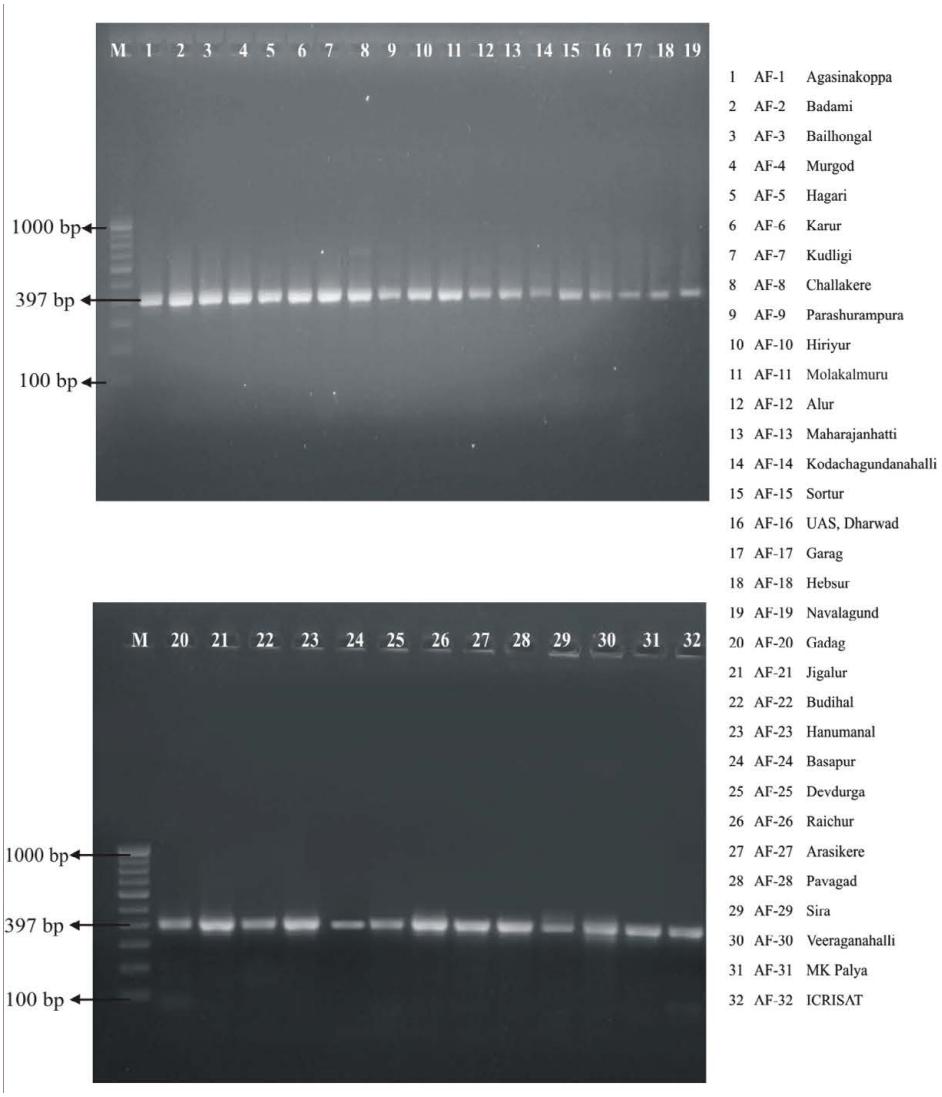

Plate 1: Identification of *A. flavus* isolates by amplification with specific primers at ITS Region

Table 3: Pre-harvest management of *A. flavus* incidence in artificial inoculation conditions at MARS, Dharwad.

Treatment	Treatment details
T_1	Seed treatment with tebuconazole @ 1 g/kg of seeds + Foliar spray with Tebuconazole @ 0.1 % at pegging stage
T_2	Seed treatment with carbendazim 25 % + mancozeb 50 % @ 3.0 g/kg of seeds + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2% at pegging stage
T_3	Seed treatment with iprodione 25% + carbendazim 25% @ 2 g/kg of seeds + foliar spray with iprodione 25% + carbendazim 25% @ 0.2% at pegging stage
T_4	Seed treatment with *Trichoderma harzianum* @ 10 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM
T_5	Foliar spray with neem oil @ 5 ml/l at pegging stage
T_6	Seed treatment with tebuconazole @ 1 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + foliar spray with tebuconazole @0.1 % at pegging stage
T_7	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2 % at pegging stage
T_8	Seed treatment with iprodione 25% + carbendazim 25% @ 2 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + foliar spray with iprodione 25% + carbendazim 25% @ 0.2 % at pegging stage
T_9	Seed treatment with tebuconazole @ 1 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage
T_{10}	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + Foliar spray with neem oil @ 5 ml/l at pegging stage
T_{11}	Seed treatment with iprodione 25% + carbendazim 25% @ 2 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1 kg/ 50 kg of FYM + Foliar spray with neem oil @ 5 ml/l at pegging stage
T_{12}	Untreated control

Table 3a: Economics of pre-harvest management of *A. flavus* incidence in groundnut at MARS Dharwad

Treatments	Treatment details	*A. flavus* incidence (%)	Reduction of *A. flavus* incidence over control (%)	Aflatoxin (µg/kg)	Yield (q/ha)
T_1	Seed treatment with tebuconazole @1g/kg of seeds + Foliar spray with tebuconazole @0.1% at pegging stage	12.50 (*3.60)	51.64	8.10	22.00
T_2	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2% at pegging stage	9.26 (3.10)	64.10	6.10	24.00
T_3	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + foliar spray with iprodione 25% + carbendazim 25% @ 0.2% at pegging stage	11.11 (3.40)	56.98	7.40	23.50
T_4	Seed treatment with *Trichoderma harzianum* @10g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM	14.81 (3.93)	42.74	9.90	19.50
T_5	Foliar spray with neemoil @ 5ml/l at pegging stage	19.44 (4.46)	24.93	14.10	17.70
T_6	Seed treatment with tebuconazole @1g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with tebuconazole @0.1% at pegging stage	1.85 (1.47)	92.59	2.40	25.10
T_7	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2% at pegging stage	1.00 (1.13)	95.80	2.40	27.20
T_8	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with iprodione 25% + carbendazim 25% @ 0.2% at pegging stage	3.24 (1.80)	87.25	3.50	24.10
T_9	Seed treatment with tebuconazole @1g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	5.56 (2.47)	78.35	4.10	25.00
T_{10}	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	4.17 (2.13)	83.69	3.70	25.20
T_{11}	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	7.41 (2.77)	71.23	4.20	24.20
T_{12}	Untreated control	25.93 (5.12)	-	19.90	16.10
	S. Em. ±	0.10		0.05	0.51
	CD (P=0.05)	0.29		0.17	1.52

*square root transformed

Table 4: Pre-harvest management of *A. flavus* incidence in natural conditions at Farmer's field, Murgod.

Treatments	Treatment details	Yield (q/ha)	Total cost of cultivation (Rs/ha)	Gross returns (Rs/ha)	Net returns (Rs/ha)	B:C ratio
T_1	Seed treatment with tebuconazole @1g/kg of seeds + Foliar spray with tebuconazole @0.1% at pegging stage	22.00	35793.00	85800.0	50007.00	2.40
T_2	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2% at pegging stage	24.00	36449.00	93600.0	57151.00	2.57
T_3	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + foliar spray with iprodione 25% + carbendazim 25% @ 0.2% at pegging stage	23.50	36626.00	89700.0	53074.00	2.45
T_4	Seed treatment with *Trichoderma harzianum* @10g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM	19.50	34630.00	76050.0	41420.00	2.20
T_5	Foliar spray with neemoil @ 5ml/l at pegging stage	17.70	34984.00	69030.0	34046.00	1.97
T_6	Seed treatment with tebuconazole @1g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with tebuconazole @0.1% at pegging stage	25.10	36423.00	97890.0	61467.00	2.69
T_7	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2% at pegging stage	27.20	37079.00	106080.0	69001.00	2.86
T_8	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with iprodione 25% + carbendazim 25% @ 0.2% at pegging stage	24.10	37256.00	93990.0	56734.00	2.52
T_9	Seed treatment with tebuconazole @1g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	25.00	37407.00	97500.0	60093.00	2.61
T_{10}	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	25.20	38063.00	98280.0	60217.00	2.58
T_{11}	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	24.20	38240.00	94380.0	56140.00	2.47
T_{12}	Untreated control	16.10	34000.00	62400.0	28400.00	
	S. Em.±	0.51				
	CD (p = 0.05)	1.52				

Table 4a: Economics of pre-harvest management of *A. flavus* incidence in groundnut at Farmer's field, Murgod.

Treatments	Treatment details	Yield (q/ha)	Total cost of cultivation (Rs/ha)	Gross returns (Rs/ha)	Net returns (Rs/ha)	B:C ratio
T_1	Seed treatment with tebuconazole @1g/kg of seeds + Foliar spray with tebuconazole @0.1% at pegging stage	21.30	35793.00	83200.0	47407.00	2.32
T_2	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2% at pegging stage	23.70	36449.00	92300.0	55851.00	2.53
T_3	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + foliar spray with iprodione 25% + carbendazim 25% @ 0.2% at pegging stage	22.30	36626.00	87100.0	50474.00	2.38
T_4	Seed treatment with *Trichoderma harzianum* @10g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM	18.30	34630.00	71500.0	36870.00	2.06
T_5	Foliar spray with neemoil @ 5ml/l at pegging stage	17.10	34984.00	66690.0	31706.00	1.91
T_6	Seed treatment with tebuconazole @1g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with tebuconazole @0.1% at pegging stage	24.50	36423.00	95550.0	59637.00	2.66
T_7	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with carbendazim 12% + mancozeb 63% @ 0.2% at pegging stage	26.30	37079.00	102570.0	65491.00	2.77
T_8	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + foliar spray with iprodione 25% + carbendazim 25% @ 0.2% at pegging stage	24.20	37256.00	94380.0	57124.00	2.53
T_9	Seed treatment with tebuconazole @1g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	23.00	37407.00	89700.0	52293.00	2.40
T_{10}	Seed treatment with carbendazim 25% + mancozeb 50% @ 3.0 g/kg of seeds + + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	24.10	38063.00	93990.0	55927.00	2.47
T_{11}	Seed treatment with iprodione 25% + carbendazim 25% @ 2g/kg of seeds + soil application of *Trichoderma harzianum* @ 1kg/ 50kg of FYM + Foliar spray with neem oil @ 5ml/l at pegging stage	23.00	38240.00	89700.0	51460.00	2.35
T_{12}	Untreated control	15.80	34000.00	61620.0	27620.00	
	S. Em.±	0.61				
	CD (p = 0.05)	1.82				

+ mancozeb 63% @ 0.2 % at pegging stage) with a benefit: cost ratio of 2.77. The lowest yield (15.80 q/ha) was recorded in T-12 (control) (Table 4a).

In both the natural and artificial inoculation conditions the *A.flavus* colonization was lower in treatments compared to control. Among the treatments, the integrated ones showed lower *A.flavus* incidence compared to individual component application. The treatments in the natural conditions showed lower aflatoxin content compared to artificial inoculation condition. This may be due to lack of drought situation at the fag end of the crop and may also due to lack of sufficient inoculum in the soil which are prerequisite for infection and aflatoxin production. Bruce [8] reported that there is a direct relationship between soil density of *Aspergillus flavus* and the incidence of groundnut colonization. Arunyanark et al. [9] reported that drought in combination with higher levels of *A. flavus* inoculum load in the soil resulted in increased kernel colonization (6 to 68%) and subsequent aflatoxin contamination (4 to183 µg/kg). From the present study it was concluded that use of molecular technique is a quick, time saving and aid in accurate identification of *A.flavus* and integrated approach is best in addressing the pre-harvest management of *A.flavus* incidence and aflatoxin content in groundnut.

Acknowledgement

Author is grateful to the **Department of Science and Technology (DST), New Delhi** for providing the Financial Assistance during the Ph.D program and **ICRISAT** for extending the facilities during Research work.

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Sardana et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00078.1

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Spatial Distribution Behaviour of Thrips in Important *Cucurbitaceous* Vegetable Crops

Sardana HR*, Bhat MN, Chaudhary H, Sureja AK, Sharma K, and Mobin Ahmad

Abstract

The individuals of a species of insects distribute themselves in their habitat with a characteristic pattern. The study of distribution pattern is of considerable ecological significance. Field sampling of an insect population without sound ecological basis can lead to erroneous estimation and conclusions. Control of the pest can be enhanced by the determining its spatial distribution and developing a sampling procedure for estimating its population.

Keywords

Spatial distribution; Field sampling; Thrips, Cucurbits

Introduction

The distribution pattern provides information about the behaviour of population and also can be used for measuring the size of population [1]. Thus the adequate knowledge of distribution of insect population gives an insight to formulate pest management strategies. The melon thrips, *Thrips palmi* Karny are one of the key pests of cucurbitaceous vegetable crops in India. Over the last 10-15 years *Thrips palmi* has rapidly become a major pest of cucurbits. Thrips feed gregariously on leaves, stems, flowers and fruits causing severe injury to cucurbits. It has a wide host range and is also cause of virus transmission. So far the research work on *Thrips palmi* has been restricted to its biology, incidence, feeding behaviour, varietal susceptibility and control [2-4]. No detailed information is available on its spatial distribution. In the present paper, efforts have been made to study the distribution pattern of thrips in major cucurbitaceous vegetable crops at various time intervals.

Materials and Methods

The unprotected cucurbitaceous crops of pumpkin, muskmelon and bottle gourd raised 2014 at the experimental farm of Indian Agricultural research Institute, New Delhi were divided each into eight uniform strata based on Harcourt [5]. Absolute counts of thrips (nymphs and adults) on one inflorescence per plant on five randomly selected plants in each stratum were recorded for each crop. Four such observations at weekly interval were recorded for pumpkin while three observations were recorded for muskmelon and bottle gourd each during May-June.

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Received: July 21, 2016 **Accepted:** August 08, 2016 **Published:** August 16, 2016

Spatial distribution

The procedure outlined by Southwood [6] was followed for determining the spatial distribution pattern. Three dispersion indices *viz*; variance to mean ratio, aggregation index (K) and mean crowding [7] were computed. Lloyd's index of patchiness, which is the ratio of mean crowding (x^*) to mean density (x) was calculated for each set of observation. The mean crowding was obtained [6] as:

$$X^* = \overline{X} + \left(\frac{S^2}{\overline{X}} - 1 \right)$$

Where, x is the thrips mean density per inflorescence per plant and S^2 is the variance. Lloyd's index equals unity in a random distribution but is greater and smaller than unity in contagious and regular distribution, respectively.

The aggregation index, K, was computed as:

$$K = \frac{\overline{X}^2}{S^2 - \overline{X}}$$

After determining the distribution pattern from dispersion parameters, the data were fitted to a negative binomial and Poisson distributions and goodness of fit tested using chi-square (χ^2) statistics.

To study the distribution further, Iwao's [8] patchiness regression, which gives a linear relationship between mean crowding (x^*) and mean density (x) over a range of different densities was fitted,

$$X^* = \alpha + \beta x$$

Where α is a constant and β the slope. The intercept, the index of basic contagion, indicates whether individual insects are dispersed in colonies and β, the coefficient of density contagiousness indicates whether the colonies were dispersed contagiously. Thus when a single individual is the basic component, α = 0, and value greater or less than 0 indicates a positive or negative association between the individuals. When β is less, equal to or greater than unity, it describes a uniform, random or aggregated distribution of basic components or colonies, respectively. Taylor's power law [9], which gives a relation between variance and mean,

$$S^2 = ax^b$$

Where, a is a constant depending upon experimental conditions, b is the coefficient of contagion, was also fitted to the data to further study the aggregation pattern.

To identify the cause of aggregation, the mean clump size (λ) was calculated using the Arbous and Kerrich [10] formula:

$$\lambda = \frac{X}{2k} V$$

Where λ is the number of individuals in aggregation for the probability level attached to v (v = function with a chi-square) distribution with 2k degrees of freedom at probability level of 0.50.

Sample size

The optimum sample number (n) of plants for a reasonably

accurate estimation of the density of thrips based on the distribution pattern, was also worked out, using Iwao's formula [11],

$$q = \frac{1}{D^2}\left(\frac{\alpha+1}{\overline{X}} + \beta - 1\right)$$

Where D is the standard error of mean.

Finally to find the appropriate transformation to normalize the data or to make the variance independent of mean, ten sets of data on thrips count were transformed by log (x+1), log (x+k/2), log{log(x+2)} [12],$\text{Sinh}^{-1}\sqrt{}$ (β – 1/ α + 1) x [13], $x^{1-b/2}$ [9] and √x+1. The means and variances were computed and correlation (r) between the variance and mean values worked out for each transformation and compared with (r) of untransformed data. In normalized data, the variance is independent of mean as shown by the correlation coefficient (r) [14].

Results and Discussion

The results on the spatial dispersion pattern of thrips in three cucurbitaceous crops *viz* pumpkin, muskmelon and bottle gourd are summarized in Table 1. The mean population of thrips (nymphs & adults) per inflorescence per plant ranged from 2.83 to 5.13. The mean population of thrips was marginally higher in bottle gourd than pumpkin and musk melon. However, the variance exceeded the mean on all dates of observations in the three cucurbits and hence the variance to mean ratio, the simplest method of showing the distribution pattern [9,15], was invariably greater than unity for three gourds viz., pumpkin, muskmelon and bottle gourd. Likewise, the index of mean crowding exceeded the mean density and thereby the Lloyd's index of patchiness was always greater than unity thereby corroborating the aggregation pattern of thrips (Table 1). This implied that the distribution pattern of thrips was of a contagious type indicating a tendency for aggregation in the spp. The values of the coefficient of dispersion, K, which measures the degree of aggregation, was less than eight in most cases, suggesting a high degree of aggregation [9].

On most of the occasions, the negative binomial distribution based on chi square (χ^2) values was found to give a better fit of probability to the data sets than the Poisson distribution (Table 1). This further confirmed the clumping pattern of the thrips distribution.

To get an insight into the type of aggregation involved i.e. whether it was an aggregation of insects in colonies or whether it was an aggregation of colonies, Iwao's [8] patchiness regression was fitted, based on the linear relationship between mean crowding and mean density, over a range of different densities $\left(X^* = \alpha + \beta^{\overline{X}}\right)$ and yielded α = 0.3103 and β = 1.1620. Here, (α) the index of basic contagious, indicates whether individual insects are dispersed in colonies and (β) the coefficient of density contagiousness indicates whether the colonies are dispersed contagiously. When a single individual is the basic component, then α = 0, and greater or less than zero value of (α) indicates a positive or negative association between individuals. When (β) is less than, equal to or greater then unity, it describes a uniform, random or aggregated distribution of the basic component or colonies, respectively. In this study, the two values obtained suggested that the thrips were distributed singly (one insect per colony) but that colonies were aggregated. The data further explained the aggregation pattern of the colonies, in term of negative binomial distribution, of about equal and fixed mean size [8]. The Taylor power law equation yielded a slope (b) value of 1.6289 indicating a clumped distribution of thrips.

For the population described by the negative binomial distribution, according to Arbous and Kerrich [9], the causes of clumping may be due to either active aggregation by the insect or some heterogeneity of the environment. These workers observed that if λ was less than 2, the distribution was due to environmental heterogeneity while λ values greater than 2 indicated that environment and insect behavior were working together. In our study, the mean clump size (λ) of aggregation was greater than 2 in all the sets of observations. Thus it may be inferred that the clumping of thrips could be due to behavior of females tending to deposit eggs in close proximity in the leaf veins & flowers and to environmental variation mainly caused by the emergence of top leaves which are the also the preferred sites for ovipositing and feeding of the thrips along with inflorescence. In cucurbits field, the females deposit eggs in leaves and flowers. Soon after hatching, the larvae start feeding on succulent leaves and growing tips and among the petals and developing ovary gregariously. This probably explains why in the field, the thrips population is observed in aggregation pattern.

A mean sample size of 44 plants was found to be optimum and appeared to be quite reliable for estimating the population of thrips in cucurbits fields (Table 2) as it was based on the dispersion behaviour of thrips. This may be adopted depending upon time, manpower,

Table 1: Different parameters for spatial distribution of thrips in pumpkin, muskmelon and bottle gourd.

Date of observation	Mean No. thrips	Variance (S^2)	Aggregation index (K)	Variance to mean ratio	Mean crowding (x*)	Lloyd's index of patchiness	-ve binomial χ^2	Poisson distribution χ^2	Mean clump size λ
Pumpkin									
27-04-14	2.83	3.58	10.68	1.26	3.09	1.09	4.94 (8)	4.43 (8)	2.49
04-05-14	3.40	7.02	3.19	2.06	4.46	1.31	15.00(11)	59.00 (11)	3.18
11-05-14	3.38	6.04	4.29	1.78	4.16	1.23	14.81(10)	30.25 (10)	3.19
18-05-14	3.40	4.96	7.41	1.45	3.85	1.13	19.37 (8)	45.00 (8)	3.11
Muskmelon									
27-04-14	2.85	6.18	2.44	2.16	4.01	1.40	14.82 (8)	25.46 (8)	2.45
04-05-14	3.15	6.34	3.11	2.01	4.16	1.32	9.12 (9)	25.70 (9)	3.01
11-05-14	4.38	7.11	7.03	1.62	5.00	1.14	19.8 (10)	9.04 (10)	4.20
Bottle gourd									
27-04-14	3.30	7.14	2.83	2.16	4.46	1.35	9.35 (9)	34.38 (9)	3.09
04-05-14	4.60	7.02	8.74	1.52	6.08	1.32	7.66 (10)	2.41(10)	4.27
11-05-14	5.13	10.52	4.88	2.05	6.18	1.20	19.51 (10)	9.84 (10)	4.92

Table 2: Average plant sample size based on Iwao's (1977) formula for thrips, *Thrips palmi* on cucurbitaceous crops.

Number of observations	Sample size (number of plants)
1	56
2	36
3	41
4	51
5	33
6	37
7	50
8	34
9	54
10	42
Mean	**44**

Table 3: Suitability of different transformations for sampling thrips population on cucurbits.

No. Of observations	Original count M	V	Log (x+1)		Log (x+k/2)		Log{log (x+2)}		$X^{1-b/2}$		$Sinh^{-1}\sqrt{\frac{\beta-1}{\alpha+1}}X$		√X+1	
1	2.83	3.58	0.53	0.04	0.90	0.01	-0.20	0.01	1.14	0.05	0.97	0.41	1.89	0.21
2	3.40	7.02	0.54	0.11	0.62	0.07	-0.20	0.03	0.98	0.30	1.16	0.81	1.98	0.43
3	3.38	6.04	0.56	0.08	0.69	0.05	-0.19	0.02	1.07	0.19	1.15	0.70	1.99	0.35
4	3.40	4.96	0.56	0.09	0.81	0.02	-0.20	0.03	1.04	0.25	1.16	0.57	2.04	0.35
5	2.85	6.18	0.48	0.09	0.52	0.08	-0.23	0.03	0.99	0.23	0.97	0.71	1.90	0.42
6	3.15	6.34	0.52	0.10	0.60	0.07	-0.22	0.03	0.99	0.27	1.07	0.73	1.96	0.39
7	4.38	7.11	0.64	0.10	0.86	0.03	-0.15	0.03	1.12	0.24	1.49	0.82	2.22	0.43
8	3.30	7.14	0.53	0.09	0.60	0.07	-0.19	0.03	1.05	0.22	1.12	0.83	1.98	0.43
9	4.60	7.02	0.68	0.07	0.93	0.02	-0.13	0.02	1.21	0.14	1.56	0.81	2.27	0.36
10	5.13	10.52	0.72	0.08	0.83	0.05	-0.11	0.02	1.20	0.19	1.74	1.22	2.42	0.47
r =	0.780 S		-0.160 NS		-0.948 S		-0.384 NS		0.434 NS		-0.692 S		0.776 S	

S = Significant at 5%
NS = Non-significant at 5%

convenience and type of study. Southwood [9] suggested that a small sample size representing 2 - 3% of plant population is reasonably good for survey and control studies. However for life table studies etc. a large sample size is required.

As the dispersion pattern of *Thrips palmi* on cucurbits is over dispersed, the need for a suitable transformation to normalize the data before an analysis of variance was felt. Six transformations were used and the mean, variance and their correlation were computed (Table 3). The correlation coefficient for untransformed data was significantly high showing the dependence of variance on the mean. Of the six transformations, three, viz; log (x+1), log [log(x + 2)] and $x^{1-b/2}$ stabilized the variance. The other transformations *viz.* $\log(x+k/2)$, $\sinh^{-1}\sqrt{\beta}-1/\sqrt{\alpha}+1.X$ and $\sqrt{x+1}$ showed dependence of variance on the mean and were not suitable for normalizing the population of thrips.

The present investigations form a basis for future studies of *Thrips palmi* on cucurbits crops. Any pest infesting the inflorescence has a direct bearing on yield loss. Therefore, precision estimates of population and sampling decisions within the limits of time and man power are essential. Spatial distribution is unique to a species in relation to its environment, knowledge of which together with the optimum sample size and a suitable transformation will provide the correct approach in field studies leading to management of *Thrips palmi* in cucurbits.

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Nanda et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00079.3

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Tagging SSR Markers Associated with Genomic Regions Controlling Anthracnose Resistance in Chilli (*Capsicum baccatum* L.)

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Abstract

Anthracnose, caused by *Colletotrichum* spp. is a serious pre- and post-harvest disease in chilli (*Capsicum annuum* L.) which is a remunerative spice-cum-cash crop of the India. An attempt was made to tag genomic regions controlling anthracnose resistance using reported microsatellite markers.Out of 60 polymorphic SSR markers screened, only four differentiated the individual constituents of resistant and susceptible bulks. Of these four, only one (HpmsE 081) was found associated with genomic regions controlling anthracnose resistance. However, the association was weak as suggested by low contribution of the marker towards the variance of response to anthracnose disease in terms of lesion size.

Keywords

Chilli; SSR markers; Anthracnose resistance; Tagging

Introduction

Chilli (*Capsicum annuum* L.) is a remunerative vegetable and spice-cum-cash crop of the Indian subcontinent. India is the largest producer accounting for 26 *per cent* of the global production followed by China. Andhra Pradesh and Karnataka together account for more than fifty *per cent* production in India. However, chilli productivity in India (1.60 t ha^{-1}) is lower than that in the developed countries such as USA and South Korea (3.4 t ha^{-1}) [1].

Among the biotic stresses that constrain the chilli production, anthracnose, caused by *Colletotrichum* spp. is a serious pre- and post-harvest disease. *C. capsici* (Syd.) Butler and Bisby, *C. gloeosporioides* (Penz.) Penz. and Sacc., *C. acutatum* (Simmonds) and *C. cocodes* (Wallr.) Hughes [2], cause anthracnose of chilli, the former two are predominant in India. Yield losses due to anthracnose in India range from 50 *per cent* [3] to 66-84 *per cent* [4] and loss in fruit quality attributes such as oleoresin, capsaicin and phenol content due to anthracnose could be 50 *per cent* [5] resulting in reduced market price.

Conventional breeding of chilli for anthracnose resistance is rather slow owing to prevalence of multiple species/strains, wide diversity and distribution, and wide variability in pathogenicity of *Colletotrichum*. SSR markers, as powerful surrogates help increase the pace and efficiency of breeding chilli for anthracnose resistance. Reported literature on identification of DNA markers linked to genomic regions controlling anthracnose resistance in chilli is scanty in India. Under this premise, the present study was conducted.

Material and Methods

Plant material

The material for the study consisted of anthracnose resistant PBC 80 and susceptible SB1 both belonging to *Capsicum baccatum*. The genotypes were crossed at the experimental plots of Department of Genetics and Plant Breeding (GPB), University of Agricultural Sciences (UAS), Gandhi Krishi Vignyana Kendra (GKVK), Bengaluru.

Methods

Resistance response of PBC 80 was confirmed by screening against seven *C. capsici* and four *C. gloeosporioides* isolates (data not shown). Seeds from the crossed 'PBC 80 × SB 1' fruits were sown to raise F_1 plants in an insect proof net house. True F_1's were selfed individually to obtain F_2 seeds. F_2 seeds of the cross were sown in nursery to raise the F_2 mapping population and 40 days old seedlings were transplanted in insect proof net house, along with their parents and F_1 by maintaining a spacing of 0.45 m between plants within a row and 0.9 m between rows. All the recommended package of practices was followed to raise a good crop.

Phenotyping F_2 population for reaction to anthracnose disease

A total of 240 F_2 plants were raised from the selfed F_1 seeds and phenotyped for reaction to anthracnose. Twenty random fruits from each F_2 plant were picked at red ripe stage and brought to the laboratory. The fruits were surface sterilised, rinsed in sterile water and inoculated with virulent strain of *Colletotrichum capsici i.e.,* '*Cc* 38' in two replications. Thereafter, the fruits were inoculated with homogenized spore suspension containing 5×10^5 spores/ml at two spots on the fruit (one µl/spot) using Hamilton micro syringe [6]. The inoculated fruits were incubated in plastic boxes with moist filter papers placed at the bottom and on top of the fruits to maintain relative humidity of over 90 *per cent* and then incubated at 27 ± 1°C for eight days (Figure 1). Disease reaction was recorded in terms of lesion size and was expressed as overall lesion diameter (OLD) across

Figure 1: Microinjection method of screening and experimental set up for screening against anthracnose.

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Received: July 27, 2016 **Accepted:** August 10, 2016 **Published:** August 16, 2016

all inoculated points on the fruits and true lesion diameter (TLD) using the following formulae

$$OLD = \frac{\sum lesion\ diameter}{Total\ \text{number of inoculated points}}$$

True lesion diameter (TLD): average of lesion diameter that are truly developed

$$TLD = \frac{\sum lesion\ \text{diamter}}{Total\ \text{number of points that developed true lesions}}$$

Genotyping

Genomic DNA was extracted from young and healthy leaves of 50 days old seedlings of parents, F_1 and F_2 plants following the extraction protocol given by Prince *et al.* (1997) with a few modifications.

Primers of 282 publically available microsatellite markers [7,8,9] were custom synthesized from Sigma genosys, Bengaluru. Reaction mixture for amplification consisted of Template DNA (12.5 ng/ µl) 2 µl, Forward primer (10 pmol/µl) 2 µl, Reverse primer (10 pmol/µl) 2 µl, 1mM each dNTP 2 µl, 10 X *Taq* buffer 1 µl, 1 U *Taq* polymerase (5U/µl) 0.2 µl.

The PCR amplified products were initially visualized on 3% agarose and where clear resolution was not observed the products were denatured and separated on 6% Polyacrylamide Gel Electrophoresis (PAGE) gel and products were visualized by silver staining.

Depending on the lesion size (mm diameter) caused by infection with virulent '*Cc* 38' isolate, the F_2 plants were categorised as resistant and susceptible following the scale modified from Hartman and Wang [10]. DNA from 10 resistant plants and ten susceptible plants were bulked. The bulks were constituted by combining equal quantity DNA (of same concentration) from selected plants, such that the final concentration of bulked DNA was made up to 12.5 ng/µl.

Sixty SSR primers which differentiated the two parents either on 3% agarose or on 6% PAGE gel were used to genotype the two bulks. Seven primers *viz.*, HpmsE001, HpmsE003, HpmsE070, HpmsE081, HpmsE097, HpmsE116 and HpmsE139 which differentiated the resistant and susceptible bulks were used to genotype individual constituents of the bulks along with resistant and susceptible parents for confirmation of polymorphism.

A total of 125 F_2 individuals, which were randomly selected including the constituents of the resistant and susceptible bulks, were genotyped using four SSR primer combinations which clearly differentiated the individual constituents of the constituting bulks. The SSR marker allele segregation was recorded as binary codes. The code '1' was assigned to the F_2 individuals which produced SSR marker amplicons size specific to the resistant parent PBC 80, '2' to those produced SSR marker amplicon specific to the susceptible parent SB 1 and '3' to those F_2 individuals that produced SSR marker amplicon specific to both parents (F_1 type), respectively.

The F_2 individuals were classified into three marker classes based on the codes.The mean lesion diameter of the individuals belonging to each of the marker classes was computed. The significance of differences among the three marker classes for mean lesion size was examined using 'F' test through one-way ANOVA approach using MS excel software.

Variance explained by the SSR marker significantly associated with genomic regions controlling response to anthracnose disease infection was computed following the method suggested by Wu et al. [11].

Broad sense heritability of the response to anthracnose infection was estimated following the method suggested by Hanson et al. [12].

The additive and dominance genetic effects of the linked SSR marker was tested following two-sample 't' test with unequal variances [11].

Inheritance pattern of anthracnose resistance

Mean lesion diameter of fruits sampled from individual F_2 plants were used to estimate skewness, the third degree statistics and kurtosis, the fourth degree statistics [13] to understand the nature of distribution and hence inheritance patternusing 'STATISTICA' software program.Genetic expectations of skewness ($-3/2\ d^2h$) reveal the nature of genetic control of the traits [14] and Kurtosis indicates the relative number of genes controlling the traits [15].

Results and Discussion

Out of 282 SSR markers screened only 60 (Table 1) differentiated the resistant (PBC 80) and susceptible (SB 1) parents indicating low level of parental polymorphism at SSR loci (21.3%), though the parents were diverse for several morphological traits. Kwon et al. [7] also reported low level of polymorphism at the SSR loci among commercial chilli varieties tested.

Bulk segregant analysis

Out of seven primers, which could differentiate the resistant and susceptible bulks, only four *viz.,* HpmsE 081 (Figure 2), HpmsE 097, HpmsE 116 and HpmsE 139 consistently differentiated the individual constituents of the two bulks. Hence these four SSR markers were used to genotype all the 125 F_2 individuals for further confirmation of their association with anthracnose resistance through single marker analysis (Figure 3). These results suggested putative association of the four SSR markers with genomic regions controlling anthracnose resistance.

Single marker analysis

Of the four SSR markers which consistently differentiated the resistant and susceptible bulks and their constituents, only one (HpmsE 081) was found associated with genomic regions controlling anthracnose resistance as indicated by significance of mean squares due to "between marker classes" (Table 2). Lower magnitudes of variance of response to anthracnose disease in terms of overall and true lesion size (explained by linked SSR marker), was amply reflected through low heritability (Table 3) suggesting weak association between the marker and the genetic determinants controlling anthracnose resistance. Voorrips et al. [16] have identified one major quantitative trait locus (QTL) with larger effects on anthracnose resistance (against *C. acutatum*) and three QTLs with smaller effects in the F_2 population (derived from *C. annuum* × *C. chinense* cross).

In single marker analysis, the distance between the linked SSR marker locus and *per cent* trait variation explained by the linked marker are confounded [17]. Further as F_2 individuals are not replicable, the SSR marker-trait (anthracnose resistance) association need to be confirmed in a replicable mapping population such as recombinant inbred lines (RILs) for effective use in marker assisted selection.

Inheritance pattern of anthracnose resistance

Positively skewed leptokurtic distribution of F_2 was observed for average OLD caused due to infection by '*Cc* 38' (Figure 4) isolate

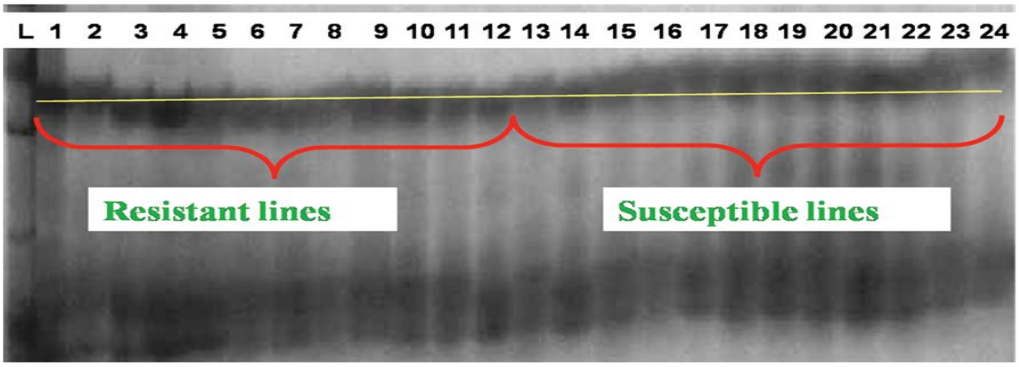

Figure 2: Primer HpmsE 081 differentiating the individual constituents of anthracnose resistant and susceptible bulks of F_2 mapping population in chilli.

Table 1: List of SSR markers polymorphic to F2 mapping population parents (PBC 80 and SB 1) in Capsicum baccatum

Sl.no	Primer	Sl.no	Primer	Sl.no	Primer	Sl.no	Primer	Sl.no	Primer	Sl.no	Primer
1	Hpms E 001	11	Hpms E 035	21	Hpms E 070	31	Hpms E 097	41	Hpms E 146	51	AA840 689
2	Hpms E 003	12	Hpms E 036	22	Hpms E 072	32	Hpms E 100	42	Hpms E 147	52	CAN 09
3	Hpms E 005	13	Hpms E 051	23	Hpms E 074	33	Hpms E 101	43	Hpms 19	53	CM 008
4	Hpms E 012	14	Hpms E 058	24	Hpms E 075	34	Hpms E 104	44	Hpms 24	54	Gpms 3
5	Hpms E 018	15	Hpms E 059	25	Hpms E 078	35	Hpms E 116	45	Hpms 13	55	Gpms 1
6	Hpms E 019	16	Hpms E 063	26	Hpms E 081	36	Hpms E 122	46	Hpms 04	56	Gpms 4
7	Hpms E 026	17	Hpms E 064	27	Hpms E 083	37	Hpms E 125	47	Hpms 1-106	57	Gpms 93
8	Hpms E 027	18	Hpms E 065	28	Hpms E 084	38	Hpms E 139	48	Hpms 1-139	58	Gpms 159
9	Hpms E 029	19	Hpms E 066	29	Hpms E 090	39	Hpms E 141	49	Hpms 1-155	59	Gpms 147
10	Hpms E 032	20	Hpms E 067	30	Hpms E 096	40	Hpms E 145	50	Hpms 1-216	60	Gpms 140

* Significant @ P = 0.05; m_2, m_0 and m_1 takes the meaning as described in the material and methods

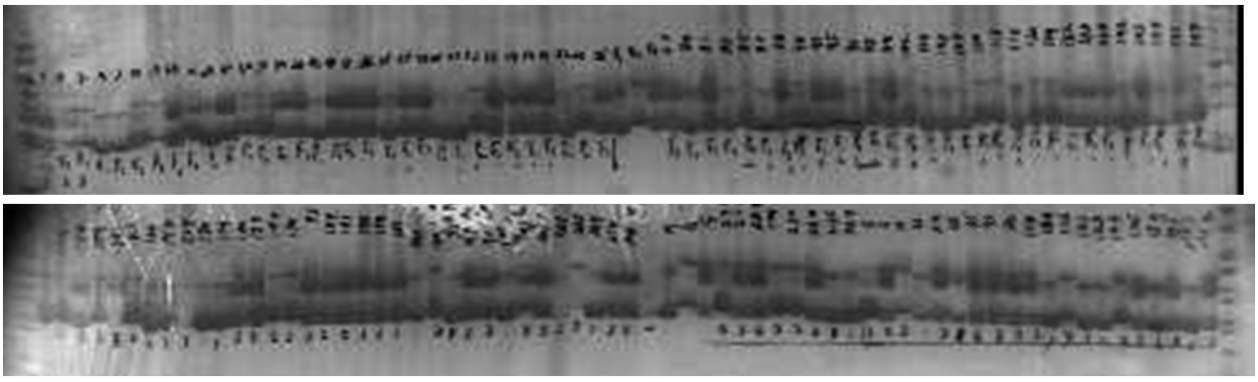

Figure 3: Segregation of SSR marker E 116 alleles among 125 F_2 mapping population individuals in chilli.

while positively skewed, platykurtic distribution of F_2 was observed for average TLD caused due to infection by '*Cc* 38' (Figure 4) isolate. Positively skewed distribution of individuals of F_2 for overall and true lesions produced in response to inoculation by '*Cc* 38' (Figure 5) is on the expected lines as all *C. baccatum* lines have been reported to have some level of resistance to anthracnose. Mild selection is expected to maximize the genetic gain. However, lepto and platy kurtic distribution of F_2 individuals with respect to overall and true lesion produced upon inoculation with '*Cc* 38' (Figures 4 and 5) indicates that fewer to large numbers of genes, respectively are involved in

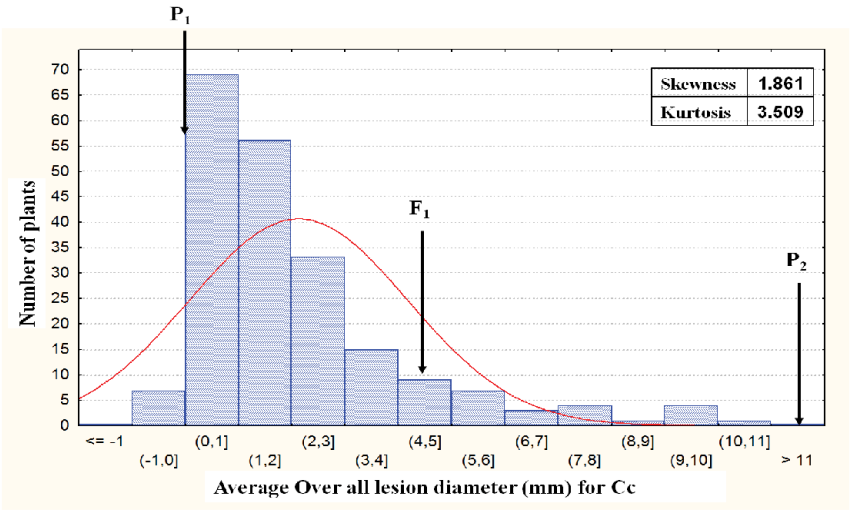

Figure 4: Distribution of intra-Capsicum baccatum F2 mapping population (PBC 80 × SB 1) individuals for average over all size of the lesion (mm) caused by *C. capsici.*

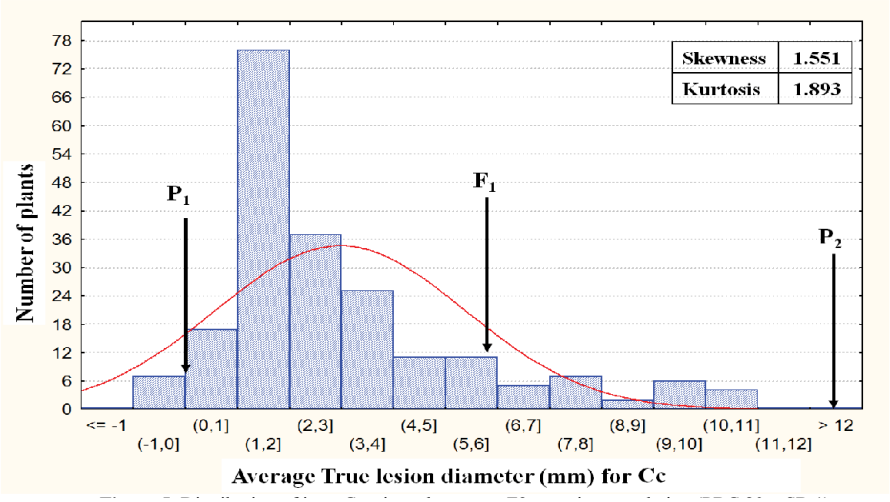

Figure 5: Distribution of intra-Capsicum baccatum F2 mapping population (PBC 80 × SB 1) individuals for average over all size of the lesion (mm) caused by *C. capsici.*

Table 2: Analysis of variance of response to anthracnose disease between and within SSR marker (Hpms E081, Hpms E097, Hpms E116, Hpms E139) classes in an intra *Capsicum baccatum* (PBC 80 × SB 1) F_2 mapping population.

Sl. No.	Response to anthracnose disease infection	Total no. of plants	SSR marker class			'F' cal
			m_2	m_0	m_1	
Hpms E081						
1.	Average OLD for Cc (mm)	121	1.56	2.94	2.47	3.88*
2.	Average TLD for Cc (mm)	121	2.11	3.60	3.49	4.70*
Hpms E097						
1	Average OLD for Cc (mm)	117	2.73	2.44	2.11	0.59
2	Average TLD for Cc (mm)	117	3.36	3.04	2.86	0.33
Hpms E116						
1.	Average OLD for Cc (mm)	123	2.89	2.15	1.92	1.94
2.	Average TLD for Cc (mm)	123	3.54	2.68	2.74	1.31
Hpms E139						
1	Average OLD for Cc (mm)	113	2.44	3.59	3.17	1.49
2	Average TLD for Cc (mm)	112	2.44	3.59	3.17	1.49

* Significant @ P = 0.05; m_2, m_0 and m_1 takes the meaning as described in the material and methods

Table 3: Estimates of variance explained by the linked SSR marker (HpmsE081), broad sense heritability and additive and dominance effect.

SSR Marker	Traits	σ^2_g	Broad sense h^2	Test statistic t_1 (additive effect)	Test statistic t_2 (dominance effect)
HpmsE 081	Average Cc OLD	0.41	0.07	0.12	0.02
	Average Cc TLD	0.59	0.09	0.14	0.05

the response to anthracnose disease infection.Polygenic inheritance of anthracnose resistance was also reported by Voorrips et al. [16]. Several researchers have assessed the resistance to be controlled by a single recessive gene [[18,19,20]. The inheritance patterns vary depending on the resistance sources and the *Colletotrichum* isolates.

Acknowledgments

We thank Kirkhouse Trust, UK for providing fellowship to the senior author and funding the research and Dr. Robert Koebner, Consultant, Kirkhouse Trust, UK for his valuable suggestions throughout the study.

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Deka et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00080.X

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Insect and Mammalian Pests of Large Cardamom (*Amomum subulatum* Roxburgh) in Sikkim Himalaya

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Abstract

Large cardamom is an important cash crop of Sikkim and Darjeeling district of West Bengal. About 3863 MT of large cardamom are produced annually from an area of 26,459 ha in Sikkim region. Insect pests are one of the constraints of large cardamom production. Surveillance was carried out in the major large cardamom growing tracts during 2010 - 12 and incidences of major pests were recorded. Minor insect pests, mammalian pests and new pests in large cardamom ecosystem were documented. Entomopathogens of leaf caterpillar (*Artona chorista*) were identified.

Keywords

Pests; Large cardamom; *Artona*; Shoot fly: Sikkim Himalaya

Introduction

Large cardamom (*Amomum subulatum* Roxburgh) belonging to family *Zingiberaceae* is an important spices crop of North East India and often referred as currency crop [1]. Now-a-days neighboring countries of India like Nepal, Bhutan and Myanmar have also started cultivation of large cardamom [2]. In Sikkim Himalayas it is cultivated since time immemorial and believed to be the native of the state [3]. Sikkim has the largest area as well as production of large cardamom in the globe and act as a big player in the world market [4]. It grows well at altitude ranges from 1000 to 2200 m MSL with well distributed rainfall spread around 200 days with a total of about 3000-3500 mm/year [5-7]. Large cardamom is a sciophyte *i.e.* the plant is grown under shade [8,9]. Crop prefers humid subtropical, semi evergreen forests hills of eastern sub-Himalayan region [10]. It is essentially a cross-pollinated crop due to the heterostylic nature of its flowers. Bumble bee *(Bombus breviceps)* is the major pollinator in large cardamom due to its high pollination efficiency attributed to its big body size and foraging habit [11,12]. In Sikkim the average productivity of large cardamom ranges from 240 to 260 kg/ha [13]. The crop grows somewhat wild in nature and damages due to insect pests are common. It is infested by various pests and diseases causing considerable amount of monetary loss [14-16]. About 3,863 MT of large cardamom are being produced annually from an area around 26,459 ha in Sikkim region [17]. Here we have presented our observations recorded on insect pests of the crop and their IPM management schedule in Sikkim Himalaya.

Materials and Methods

Surveillance was carried out in major large cardamom growing tracts of Sikkim and Darjeeling district of West Bengal during 2010-12. Data were recorded based on two different types of surveillance *viz.*, fixed plot and roving surveillance. Fixed plot surveillance was conducted at Kabi (1567 m amsl), North Sikkim and Pangthang (1952 m amsl), East Sikkim at monthly interval.

Roving surveillance was carried out in four districts of Sikkim, Darjeeling and Kalimpong sub division in Darjeeling district of West Bengal. Data recorded in two gardens from each district / sub divisions and per cent incidence were recorded. Roving surveillance was carried out twice in a year in the months of April - May and October - December.

In both the surveillance, twenty five clumps of large cardamom per garden were selected through random sampling and pests of major and minor importance recorded. Numbers of infested tillers per clumps were noted and per cent incidence for important pests was derived. Natural enemies (entomopathogens) associated with *Artona chorista* in field condition were recorded and pathogenicity test was carried out.

Results and Discussion

In the fixed plot surveillance, occurrence (per cent) of three important insect pests *viz.*, stem borer (*Glyphipterix sp.*), shoot fly (*Merochlorops dimorphus)* and leaf caterpillar (*Artona chorista*) was recorded. *M. dimorphus* was recorded highest among the different pests recorded in the surveyed areas (Table 1). It was 57.00 per cent in Pangthang and 46.30 per cent in Kabi. Roving surveillance was carried out in four districts of Sikkim, Darjeeling and Kalimpong sub division in Darjeeling district of West Bengal. Data on pest incidence and traditional practice of pest management was recorded. Occurrence of shoot fly (*M. dimorphus*) was recorded the highest (44.00 per cent) among the other major pests recorded during roving surveillance followed by leaf caterpillar (*A. chorista*) and stem borer (*Glyphipterix* sp.). From the data it is revealed that occurrence of *M. dimorphus* and *Glyphipterix* sp. was highest in East Sikkim than other districts / sub division of West Bengal. Infestation of *A. chorista* was observed more in Darjeeling district of West Bengal than other surveyed areas (Table 2). In both the surveillance incidence of

Table 1: Incidence of insect pests at fixed plots in Sikkim region during 2010 -12.

Pests	Incidence percent (Range)	
	Kabi	Pangthang
Stem borer (*Glyphipterix* sp.)	0-16	0-20
Shoot fly (*M. dimorphus*)	16-64	16-72
Leaf caterpillar (*A .chorista*)	0-24	0-16

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Received: July 27, 2016 **Accepted:** August 10, 2016 **Published:** August 17, 2016

Table 2: Incidence of insect pest in large cardamom field in different districts of Sikkim and Darjeeling district of West Bengal during 2010-12.

Name of the districts/ Sub Division	Incidence (percent) – Range		
	Insect pests		
	Stem borer (*Glyphipterix sp.*)	Shoot fly (*M. dimorphus*)	Leaf caterpillar (*A. chorista*)
North	12-20	32-40	8-12
East	16-24	40-48	12-16
South	4-12	36-40	0-12
West	8-20	32-40	8-16
Kalimpong	4-8	12-28	0 - 4
Darjeeling	4-16	28-44	16-28

Table 3: Wild mammalian pests of large cardamom in Sikkim region during 2010-12.

Name of the pests	Vernacular name	Period of occurrence	Name of the infested parts	Places
Rodents	Musha	Aug-Dec	Spikes / capsules	Kabi, Pangthang and Dzongu
Himalayan palm civet	Kala	Aug –Dec	capsules	Kabi and Pangthang
Indian wild boar	Bongdel	April- Sept	Clumps	Kabi
Musk deer	Kasturi Mirga	Aug-Dec	Spikes and capsules	Dzongu and Kabi
Monkey	Bandar	April-Sept	Clumps	Kewzing and Namchi

Table 4: New pest recorded in the cardamom ecosystem in Sikkim during 2010-12.

Sl. No.	Pest	Host	Remarks
1	Aphid (*Mollitrichosiphum* sp.)	Utis (*Alnus nepalensis*)	Utis is an important shade tree of large cardamom ecosystem

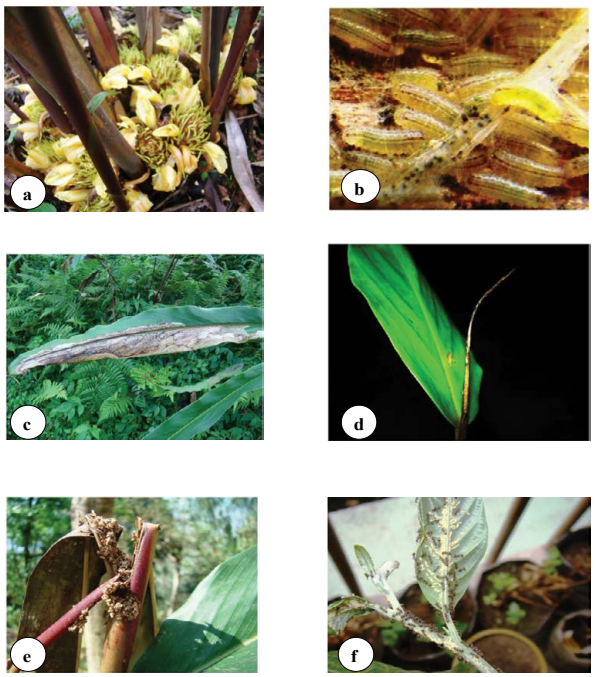

Plate 1: Photographs of insect and pests of large cardamom at Sikkim Himalaya. (**a.** Large cardamom flowering clump; **b.** Congregation of *A. chorista;* **c.** Large cardamom leaf damaged by *A.chorista*; **d.** *M. dimorphus* infested shoot tip*;* **e.** Symptoms of *Glyphipterix* sp. infestation*;* **f.** *Mollitrichosiphum* sp . on *Alnus* twig).

Table 5: Traditional practices of pest management in Sikkim.

Sl./No.	Traditional practice	Against pest
1.	Collection and burning of infested plant parts	Leaf caterpillar, stem borer and shoot fly.
2.	Leave extract – Titepati, Chillowney, Indreni and Tobacco	Chewing and sucking Pests
3.	Field exposures to Sun shine & Bird	Soil insects mainly white grub
4.	Application of ash and ash solution	Ants and aphids
5.	Covering of Cardamom clumps with dried leaves / small branches	Mammalian Pests *viz*., civet, wild boar and deer
6.	Crop rotation	White grub and other soil borne insects
7.	Drainage / Flooding	Soil insects – white grubs
8.	Kerosene	House hold pests / Ants
9.	Mint leaves	House hold pests / House fly

mammalian pests and also a new pest in cardamom ecosystem was recorded. Mammalian pests became a cause of concern in some areas. Presence of five mammalian pests was recorded based on damage caused by them and identified with the help of local farmers (Table 3). Utis (*Alnus nepalensis*) is an important shade tree for large cardamom [18]. An aphid species is commonly observed in the twigs of the tree. The identification of the pest was confirmed from Network Project on Insect Biosystematics-NPIB), Indian Agricultural Research Institute, New Delhi as *Mollitrichosiphum* sp. (Registered in IARI, New Delhi as RRS NO. 2133-2162/11) (Table 4 and Plate 1). Occurrence of some other minor insect pests' *viz*., lace wing bug, fruit borer, aphid, mealy bug, hairy caterpillar, scale insects, grass hopper, rhizome weevil and thrips were also recorded during the surveillance. Attempt was also made to find out the association of natural enemies with these pests. Dead caterpillars of *A. chorista* was observed in the field conditions and collected for isolating the associated pathogens. Four entomopathogens namely *Cladosporium* sp., *Metarrhizium* sp., *Penicillium* sp. and *Verticillium* sp. were isolated and identified. In the pathogenicity test *Cladosporium* sp. was found promising under laboratory conditions. Large cardamom is basically grown organically in the Sikkim Himalayan region. Nine different practices are adopted by the farmers to manage the agricultural and house hold pests in this region (Table 5). These traditional practices may serve as important input for valid scientific investigation, standardization and large scale use in integrated pest management.

Acknowledgements

The authors are thankful to the large cardamom farmers of Sikkim and Darjeeling district of West Bengal, who provided valuable information and cooperation during the course of study. They are also thankful to IARI, New Delhi for the help to identify the insect pest.

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Gundappa et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00081.1

Vegetos- An International Journal of Plant Research

Research Article

A SCITECHNOL JOURNAL

Appraisal of Thrips Population Dynamics in Mango using Weather Based Indices

Gundappa*, Adak T and Shukla PK

Abstract

The aim of this investigation was to assess the dynamics of mango thrips population across 20 mango orchards based on thermal indices under subtropical Lucknow condition during 2013 and 2014. Wide variations in thrips populations were observed across different critical crop phenological stages and seasons. The thrips population was observed between 13th SMW to 24th SMW during 2013 cropping season, whereas it was found from 15th SMW to 28th SMW in the subsequent year. The mean thrips population across standard weeks was highest (6.18 ± 0.14) during 15th SMW followed by 3.13 ± 0.15 in 16th and 1.87 ± 0.06 in 17th SMW during 2013, whereas in the next year (2014), the highest values were 4.67 ± 0.09 followed by 4.19 ± 0.10 and 4.07 ± 0.15 in 16th, 17th and 18th SMW respectively. Step wise regression analysis revealed that maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity, sunshine hours and evaporation could explained 55 per cent variations in thrips population. Different thermal indices *viz*., growing degree days (GDD), heliothermal units (HTU), photothermal units (PTU), were cumulated up to peak population density and a positive and significant correlation was revealed. The best fit polynomial regression analysis indicated that thrips population could be predicted up to 95 per cent using these thermal indices. Thus, it was concluded that use of all these robust indices may be useful in assessing the pest-weather dynamics of mango growing region and serve as a basis for real time pest management advisory.

Keywords

Thermal indices; GDD; Mango; Thrips; Prediction model

Introduction

Mango (*Mangifera indica* L.), one of the major fruit crops of India, is known as the king of fruits for its sweetness, excellent flavour, delicious taste and high nutritive value [1]. It is attacked by several pests during its vegetative and reproductive phases [2]. During the reproductive phase of the crop, pests like hoppers and thrips pose a threat to mango production. Under the era of changing climatic situation, thrips are becoming serious pest on mango. Several species of thrips are observed in mango orchards in Florida, the Caribbean, Central and South America, and Asia [3-5]. Among the thrips species, *Scirtothrips dorsalis* and *Thrips hawaiiensis* were recorded as severe pests of various vegetables, fruits and ornamental crops in eastern Asia [6,7]. In India, *Thrips palmi* was recorded on mango inflorescence [8], from Andhra Pradesh three species of thrips *viz*., *Megalurothrips distalis* (Karny), *Thrips hawaiiensis* and *Haplothrips tenuipennis* are reported on mango [9-11]. Two species of thrips *Scirtothrips mangiferae* Hood and *S. dorsalis* Hood were reported by Kumar and Bhatt [12] respectively, from Gujarat on mango. Kumar et al. [13] reported from Cuttack that *Scirtothrips mangiferae*, *S. dorsalis*, *Rhipiphorothrips cruentatus* Hood are occurring on mango. From Haryana *R. cruentatus* was reported by Dahiya and Lakra [14]. Patel et al. [15] reported three species of thrips on mango rootstock viz., *Pantachaetothrips* sp., *Selenothrips rubrocinctus* Giard, *Caliothrips impurus* Priesner, besides *Scirtothrips dorsalis*. From Kerala Ananthakrishnan and Muraleedharan [16] reported *Selenothrips rubrocinctus* on mango. Recently *Frankliniell schultzei* and *Thrips subnudula* reported on inflorescence of mango from Tamil Nadu [17]. Thrips pose an increasing threat to mango production, which can cause considerable economic loss in mango orchards. Indiscriminate use of synthetic insecticides for routine thrips control is detrimental to non-target organisms and causes pesticide residues on food and in the environment. Other destructive consequences include the development of resistant pest populations, pest resurgence and the outbreak of secondary pest infestations [18,19]. To minimize harmful effects of synthetic pesticides, development of non-polluting plant protection strategies is necessary. For rational use of synthetic pesticides forewarning/prediction models for the destructive pest like thrips are need of the hour.

Thermal characteristics may vary among species, populations, developmental stages, and with other ecological factors such as food source [20-23]. This effect of temperature can be described by specific rate functions of temperature for development (thermal models), which are used in predicting insect pests interaction with biotic and abiotic factors [24]. The pest forecasting models facilitate better preparedness to combat outbreaks of serious insect pests by developing effective pest management strategies well in advance. The temperature-response phenology models simulate the variability in insect development times within a population based on the detailed laboratory assessments of the insect's life history and thus, can provide better results on future pest activity [25]. In this context this study aimed to develop weather based prediction models for mango thrips.

Materials and Methods

A field experiment was conducted for two consecutive seasons (2013 and 2014) on a *Typic ustochrepts* soil in Lucknow, Uttar Pradesh, India at 22 locations. The climate of the experimental site is semi-arid subtropical with hot dry summers and cold winters. Orchards of Mango cv. *Dashehari* of 20-35 years age were selected with planting 7.5×7.5 to 10×10 mt. Each orchard had at least 25 trees. Data on thrips population was recorded on weekly basis from 5 randomly selected trees in four direction of the tree. Thrips count was taken after gently tapping shoot or panicle by holding white paper in the palm. For analysis, mean count per shoot or panicle was taken and expressed as number of thrips per shoot or panicle. Daily weather data of temperature (maximum and minimum), relative humidity (morning and evening), rainfall, wind speed, bright sunshine hours

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Received: July 28, 2016 **Accepted:** August 10, 2016 **Published:** August 17, 2016

and evaporation rates were recorded in the agromet observatory located within the experimental site. Temperature, solar radiation and day length data were used to compute agro climatic models. These models were calculated as follows:

Growing Degree days (GDD) = $\Sigma(T_{Max} + T_{Min}) /2\text{-}T_{Base}$

Where T_{Max} and T_{Min} are the maximum and minimum temperatures (^{0}C) of the day and T_{Base} is base temperature which was taken as 15^{0}C [26].

Heliothermal units (HTU) = GDD × actual bright sunshine hour (n)

Photothermal units (PTU) = GDD × day length (N)

Where N indicates maximum possible bright sunshine hours or day length and calculated as

$N = (24/\pi) \times Ws$

Ws is the sunset hour angle (Radian) = Arc Cosine [-tan (Φ) × tan (σ)]

Φ= Latitude in radian,

σ = Solar declination in radiation, calculated as follows

$\sigma = 0.409 \times \text{Sine}\ [(2 \times \pi \times J)/d\text{-}1.39]$

Where J= Julian days (1 to 365/366) and d = No. of days in the year

These models were computed on daily basis taking 1st September as base for each year since mango is harvested during June and July in Northern India and post harvest vegetative phase started. The thrips occurrence was also plotted against these models and cumulated up to peak/maximum thrips incidence and severity for generating regression based prediction models. Statistical analysis viz., regression equations, were carried out using MS Excel and SPSS packages (Version 12.0). The required statistically significant graphs were drawn using MS Excel/Power Point software packages.

Results and Discussion

Weather situation during study period

Weather parameters particularly maximum and minimum temperatures, rainfall, pan evaporation, relative humidity etc. has profound influence on mango crop phenology in turn pest-weather dynamics. Henceforth, analyses of weather paramneters are essential in assessing the pest population dynamics. The analysis showed maximum temperature ranged between 29.8 to 40.3^{0}C during 2013 crop season and 25.7 to 43.4^{0}C during 2014 crop season at the time of first appearance of thrips population on mango and its peak values. However, the minimum temperature was recorded lower in 2014 season as compared to 2013. A range of 9.5 to 26.8^{0}C was observed in both the seasons. The rainfall was widely distributed in 2013 than 2014 fruiting season. Higher pan evaporation was recorded in 2014 as compared to 2013 (Figure 1).

Population dynamics of mango thrips

A wide variation in thrips occurrence on the mango crop at its different phenological stages was revealed in 20 mango fixed orchards during two consecutive mango cropping season (2013 and 2014). The first appearance of thrips population was observed on 13th standard meteorological week (SMW) and 15th SMW in 2013 and 2014 fruiting season respectively (Table 1). The infestation of thrips on mango crop observed till 24th SMW during 2013 and up to 28th SMW during 2014. Among the 20 mango orchards, highest thrips population density (13.6 /panicle) was recorded in 15th SMW at Kakori orchard (II), followed by 10.5/ panicle and 10.3/panicle in Kakori (I) and NB Dhanewa (I) orchard in 2013 season. The higher values of thrips population density were recorded in 15th and 16th SMW in 2013 while in the next season, it was observed from 17th to 20th SMW. Some of the mango orchards viz., Malihabad (9.45 and 9.15/panicle), Kanar-II (9.4/panicle) [21] and Allupur-I (8.8/panicle) showed higher thrips infestation at flowering and initial fruit setting stage. In 2014 mango season, highest thrips infestation was recorded in CISH Block II as 13.0 /panicle followed by 10.1 /panicle (Allupur (II). Mango orchards

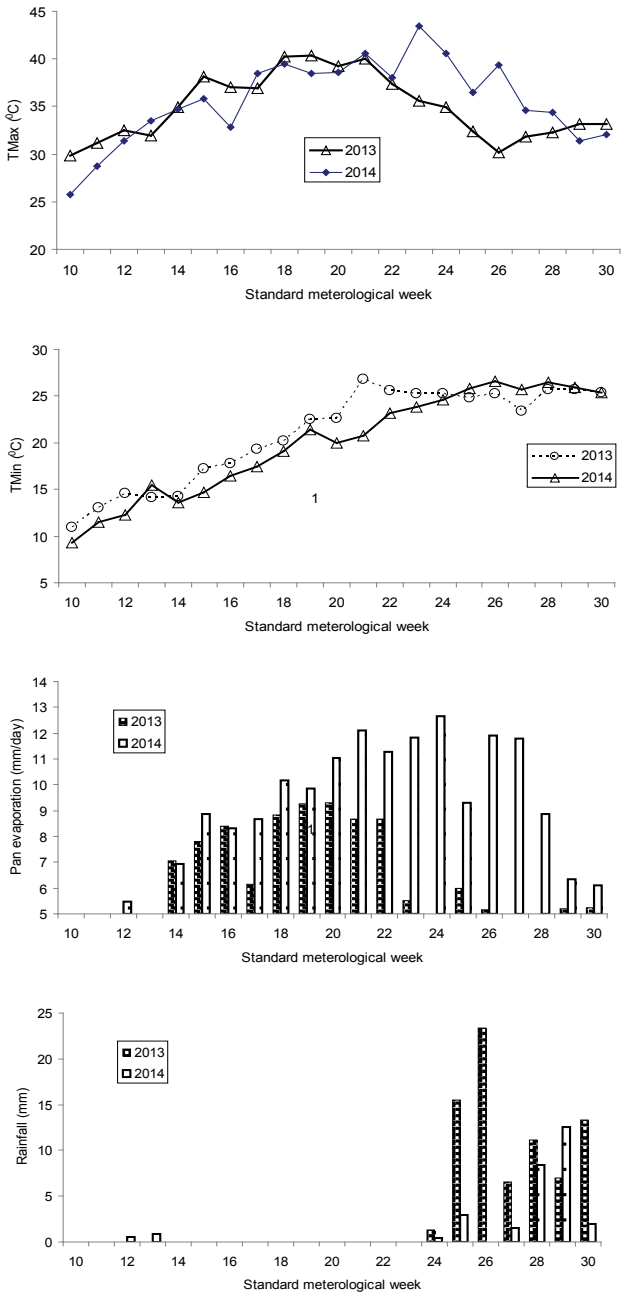

Figure 1: Weather dynamics during study period (2013-14).

of CISH Block III had thrips density of 8.95 to 9.3/panicle followed by Ulrapur-II (8.85/panicle) and Mahmood Nagar-I (8.75 /panicle). Such wide spatio-temporal variations in thrips populations across 20 mango orchards and seasons may be due to differences in cultural practices adopted by the mango growers and weather dynamics. The mean thrips population across standard weeks was highest (6.18 ± 0.14) during 15th SMW followed by 3.13 ± 0.15 in 16th and 1.87 ± 0.06 in 17th SMW during 2013, whereas in the next year (2014), the highest values were 4.67 ± 0.09 followed by 4.19 ± 0.10 and 4.07 ± 0.15 in 16th, 17th and 18th SMW respectively (Table 2). Although there were significant variations in thrips population in SMW and orchards, a range of 0.15 to 13.6 in 2013 and 0.15 to 13.0 in 2014 was found. Peak occurrence of thrips was found during the flowering and fruiting developmental stages of mango. Appearance of thrips population on mango was delayed one week during the year 2014 compared to 2013 (Figure 2), this is attributed to the maximum temperature which was higher and influenced the faster growth and development of thrips. Higher temperature also play key role in the development of vegetative as well as reproductive growth of the mango. Prolonged lower night temperature (minimum temperature) may be responsible factor for the longer stay of thrips on mango for one more month in 2014 cropping season. The peak infestation of thrips on mango was synchronized with the flowering, fruit development and vegetative flushes of mango in the Lucknow region. Changes in the phenology of crop may also influence the pest occurrence. The impact of temperature and rainfall on the mango crop phenology was observed in Lucknow region [27] and pest-weather dynamics during critical crop phenological stages was also reported by Ravishankar et al. [28]. It is obvious that abiotic factors had profound influence on the dynamics of thrips population across regions. In a study in

Table 1: Mean density of mango thrips population in 20 orchards during 2013 and 2014 at Lucknow, Uttara Pradesh.

Year	2013												2014												
	Standard meteorological weeks																								
Location	13	14	15	16	17	18	19	20	21	22	23	24	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Malihabad (Fixed I)	0	4.55	9.15	0.85	0.65	0.4	1	0.5	1	1.1	0	0.4	0	3.95	4.25	4.9	1.75	1.25	0.6	1.2	1	0	0.65	0.75	0.4	0
Malihabad (Fixed II)	0	5	9.45	2.15	0.55	0.25	0	0.8	0.65	1	0	0	1.05	4.45	4.6	6.95	1.45	0.5	0.4	1	1	0.4	0.5	0	0.5	0
Navipana (Fixed I)	0	3.35	3.95	1.15	0.6	0	0	0	0.6	0.25	0	0.6	6.9	5.05	2.35	0.7	0.75	0	0.8	0.65	1.5	0.4	0	0.65	0	0
Navipana (Fixed II)	0	5.85	6.2	3.1	0.75	0	0	0.35	1.2	0.5	0	0.85	6.75	6.6	2.5	1.85	1.75	1	0.75	1	1	0.65	0	0	0	0
Methe Nagar (Fixed I)	0	1.35	5.65	3.45	2.35	0.6	0.15	0.95	1.05	0	0.7	1	0	6.4	3.3	0.95	2.8	1	1	0	0.75	0.6	0	0	0	0
Methe Nagar (Fixed II)	0	2.75	5.6	0.6	2.05	0.8	0	0.55	1.3	2.5	1.15	1.15	2.7	5.5	4.7	3.65	1.2	0.5	0.25	0.8	1.15	0	0	0	0	0
Hafizkhera (Fixed I)	0	0	5.3	1.85	4.25	0.85	0.35	1.15	0.95	0.55	0.25	1.05	1.4	5.7	3.15	0.15	0.5	1	0.5	0.85	1.15	0	0	0	0	0
Hafizkhera (Fixed II)	0	0	4.25	1.55	4.8	1.2	0.25	1.3	1.05	0.65	0.45	0.85	3.1	5.3	3.15	2.75	3	1.15	3.7	0.9	0.75	0.85	0.6	0	0	0
Mahmood Nagar (Fixed I)	0	6	6.9	2.75	1.8	1.15	0.4	0.6	1	1	0	0.4	1.05	3.65	4.55	8.75	2.85	0.65	0.75	1.25	0.75	0	0	0.4	1.1	0
Mahmood Nagar (Fixed II)	0	2.15	7.55	1.2	0.25	0.9	0.65	0.75	1	0.75	0	0	0	4.95	2.75	5.2	1.8	0	0.6	1.15	0	1	0	0.4	0	0
Kakori (Fixed I)	0	6.4	13.6	10.5	3.25	0.85	0.7	0.65	2.5	1	0.9	0	0	0	4.3	7.3	3.25	0	0.4	1	0.6	0	0	0	0	0
Kakori (Fixed II)	0	0.7	8.15	4.55	1.35	1.55	0.95	0.75	1.15	1	0.95	0.25	2.7	5.05	5.95	4.4	3.3	1	1.1	1.55	1.25	1.15	0.9	0.9	1	0
Ulrapur (Fixed I)	0	3.95	1.1	1.4	2.5	0.6	0	0.7	1.25	1.05	0.8	0	5	2.9	7.85	4	1.45	0.8	3	1.25	0.6	0.35	0.4	0	0	0
Ulrapur (Fixed II)	0	3.75	6.45	1.2	3.1	0.25	0.35	0.35	0.25	0.65	0.6	0.4	2.65	3.05	8.85	0	1.3	0.65	0.35	1.65	1	0	0	0	4	0
Kanar (Fixed I)	0	1.1	4.5	5.8	2.3	0.7	1.1	0.65	0.65	0.6	1	0.4	0	5.05	4.4	4.6	1.1	1	0.5	1	0.9	0.9	0	0.25	0	0
Kanar (Fixed II)	0	1.55	7.3	9.4	0.8	0.55	0.55	0.6	1.15	1.5	1.35	0	1.1	4.1	4.85	3.4	1.55	0.25	1.3	0.5	0	0.75	0.75	0	0	0
NB Dhanewa (Fixed I)	1.6	2.75	10.3	1.65	1.95	0.6	1.45	1.05	0.5	1.2	0	1.1	5.1	8.2	1.75	1.5	1	1.25	1.65	0.75	0.75	0.8	0.85	0.4	0	0
NB Dhanewa (Fixed II)	1.75	2.65	6.6	2.25	1.05	0.25	2.25	0.6	1.05	0.55	0	0.75	5.95	5.55	2.4	0.75	0	0	1.15	0	0.85	0	0	0.65	0	0
CISH Block-III (Fixed I)	3.35	7.15	1	1.2	2.25	0.25	2.95	6.2	1.4	1.35	1.65	0	7.4	6.65	8.95	9.3	7.45	6.5	5.35	2	0	0	1.35	0	1.4	0.4
CISH Block-II (Fixed II)	0	1.4	3.8	2.7	3.05	1.85	0.4	0.6	6.2	2.25	0.75	0	1.5	3.9	2.85	3.15	4.1	13	2.75	4.65	1	1	0.9	4.25	0	0
Allupur (Fixed I)	0	1.05	4.9	0.65	0.85	0.8	0.4	1.05	0.85	1.4	0.75	0	0	1.7	2.55	5.15	0.65	0.35	0.95	1.35	2	0	0.65	0.9	0.4	0
Allupur (Fixed II)	0	1.55	4.2	8.8	0.55	1.25	0	0.5	0.6	1	0.8	0	1.95	4.95	2.1	10.1	5.25	2.55	2.5	1.6	1.25	0	0.75	1.15	0	0.9

Table 2: Univariate statistical analysis for the population of mango thrips across the standard meteorological weeks (SMW) during 2013 and 2014 at Lucknow, Uttara Pradesh.

			2013					2014	
SMW	**Mean**	**Range**	**Skeweness**	**Kurtosis**	**SMW**	**Mean**	**Range**	**Skeweness**	**kurtosis**
13	0.30 ± 0.04	3.35-1.60	2.91	8.43	15	2.56 ± 0.13	7.40 - 1.05	0.74	-0.79
14	2.95 ± 0.11	7.15-0.70	0.50	-0.83	16	4.67 ± 0.09	8.20 - 1.70	-0.69	1.51
15	6.18 ± 0.14	13.60-1.00	0.51	1.04	17	4.19 ± 0.10	8.95 - 1.75	1.23	0.82
16	3.13 ± 0.15	10.50-0.60	1.61	1.55	18	4.07 ± 0.15	10.10 - 0.15	0.52	-0.54
17	1.87 ± 0.06	4.80-0.55	0.74	-0.11	19	2.19 ± 0.09	7.45 - 0.65	1.57	2.96
18	0.71 ± 0.02	1.85-0.25	0.61	0.18	20	1.56 ± 0.14	13.00 - 0.35	3.45	12.60
19	0.63 ± 0.04	2.95-0.15	1.85	3.50	21	1.38 ± 0.07	5.35 - 0.40	1.80	3.06
20	0.94 ± 0.06	6.20-0.35	4.25	19.19	22	1.19 ± 0.05	4.65 - 0.40	2.64	10.17
21	1.24 ± 0.06	6.20-0.25	3.76	15.67	23	0.88 ± 0.02	2.00 - 0.60	-0.12	1.09
22	0.99 ± 0.03	2.50-0.25	1.02	1.67	24	0.40 ± 0.02	1.15 - 0.35	0.38	-1.51
23	0.55 ± 0.03	1.65-0.25	0.38	-0.81	25	0.38 ± 0.02	1.35 - 0.40	0.60	-0.79
24	0.42 ± 0.02	1.15-0.25	0.46	-1.35	26	0.49 ± 0.05	4.25 - 0.25	3.54	14.40
	-	-	-	-	27	0.40 ± 0.05	4.00 - 0.40	3.33	12.52
	-	-	-	-	28	0.06 ± 0.01	0.90 - 0.40	3.76	14.53

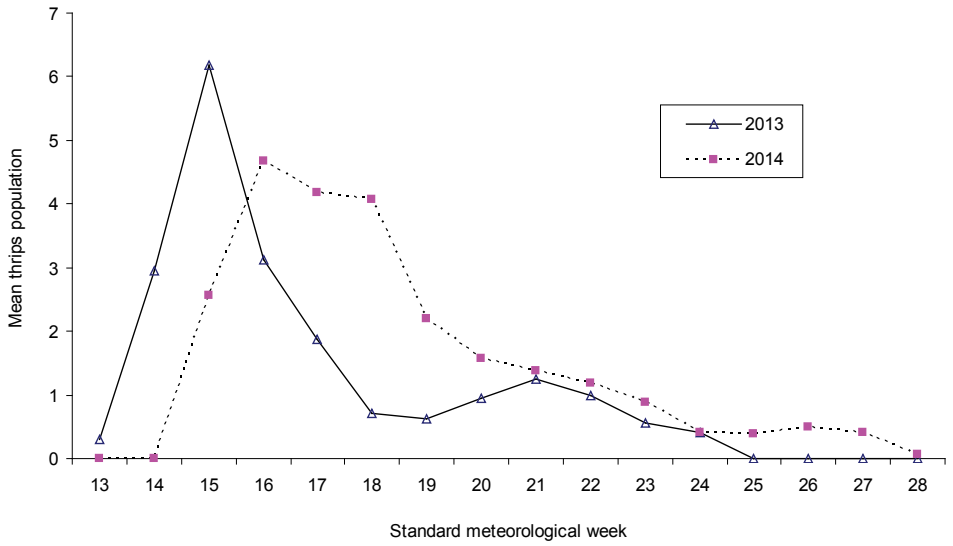

Figure 2: Mean thrips population across different standard meteorological weeks during the study period (2013-14).

Malaysia, three peak populations of mango thrips were recorded and the population was significantly influenced by abiotic factors like temperature, relative humidity on thrips population dynamics [29].

Thrips population relationship with weather parameters

For development of efficient pest management approach, understanding of association between pest population and weather parameters is pre-requisite for any particular location [30-33]. Many workers have reported that weather factors as one amongst the major contributing factor to the outbreak of pests on mango across the country, workers have described the relationship of ecological factor with the pest population under specific location [34-37]. Weather variables including rainfall, temperature, relative humidity and wind have been reported as important factors that significantly affect thrips population [38,39]. In our study thrips population was found negatively correlated with maximum relative humidity (r = -0.792**) and minimum relative humidity (r = -0.670**) during the year 2013. Whereas during the year 2014 thrips population was found negatively correlated with minimum temperature (r = -0.865**), minimum relative humidity (r = -0.634**), maximum relative humidity (r = -0.577**) and evaporation (r = -0.581**) (Table 3). Similar results were reported by Duraimurugam and Jagadish [40], Shukla [41], Panickar and Patel [42], Nandini et al. [43] and Vanisree et al [44], Meena et al. [45] on various crops. Step wise regression analysis revealed that maximum temperature, minimum temperature and maximum relative humidity, minimum relative humidity, sunshine

hours and evaporation could explained 55 per cent variations in thrips population ($p<0.009$) (Table 4). Similar finding was reported in case of coffee borer [46] where correlation studies indicated that different weather parameters influence pest population differently across seasons.

Relationship between thermal indices of mango *vis-à-vis* thrips population

Different thermal indices (growing degree days, Photothermal units and heliothermal unit) were calculated for mango based on the weather data of experimental station. It was estimated that the thermal heat accumulation (GDD) and two other indices (HTU and PTU) were 1007.28^{0}Cday, and 8951.67 and 12608.22 at the time of peak occurrence of thrips population during 2013 while the corresponding values were 1123.04, 8273.42 and 14256.98 of GDD, HTU and PTU respectively during 2014 crop season (Table 5). The significantly positive relationship was observed between thermal indices of mango viz., growing degree days (r = 0.946**), Photothermal units (r = 0.915**) and heliothermal unit (r = 0.940**) with the thrips population. The different thermal indices of mango along with thrips population were subjected for the regression analysis to develop prediction models. This revealed that different regression equation (Linear, Logarithmic and exponential) had explained good amount of variation. During the year 2013 exponential model had explained highest variation for GDD ($y = 0.0421e^{0.0024x}$; R^2=0.97**), HTU ($y = 0.0394e^{0.0003x}$; R^2=0.90**) and PTU ($y = 0.0616e^{0.0002x}$; R^2 = 0.90**) compare to linear and logarithmic models. During the year 2014, compared to exponential and logarithmic models linear models for GDD ($y = 0.0033x - 3.8391$; $R^2 = 0.91$**), HTU ($y = 0.0004x - 3.6391$; $R^2 = 0.90$**) and PTU ($y = 0.0002x - 3.3693$; $R^2 = 0.90$**) had explained highest variation (Table 6). Stepwise regression analysis between different thermal indices and thrips population had explained considerable amount of variation. Growing degree days of mango along with photothermal units ($y = 0.046X_1-0.002X_3-9.56$; R^2= 0.97**) had explained about 97 per cent of variation. All thermal indices parameters together had also explained up to 97 per cent of variation (Thrips population = 0.042×GDD - 0.0003×HTU - 0.002×PTU - 8.97; R^2 = 0.97**) (Table 7). It indicated that GDD and PTU are important thermal indices play a key role for prediction of mango thrips population. Based on three years study (2011-14) at Lucknow, Uttar Pradesh Region with 20-35 years old mango orchards of *cv. Dashehari*, it was found that mango hopper populations could be predicted 80 to 90% using thermal indices [47,48].

Table 3: Correlation coefficients (r) between mango thrips population and weather parameters.

Weather parameter	2013	2014
Temperature (Min)	NS	-0.865**
Relative humidity (Max)	-0.792**	-0.634*
Relative humidity (Min)	-0.670*	-0.577*
Evaporation	NS	-0.581*
**. Correlation is significant at the 0.01 level (2-tailed).		
*. Correlation is significant at the 0.05 level (2-tailed).		

Table 4: Step-wise regression analysis for mango thrips population with weather parameters.

Regression equation	**R^2**	**F**	**Sig.**
$y = 8.76+0.17X_1-0.22X_2-0.09X_3+0.02X_4-0.29X_5-0.10X_6$	0.55	3.99	p<0.009
$y= 8.91+0.12X_1-0.25X_2-0.08X_3+0.03X_4-0.24X_5$	0.54	4.85	p<0.004
$y=9.48+0.03X_1-0.23X_2-0.09X_3+0.05X_4$	0.52	5.76	P<0.002
$y=10.77-0.05X_1-0.12X_2-0.06X_3$	0.50	7.60	p<0.001
$y=4.23++0.08X_1-0.27X_2$	0.44	9.24	p<0.001
Y= Population ; X_1 = Temperature (max) ; X_2= Temperature (min); X_3 = Relative humidity (max); X_4= Relative humidity (min); X_5= Sunshine hours; X_6= Evaporation			

Table 5: Thermal indices during the incidence of mango thrips across two consecutive seasons.

	2013				2014				
Std. week	Thrips Population	GDD	Helio thermal unit	Photothermal Unit	Std. week	Thrips Population	GDD	Helio thermal unit	Photothermal unit
13	0.30	870.0214	6694.368	10567.99	15	2.56	1052.11	9675.61	13168.96
14	2.95	930.3071	9265.216	11473.83	16	4.67	1123.04	8273.42	14256.98
15	6.18	1007.286	8951.676	12608.22	17	4.19	1197.54	12301.28	15408.25
16	3.13	1096.25	8888.737	13917.22	18	4.07	1295.87	12484.18	16883.10
17	1.87	1178.986	10749.92	15169.45	19	2.19	1395.37	12743.66	18388.41
18	0.71	1283.707	14010.01	16724.74	20	1.56	1499.96	15589.56	19968.88
19	0.63	1391.707	13303.82	18340.2	21	1.38	1606.84	15983.27	21580.19
20	0.94	1505.086	15319.32	20037.27	22	1.19	1714.41	17596.56	23190.68
21	1.24	1623.95	15449.41	21810.18	23	0.88	1835.74	18933.47	24967.19
22	0.99	1753.864	15990.74	23724.45	24	0.40	1966.50	15888.69	26840.67
23	0.55	1859.586	13759.69	25291.35	25	0.38	2079.82	8703.79	28432.51
24	0.42	1969.121	11779.05	26876.4	26	0.49	2200.33	18683.86	30067.42
					27	0.40	2318.12	9914.10	31600.91
					28	0.06	2420.79	10717.02	32857.57

Table 6: Linear and non- linear regression analysis for prediction of thrips population as a function of thermal indices.

	2013			2014		
	Equation	R^2	r	Equation	R^2	r
GDD						
Linear	y = 0.004x - 3.7961	0.75	0.867	y = 0.0033x - 3.8391	0.91	0.958
Logarithmic	y = 5.067ln(x) - 34.772	0.67	0.802	y = 5.2482ln(x) - 37.092	0.85	0.925
Exponential	$y = 0.0421e^{0.0024x}$	0.97	0.986	$y = 0.0146e^{0.0025x}$	0.88	0.939
HTU						
Linear	y = 0.0004x - 3.5193	0.60	0.776	y = 0.0004x - 3.6391	0.90	0.948
Logarithmic	y = 4.4634ln(x) - 40.121	0.52	0.724	y = 5.0615ln(x) - 46.168	0.84	0.917
Exponential	$y = 0.0394e^{0.0003x}$	0.90	0.950	$y = 0.0169e^{0.0003x}$	0.86	0.932
PTU						
Linear	y = 0.0003x - 3.1569	0.75	0.866	y = 0.0002x - 3.3693	0.90	0.951
Logarithmic	y = 4.3919ln(x) - 41.188	0.65	0.812	y = 4.6993ln(x) - 45.193	0.83	0.912
Exponential	$y = 0.0616e^{0.0002x}$	0.97	0.986	$y = 0.02e^{0.0002x}$	0.89	0.943

y= Population; GDD – Growing degree days; HTU- Heliothermal units; PTU- Photothermal units;

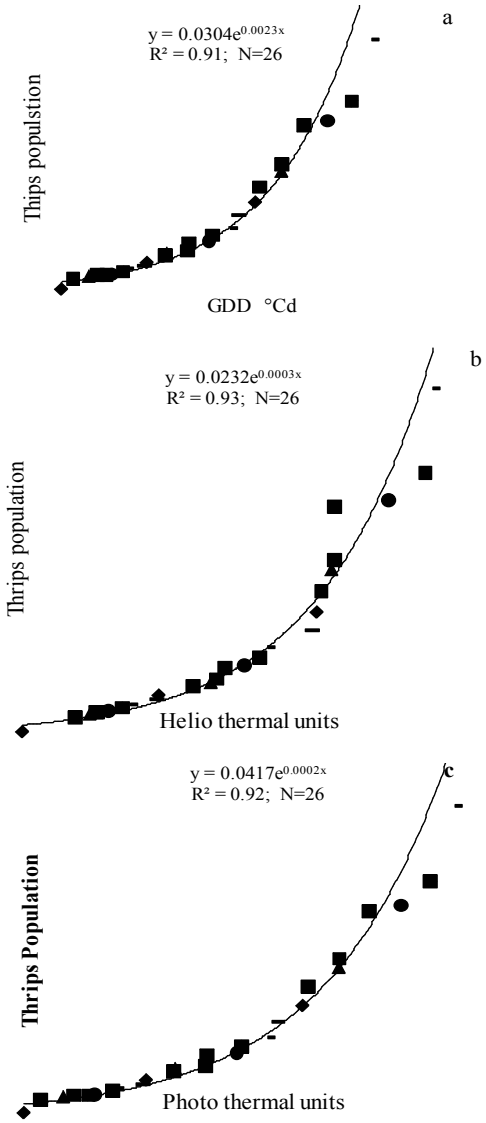

Figure 3: Prediction models for mango thrips using different thermal indices.

Table 7: Step-wise regression analysis between the thrips population and weather based indices.

Linear Regression equation	R^2	F	Sig.
$y = 0.003X_1$-3.58	0.89	205.38	p<0.000
$y = 0.00042X_2$-3.73	0.83	123.35	p<0.000
$y = 0.00023X_3$-3.07	0.88	181.00	p<0.000
$y = 0.009X_1$-0.0007X_2-2.93	0.93	166.36	p<0.000
$y = 0.046X_1$-0.002X_3-9.56	0.97	375.27	p<0.000
$y = 0.00068X_2$-0.0005X_3-1.74	0.91	117.03	p<0.000
$y = 0.042\ X_1$-0.0003X_2-0.002X_3-8.31	0.97	311.80	p<0.000

y= Population X_1 = Growing degree days (GDD) ; X_2= Heliothermal units (HTU); X_3 = Photothermal units (PTU)

Prediction models for mango thrips

Positive relation between thrips populations up to its peak value was observed against these thermal indices, the progressive changes in peak thrips incidence and population. Henceforth, cutting across the years, the peak thrips population were pooled (Figure 3), and was predicted by exponential regression equations of following types;

Mango thrips population = $0.0304e^{0.002\times GDD}$ (n = 26, R^2 = 0.91**)

Mango thrips population = $0.0232e^{0.0003\times HTU}$ (n = 26, R^2 = 0.93**)

Mango thrips population = $0.0417e^{0.0002\times PTU}$ (n = 26, R^2 = 0.92**)

The thermal indices during the reproductive stages particularly, during flowering and fruit set, varied across the seasons and thereby influences the thrips populations. Normally with the progress of reproductive phase in mango *viz.*, flowering, panicle emergence, fruit set and development, hopper started shoots up based on exiting hydrothermal conditions. Application of thermal indices in this study confirmed that variations in thrips populations may be predicted >93 %. Thus, it was concluded that use of all these robust indices may be useful in assessing the pest-weather dynamics of region [48]. Based on all these information, region specific crop simulation dynamics models may be developed to predict and forecast the thrips population so that farmers can adopt control measures well in advance to save the fruit crop being lost.

Acknowledgements

Authors are thankful to the Director, CISH, Lucknow for providing facilities for carrying out the work. Thanks are also due to Indian Council of Agricultural

Research, New Delhi for providing financial assistance under National Initiative of Climate Resilient Agriculture (NICRA) Project entitled, "Understanding the changes in host pest interactions and dynamics in mango under climate change scenario", which facilitated carry out this study.

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Hassanen et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00082.3

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Leaf Dust Accumulation and Air Pollution Tolerance Indices of Three Plant Species Exposed to Urban Particulate Matter Pollution from a Fertilizer Factory

GHassanen RA, Morsy AA and Saleh HA*

Abstract

To assess the dust interception efficiency and air pollution tolerance index (APTI) of three plant species *Cyperus alopecuroides*, *Pluchea dioscoridis* and *Ricinus communis* near fertilize factory in Abo-Zabaal village (Egypt) the present study was undertaken. The air quality has been measured and air pollution index was calculated for the different sites. Also relative water content, total chlorophyll, leaf extract pH and ascorbic acid content were measured. The highest dust deposition was detected in *Pluchea dioscoridis* (0.29) and *Ricinus communis* (0.19) and the values were slightly higher in winter than in summer. The APTI highest values were observed in *R. communis* (25.3) and the lowest ones were recorded in *C. alopecuroides* (12.16) and the values were slightly higher in summer than in winter. Thus plants can be used to intercept dust particles which are of potential health hazards to humans.

Keywords

Air pollution; APTI; Particulate matter; Dust deposition

Introduction

Air Pollution can be defined as the human introduction into the atmosphere of chemicals, particulate matter or biological materials that cause harm or discomfort to humans, or other living organism or damage the environment [1]. Air pollution is a major problem arising mainly from industrialization [2].

Particulate matter (PM) has been widely studied in recent years and the United Nations estimated that over 600 million people in urban areas worldwide were exposed to dangerous levels of air pollutants [3]. Atmospheric PM with aerodynamic diameter <10 μm (PM10) or <2.5 μm (PM2.5) are of considerable concern for public health [4-6]. It has been established that leaves and exposed parts of a plant generally act as persistent absorbers in a polluted environment [7].

Dust particles affect leaf biochemical parameters, bringing about some morphological symptoms. The extent of such effects depends on plant tolerance toward dust particles and on the chemical nature of the dust [8].

Sensitivity and response of plants to air pollutants is variable. The plant species which are more sensitive act as biological indicators of air pollution. The response of plants to air pollution at physiological and biochemical levels can be understood by analyzing the factors that determine resistance and susceptibility. Using plant, as indicator of air pollution is the possibility of synergistic action of pollutants [9].

Response of plants towards air was assessed air pollution tolerance index (APTI) which denotes capability of a plant to combat against air pollution. APTI determination of plants is important because in recent century by increasing industrialization, danger of desertification due to air pollution is threatening the environment. Screening of plants for their sensitivity/tolerance level to air pollutants is important because the sensitive plants can serve as bio- indicator and the tolerant plants as sink for controlling air pollution in urban and industrial areas [10].

The aim of this study is to determine the amount of dust accumulation on leaves and the APTI values of three plant species collected from different distance near Abo Zabal fertilizers factory, Egypt during winter and summer seasons.

Materials and Methods

Study area

The chemical fertilizer production company is installed on a site of 284,000 m^2, 30 km North East of Cairo El Maahd road at El Esmalia canal. Six sites were selected for the study Figure 1. One site was in front of the factory (site F) at 30°16'31.45" N and 31°22'51.67" E and another four sites were downwind direction and separated from each other and from the site at the factory with one kilo (Site 1 at 30°16'18.15"N and 31°22'23.53"E, 2 at 30°16'1.05"N and 31°21'53.83"E, 3 at 30°15'40.10"N and 31°21'24.45" E and 4 at 30°15'25.56"N and 31°21'14.55"E) and the another site was in the other direction (upwind) from the factory as control (site C) 30°18'0.85"N and 31°23'31.55" E.

The leaves of the following species were used for the determination of different parameters

Cyperus alopecuroides (Family *Cyperaceae*): With leaves up to as long as stem, stiff below, curved and somewhat flexuous above; soft; blades 15 mm wide, margins smooth.

Pluchea dioscoridis (Family *Asteraceae*): Its leaves are hairy, simple, lanculate, acute, serrate and sessile leaves which either elliptic or oblong and tapering towards the base.

Ricinus communis (Family *Euphorbiaceae*): The leaves are alternate, orbicular, palmately loped, 1–6 cm broad, with 6–11 toothed lobes, glabrous; long petiole.

Air quality analysis (SO_2, NO_x and suspended particulate matter (SPM))

Sampling and measurements were based on environmental protection agency [11] and American Standard test methods (ASTM:

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Received: February 25, 2016 **Accepted:** August 10, 2016 **Published:** August 17, 2016

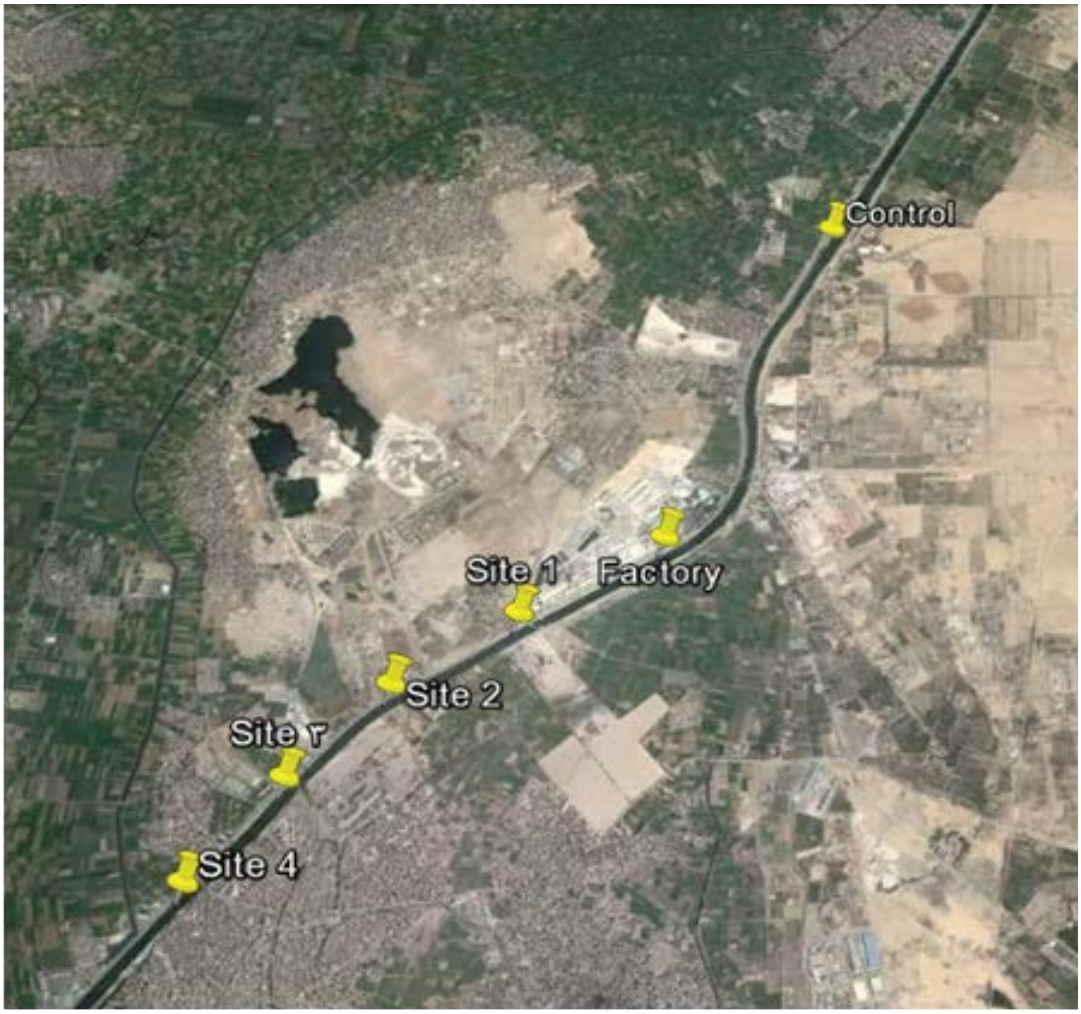

Figure 1: The study area showing the different sites.

ASTM D6216 and NOISH 0600) using Miran Gas Analyze and Thermo Dust meter [12].

Air pollution index (API): was calculated as described by Rao and Rao [13].

API=1/3 [(SPM)/(S_{SPM})+(SO_2)/(S_{SO2})+(NO_X)/(S_{NOx})] X 100 where S_{SPM}, S_{SO2} and S_{NOx} represent the ambient air quality standards for SPM, SO_2 and NO_x.

Index value Remarks (Ambient air quality standards taken for calculation of air pollution index 140 µg/m^3 for SPM, 60 µg/m^3 for SO_2 and 60 µg/m^3 for NO_x) 0-25 Clean air; 26-50 Light air pollution; 51-75 Moderate air pollution 76-100 Heavy air pollution; >100 Severe air pollution.

The leaf dust deposition was calculated and the result expressed as Dust accumulation per hour (gm^{-2} leaf area) [8].

Relative leaf water content (RWC) measured following the method described by Liu and Ding [14], Total chlorophyll content (TCH) was done according to the method described by Maclachlan and Zalik [15]. For determine the leaf extract pH, 5 g of the fresh leaves was homogenized in 10 ml deionized water, and then filtered and the pH of leaf extracts was determined by Hanna GLP pH bench meter.

Ascorbic acid content (expressed as mg/g) was measured using spectrophotometric method described by Heath and Packer [16].

The air pollution tolerance indices of three plants species were determined following the method of Singh and Rao [17]. The formula of APTI is given as follow:

APTI=[A(T+P)+R]/10 Where A=Ascorbic acid content (mg/g), T=total chlorophyll (mg/g), P=pH of leaf extract, and R=relative water content of leaf (%).

(0 to 1=most sensitive; 2 to 16=sensitive; 17 to 29=intermediate; 30to100=tolerant).

Statistical analysis and data confirmation

Data were statistically analyzed ANOVA one way. The computations were done by using SPSS software Version (17.0). Values presented are means ± standard deviation (SD) of three replicates.

Results

The air pollution index for the six sites Table 1 showed that three sites (F, 1 and 2) were under severe air pollution (187.14, 153.8 and 128.33) and these sites the nearest to the fertilizer factory, and one site (3) was under heavy air pollution (92.5), and the control site and the 4Km downwind site (site 4) were light air pollution (42.46 and 49.76).

Dust accumulation on the surface of the leaves of *Cyperus alopecuroides, Pluchea dioscoridis* and *Ricinus communis* recorded in Table 2. The values were higher at the factory site (F) and decreased by increase the distance downwind from the factory. The values were slightly higher at winter than at summer and the highest values was on the leaves of *Pluchea dioscoridis* then *Ricinus communis*. *Cyperus alopecuroides*, is the least accumulator.

It is appear from Table 3 that the relative water content increased in sites F, 1, 2 and 3 if compared with sites C and 4, and the increase in values in the three plant species were higher in summer than in winter and the highest increase were in *R. communis*.

The study showed a change in chlorophyll a+b content recorded in (Table 4). The highest values were at sites C and 4 and the lowest were recorded at sites F, 1, 2 and 3. Total chlorophyll content values decreased as the distance from the factory decreased, and the highest reduction in chlorophyll was in *C. alopecuroides* followed by *R. communis* then *P. dioscoridis*.

Remarkable decrease in pH is shown in (Table 5). The value under the influence of the factory was recorded; the pH values were slightly acidic in sites F and 1 and alkaline to slightly alkaline in the other four sites. The lowest values recorded in *P. dioscoridis*. The values were higher in summer than winter.

Ascorbic acid content (Table 6) increased in the three species after exposure to air pollution, and the highest increases in values were recorded in *C. alopecuroides*.

Air pollution tolerance index recorded in (Table 7). Between the three plant species *R. communis* had the highest value of APTI. All the three species average of the APTI for the six sites were intermediate except *C. alopecuroides* was sensitive during winter.

Discussion

Plant leaves adsorb and, in smaller quantities, absorb particulate and gaseous pollutants [18]. Several studies have evaluated different plant species for their capacity in capturing air pollutants [19]. Dust interception and its accumulation in different plant species depends on various factors, such as leaf shape and size, orientation, texture, presence/absence of hairs, length of petioles etc., weather conditions and direction and speed of wind and anthropogenic activities [20].

The highest values of dust accumulation was shown on the leaves of *P. dioscoridis* which can be explained by the presence of hair on leaves with short leaf petiole that reduces movement of leaves in wind, while the lowest values were on *C. alopecuroides* which may be explained by the smooth texture of the long leaves that help the leaves to flutter during wind, and the vertical position of the leaves which prevents dust retention and in case of *R. communis* it lies in between as it has no hair to hold the dust and its texture not smooth

Table 1: Ambient air quality and air pollution index for different sites during summer and winter of 2012.

Sites		Season	Air pollution parameters				Air pollution index	
			CO mg/m³	SO_2 µg/m³	NO_2 µg/m³	SPM10 µg/m³		
Control	C	Summer	3	40	33	50	42.46	Light air pollution
		Winter	2.8	37	32	52	40.7	Light air pollution
factory	F	Summer	5.5	166	98	170	187.14	Severe air pollution
		Winter	5.2	157	96	178	182.9	Severe air pollution
Km distance downwind from factory	1	Summer	4.2	122	95	140	153.8	Severe air pollution
		Winter	4.1	119	91	147	151.67	Severe air pollution
	2	Summer	3.8	100	89	98	128.33	Severe air pollution
		Winter	3.4	93	84	100	122.14	Severe air pollution
	3	Summer	3.4	70	64	76	92.5	Heavy air pollution
		Winter	3.2	67	56	82	87.85	Heavy air pollution
	4	Summer	3.1	44	40	55	49.76	Light air pollution
		Winter	3	42	37	56	47.22	Light air pollution

Table 2: Mean values of Dust accumulation per hour (g m^{-2} leaf area) on the surface of leaves from the plant species (*C. alopecuroides, P. dioscoridis* and *R. communis*) at different sites (Values were represented as mean ± SD and the values with same letter in the same row are not significant).

Dust accumulation per hour (gm^{-2} leaf area)							
Plant species	Season	Sites					
		Control	Factory	Km distance downwind from the factory			
		C	F	1	2	3	4
C. lopecuroides	Summer	0.003 ± 0.001d	0.052 ± 0.005a	0.027 ± 0.005b	0.009 ± 0.0005c	0.004 ± 0.0001d	0.003 ± 0.0001d
	Winter	0.003 ± 0.001d	0.052 ± 0.005a	0.027 ± 0.005b	0.009 ± 0.0005c	0.004 ± 0.0001d	0.002 ± 0.0001d
P. dioscoridis	Summer	0.02 ± 0.006c	0.28 ± 0.003a	0.1 ± 0.01b	0.09 ± 0.006bc	0.05 ± 0.006c	0.02 ± 0.008c
	Winter	0.02 ± 0.004c	0.29 ± 0.001a	0.15 ± 0.01b	0.1 ± 0.004bc	0.07 ± 0.006c	0.03 ± 0.008c
R. communis	Summer	0.007 ± 0.002c	0.18 ± 0.02 a	0.072 ± 0.005b	0.037 ± 0.003b	0.032 ± 0.003 b	0.0125 ± 0.003c
	Winter	0.006 ± 0.002c	0.19 ± 0.02a	0.08 ± 0.005 b	0.05 ± 0.003b	0.03 ± 0.003b	0.009 ± 0.001c

Table 3: Relative leaf water content (RWC) percentage of the three pant species from different sites during summer and winter of 2012 (Values were represented as mean ± SD and the values with same letter in the same row are not significant). The percentage between every site and control were shown below the values.

Relative leaf water content (RWC) percentage							
Plant species	**Season**	**Sites**					
		Control	**Factory**	**Km distance downwind from the factory**			
		C	**F**	**1**	**2**	**3**	**4**
C. alopecuroides	Summer	76.3 ± 1.2c	92.1 ± 2.1a 20.7%	85.1 ± 0.8b 11.1%	81.1 ± 0.9b 6.2%	79.1 ± 0.3c 3.6%	75.6 ± 1c -1%
	Winter	79.9 ± 0.9c	94.3 ± 0.7a 18%	87.1 ± 0.4b 9%	82.3 ± 0.3c 3%	80.2 ± 1c 0.37%	78.3 ± 0.7c -2.1%
P. dioscoridis	Summer	65.9 ± 1.1e	85.2 ± 0.3a 29.2%	81 ± 0.7b 22.9%	77.4 ± 0.9c 17.5%	70.1 ± 0.6d 6.4%	66.3 ± 0.5e 0.6%
	Winter	70.3 ± 0.7d	87.1 ± 0.8a 23.8%	82.4 ± 1.2b 17.2%	78.1 ± 0.9c 11.1%	70.4 ± 0.5d 0.14%	70.2 ± 1.2d -1%
R. communis	Summer	56.3 ± 1.6d	81.1 ± 1.4a 44%	72.1 ± 0.9b 28%	66.9 ± 1.1c 18.8%	60.8 ± .3d 8%	57.2 ± 1.2d 1.5%
	Winter	61.2 ± 0.7d	83.2 ± 1a 35%	74.2 ± 0.9b 21.1%	70.3 ± 0.7c 14.8%	62 ± 1d 1.3%	60.1 ± 0.5d -1.1%

Table 4: Total chlorophyll content (mg/g fresh wt.) of the three pant species from different sites during summer and winter of 2012 (Values were represented as mean ± SD and the values with same letter in the same row are not significant). The percentage between every site and control were shown below the values.

Total chlorophyll content (mg/g fresh wt.)							
Plant species	**Season**	**Sites**					
		Control	**Factory**	**Km distance downwind from the factory**			
		C	**F**	**1**	**2**	**3**	**4**
C. alopecuroides	Summer	14.7 ± 0.3a	6.2 ± 0.2c -57.8%	6.6 ± 0.3c -55.1%	8.2 ± 0.1bc -44%	10 ± 0.1b -31.9%	13.6 ± 0.1a -7.5%
	Winter	14.06 ± 0.3a	5.2 ± 0.2c -63%	6 ± 0.3c -57.3%	7.6 ± 0.1bc -46%	9.66 ± 0.4b -31.3%	13.6 ± 0.7a -3.2%
P. dioscoridis	Summer	22.7 ± 0.15a	11.6 ± 0.2c -48.9%	12.9 ± 0.15c -43.25	13.1 ± 0.23c -42.3%	17.2 ± 0.3b -24.2%	21.5 ± 0.1a -5.3%
	Winter	21.9 ± 0.15a	10.6 ± 0.2c -51.6	11.9 ± 0.15c -45.7%	12.6 ± 0.23c -45%	16.63 ± 0.2b -24%	20.8 ± 0.6a -5%
R. communis	Summer	19.3 ± 0.2a	8.7 ± 0.3c -54.9	9.3 ± 0.34c -51.8%	10.4 ± 0.05bc -46.1%	12.5 ± 0.17b -35.2	18.2 ± 0.4a -5.7%
	Winter	17.24 ± 0.2a	7.9 ± 0.3c -54.2	8.6 ± 0.34c -50.1%	10 ± 0.05bc 41.9%	11.9 ± 0.4b -30.9%	18.2 ± 0.9a -5.7%

Table 5: Leaf extract pH of three plant species taken from different sites during summer and winter of 2012. (Values were represented as mean ± SD and the values with same letter in the same row are not significant). The percentage between every site and control were shown below the values.

Plant species	**season**	**Sites**					
		Control	**Factory**	**Km distance downwind from the factory**			
		C	**F**	**1**	**2**	**3**	**4**
C. alopecuroides	Summer	7.81 ± 0.2a	6.2 ± 0.2a	6.75 ± 0.5a	7.1 ± 0.3a	7.61 ± 0.13a	7.97 ± 0.1a
	Winter	7.85 ± 0.1a	6.25 ± .15a	6.83 ± 0.2 a	7.2 ± 0.19 a	7.73 ± 0.12a	8 ± 0.13a
P. dioscoridis	Summer	8.13 ± 0.3a	5.72 ± 0.2b	6.7 ± 0.3a	7.36 ± 0.2a	7.85 ± 0.2a	8.2 ± 0.2a
	Winter	8.19 ± 0.2a	5.81 ± 0.2b	6.91 ± 0.21a	7.61 ± 0.4a	7.91 ± 0.19a	8.3 ± 0.1a
R. communis	Summer	8.03 ± 0.5a	4.9 ± 0.15b	5.33 ± 0.3b	6.91 ± 0.13a	7.71 ± 0.21a	8.15 ± 0.3a
	Winter	8.12 ± 0.1a	5.1 ± 0.1b	5.37 ± 0.5b	6.97 ± 0.05a	7.98 ± 0.26a	8.2 ± 0.2a

to prevent dust retention. The influence of leaf characteristics on dust accumulation have also been studied [21,22].

The values of dust accumulation were slightly higher in winter than at summer. Prajapati and Tripathi [8] explained high dust accumulation in the winter season as wet surfaces of leaves may help in capturing dust. High wind speed may be the reason for the relatively lower dust accumulation in the summer than in winter.

As an important physiological factor, RWC is affected by the air pollution directly. It was proved that when plants suffered from air pollution, their stomatal density would increase [23], which led a decrease of the water content in plant tissues [24]. Thus higher water content within a plant body can help to maintain its physiological balance and enhance plants tolerance ability under the stress conditions (Agarwal and Tiwari, 1997). The increase in relative water content in polluted sites than the control had been recorded by Agbaire and Esiefarienhe [25], Gharge and Menon [26] and Rai et al. [27]. The same was recorded in this study. The values in winter are higher than summer that might be associated with a decrease in moisture availability.

Air pollution has strong influence on TCH in plant leaves, such as SO_2, an important pollutant affecting plants' health. In general, higher

Table 6: Ascorbic acid content (mg/g fresh wt.) of the three plant species taken from different sites during summer and winter of 2012. (Values were represented as mean ± SD and the values with same letter in the same row are not significant). The percentage between every site and control were shown below the values.

Ascorbic acid content (mg/g fresh wt.)							
Plant species	**Season**	**Sites**					
		Control	**Factory**	**Km distance downwind from the factory**			
		C	**F**	**1**	**2**	**3**	**4**
C. alopecuroides	Summer	2.18 ± 0.072c	10.14 ± 0.14a 365.1%	8.60 ± 0.25b 294.4%	7.70 ± 0.05b 253.2%	3.92 ± 0.07c 79.8%	2.82 ± 0.08c 29.3%
	Winter	1.88 ± 0.13c	8.12 ± 0.30a 331.9%	7.11 ± 0.05a 278.2%	5.90 ± 0.44b 213%	3.02 ± 0.50c 60.6%	2.00 ± 0.21c 6.4%
P. dioscoridis	Summer	3.54 ± 0.26c	6.38 ± 0.25a 80%	5.80 ± 0.20a 63.8%	5.27 ± 0.30ab 48.8%	4.90 ± 0.20b 38.4%	4.00 ± 0.20bc 12.9%
	Winter	3.11 ± 0.33c	6.12 ± 0.30a 96.7%	5.30 ± 0.37a 70.4%	4.50 ± 0.55ab 44.6%	4.20 ± 0.10b 35%	3.21 ± 0.11c 3.2%
R. communis	Summer	4.75 ± 0.25d	12.66 ± 0.15a 166.5%	11.10 ± 0.1b 133.6%	8.82 ± 0.09c 85.6%	7.54 ± 0.28c 58.7%	5.08 ± 0.15d 6.9%
	Winter	3.21 ± 0.23d	9.30 ± 0.51a 189.7%	8.01 ± 0.36ab 149.5%	6.31 ± 0.40b 96.5%	4.90 ± 0.32c 52.6%	3.50 ± 0.21d 9%

Table 7: Air pollution tolerance index of the three plant species taken from different sites during summer and winter of 2012. (I=intermediate and S=sensitive)

Plant species	**season**	**Sites**						**Average**
		Control	**Factory**	**Km distance downwind from the factory**				
		C	**F**	**1**	**2**	**3**	**4**	
C. alopecuroides	Summer	12.53 S	21.78 I	19.99 I	19.89 I	14.81 S	13.64 S	Intermediate
	Winter	12.76 S	18.72 I	17.83 I	16.96 S	13.27 S	12.16 S	Sensitive
P. dioscoridis	Summer	14.62 S	19.57 I	19.37 I	18.52 I	19.19 I	18.51 I	Intermediate
	Winter	16.38 S	18.75 I	18.21 I	16.9 S	17.34 I	16.36 S	Intermediate
R. communis	Summer	18.61 I	25.32 I	23.44 I	21.95 I	21.31 I	19.1 I	Intermediate
	Winter	14.2 S	20.41 I	18.61 I	17.73 I	15.84 S	15.25 S	Intermediate

concentration of SO_2 will reduce the leaf TCH [28]. Photosynthesis was reduced in plants when the leaf pH was low [27]. Our results indicate that the TCH value in plant leaves decreased with higher concentration of SO_2 which is in agreement with previous studies [29,30].

The result of pH of the leaf extract was acidic in severe air pollution at the factory. The same result was recorded by Bakiyaraj and Ayyappan [10] and Nayak et al. [31]. According to Zhen [32], when plants are suffering from air pollutants (especially SO_2), their cellular fluid would produce massive H^+ to react with SO_2, which enters through stomata and intercellular space from air, so that H_2SO_4 is generated and then leaf pH reduces.

Ascorbic acid is a strong reluctant and it activates many physiological and defense mechanism. Its reducing power is directly proportional to its concentration [33]. Ascorbic acid content increased in the three plant species at the sites of high and severe air pollution. The same was reported by Agbaire and Esiefarienhe [25], Gharge and Menon [26] and Rai et al. [27].

The air pollution tolerance index (APTI) plays a significant role to determine resistivity and susceptibility of plant species against pollution levels. APTI is used to rank plant species in order of tolerance to air pollution [31].

The three plant species found to be intermediate tolerant at the sever air pollution at the factory site and the highest was *R. communis* which may be related to high ascorbic acid (AA) and total chlorophyll (TCH) than two species, the lowest was *C. alopecuroides* which can be related the low values of AA and TCH as suggested by Kuddus, et al., [34] that high AA and TCH related to tolerance species, so we can planted *R. communis* around the fertilizer factory for creating green environment and decrease the air pollution effect.

In conclusion plants can be used to intercept dust particles which are of potential health hazards to humans. The dust interception capacity of different leaves depends on leaf structure, phyllotaxy, presence/absence of hairs, presence of wax on leaf surface, length of petioles, and canopy structure. Plants with a waxy coating, rough leaf surfaces, and short petioles tend to accumulate more dust than plants with long petioles and smoother leaf surface.

APTI determinations are of importance because with increased industrialization, there is increasing danger of desertification due to air pollution. The results of such studies are therefore handy for future planning and may be helpful to bring out possible control measures. It is worth noting that combining a variety of parameters gave a more reliable result than when based on a single biochemical parameter.

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Anil Kumar et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00083.5

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Combining Ability and Heterosis Study for Fibre Yield and Yield Attributing Characters in Tossa Jute (*Corchorus olitorius* L)

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Abstract

Present study was carried out to estimate combining ability and heterosis for fibre yield and yield attributing characters among diverse tossa jute germplasm lines and in 21 F_1 hybrids derived from 7 x 7 half diallel mating design. Analysis of variance revealed significance of parent's *vs* hybrids for most of the characters indicating presence of heterosis. High *gca* effects for fibre yield, plant height and green weight was recorded by OIN-255. High significant *sca* effects for fibre yield were recorded in OMU-19 x OMU-27 (19.53**) and OIN-255 x OEX-32 (15.50*) crosses. Variances for general combining ability and specific combining ability were significant for few characters indicating additive and non-additive gene actions for those characters. High standard heterosis for fibre yield was recorded by OMU-19 x OMU-27 (41.01**) and OIN-255 x OEX-32 (32.47*) crosses. Genetic parameters indicated that plant height, green weight and stick weight were controlled by additive gene action and basal diameter and fibre yield controlled by non-additive gene action.

Keywords

Combining ability; Heterosis; Fibre yield; Genetic parameters

Introduction

Jute is a major bast fibre crop mainly grown in India and Bangladesh. In India, Jute is mainly grown in West Bengal, parts of Bihar, Assam and Orissa. India produces raw jute fibre of 11.41 million bales from 0.86 million ha of area with an average productivity of 23.72 q/ha in 2013– 14 (www.jutecomm.gov.in). In the year 2013-14 India exported raw jute of 216 M. tonnes worth 1880 crores even though the yields of cultivated varieties have been plateaued, as most of the released varieties were developed from few selected parents [1]. So there is an urgent need to increase the yield levels per unit area as the crop area is decreasing year by year and facing stiff competition from other highly remunerative crops.

Diallel mating designs provide information regarding combining ability, heterosis and genetic parameters useful in planning future breeding programmes. Based on the combining ability analysis, characters with high GCA effect indicate to additive gene effects and high SCA effect indicates dominant gene effects controlling those characters. And non-significance of GCA and SCA effects indicated the presence of non-allelic interactions controlling those characters [2] (Fehr, 1993). But understanding the genetic parameters for fibre yield and yield attributing characters will allow the breeders to precisely execute their breeding and selection programmes. Current study was managed to estimate the genetic parameters and the mode of inheritance for fibre yield and yield attributing traits of *Corchorus olitorius* in a set of half diallel crosses.

Materials and Methods

Seven germplasm lines were selected based on diversity analysis and used to make 7 X 7 half diallel mating design. Twenty one F_1s along with parents and checks were sown in randomized complete block design with three replications. Recommended package of practices were applied to raise a healthy crop. Crop was harvested at 120 days after sowing and data for plant height, basal diameter and green weight were recorded. Data for fibre yield and stick weight was recorded after retting. The data were analyzed using INDOSTAT statistical software, Hyderabad, India, for combining ability, heterosis and genetic parameters based on Griffin's numerical (Table 1) and Haymen's graphical approach (Table 5).

Results and Discussion

Analysis of variance revealed significant differences among treatments for all characters except for basal diameter. Parent's *vs* hybrids were significant for all the characters except for basal diameter indicating the presence of heterosis for those characters.

Significant GCA effects were observed for each fiber yield and yield attributing characters except basal diameter (Table 2). The estimated GCA effect for the seven parents significantly varied for both fiber yield and yield attributing characters. Among the genotypes, OIN-255 was the best general combiner for fiber yield, plant height and green weight. OMU-27 was also good combiner for plant height and stick weight. OMU-07 recorded negative combining ability effects for plant height, green weight, stick weight and fibre yield. Variance due to GCA were lower than the corresponding SCA variance for all the characters indicating the presence of non-additive gene action controlling the traits. The lower ratio of σ2 GCA/ σ2 SCA indicates a predominance of non-additive gene action (dominant or epistasis) in the inheritance of traits [3] (Sprague and Tatum, 1942). Non-additive gene action for fibre yield and yield attributing characters were earlier reported [4-7].

For fibre yield SCA effects were significant for only two crosses, those best specific combinations were OMU-19 x OMU-27 (19.53**) and OIN-255 x OEX-32 (15.50*) (Table 3). For plant height OMU-07 x OIN-255 (39.23*) and for green weight OMU-19 x OMU-27 (461.95**) and OIJ-211 x OEX-29 (385.15**). Some of these crosses (OIN-255 x OEX-32 for fibre yield, OMU-07 x OIN-255 for plant height) were related to their parents' GCA effects; at least one of their parents had high or average GCA effects for particular traits. Similar kinds of results were also reported [4,6-9].

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Received: July 27, 2016 **Accepted:** August 16, 2016 **Published:** August 23, 2016

Table 1: Analysis of variance for fibre yield and yield attributing traits.

Source	df	Plant height	Basal diameter	Green weight	Stick weight	Fibre yield
Replications	2	2040.33	10.57	211318*	1749.34	123.54
Treatments	27	2023.63**	3.67	142478.4**	4292.66**	436.14**
Parents	6	4601.95**	1.34	135915.6	3420.31*	194.66
Hybrids	20	759.09	4.18	127467.2*	3539.21**	265.34**
Parents vs Hybrids	1	11844.62**	7.3	482079.6**	24595.67**	5301.08**
Error	54	796.21	4.14	62967.71	1406.12	114.56
Total	83	1225.47	4.14	92407.34	2353.38	219.39

Table 2: General combining ability for fibre yield and yield attributing traits.

Parent	Plant height	Basal diameter	Green weight	Stick weight	Fibre yield
OMU-19	0.95	0.03	-4.04	4.09	2.42
OMU-07	-23.54**	-0.12	-102.37*	-25.69**	-5.00*
OIN-255	10.11*	0.23	134.13**	12.79	4.49*
OIJ-211	1.49	0.27	-9.98	-3.79	-2.07
OMU-27	12.87*	0.2	73.73	14.59*	3.28
OEX-29	2.4	-0.27	-64.52	-1.07	-3.29
OEX-32	-4.29	-0.33	-26.93	-0.93	0.16
SE (g)	18.64	1.35	165.76	24.77	7.07
SE (gi-gj)	28.47	2.05	253.20	37.84	10.80
σ^2 GCA	112.15	-0.09	4153.37	126.26	8.611
σ^2 SCA	237.63	0.03	23395.91	912.39	115.67
σ^2 GCA/ σ^2 SCA	0.47	-2.89	0.17	0.13	0.07
σ^2 A	224.31	-0.18	8306.74	252.53	17.22
σ^2 D	237.63	0.03	23395.91	912.39	115.67

However, some of the best specific combinations (OMU-19 x OMU-27 for fibre yield, OMU-19 x OMU-27 for green weight) were obtained from parents having poor and negative GCA effects. Similar kinds of results were reported by Kumar and Palve [6] and Sengupta [7].

Heterosis

Mean performance, specific combining ability and standard heterosis (Over JRO 2014) values were presented in Table 4. High mean fibre yield was recorded by OMU-19 x OMU-27 (16.73g) and OIN-255 x OEX-32 (15.72g) and best check JRO 204 fibre yield was 11.86 g. High significant and positive standard heterosis for fibre yield was recorded by OMU-19 x OMU-27 (41.01**) and OIN-255 x OEX-32 (32.47*) and these crosses were recorded high mean values and high SCA effects. Yield is cumulative effect of other yield attributing characters and this phenomenon can be observed in high yielding F_1s OMU-19 x OMU-27 and OIN-255 x OEX-32 which recorded high mean values for yield attributing characters like plant height, basal diameter, green weight and stick weight.

No hybrid was recorded significant positive standard heterosis for plant height, Basal diameter, similar reports of non-significant heterosis was by Basak and Dana [10] and green weight indicating that all hybrids were on per with the best check for these characters.

Estimates of genetic parameters D (Additive effects) and H1 (Dominance effects) were significant for plant height (1253.77* and 1201.24*) (Table 5), this indicates the presence of both additive and non-additive gene interactions governing the character. For the characters green weight (111143.00*), stick weight (4028.60*) and fibre yield (398.66*) H1 was only significant indicating the presence of non-additive gene interactions. Average degree of dominance for basal diameter (1.39), green weight (2.22), stick weight (2.46) and fibre yield (3.87) recorded more than unit value, so these characters may be governed by over dominance, whereas plant height recorded 0.98 which is equal to one indicating presence of complete dominance. Plant height (2.99) and stick weight (1.37) recorded more than unit value for the ratio of dominance/ recessive genes indicating these characters having excess of dominant genes in the parents whereas, green weight (1.09) and fibre yield (1.07) were recorded near to the value of one indicating equal proportion of dominance and recessive genes in the parents, whereas the character basal diameter recorded value less than one (0.40) indicating presence of excess of recessive genes in the parents. For the characters plant height (0.19), basal diameter (0.28), stick weight (0.22), and fibre yield (0.23) positive and negative alleles were unequally distributed (H1/H2 values) and for green weight (0.25) positive and negative alleles were equally distributed in the parents.

Environment component was significant for all the characters under study, indicating influence of environment on character expression. And these results were supported by low heritability for all the characters. Number of dominant gene groups (h2/H2) for plant height (2.27), basal diameter (0.30), green weight (0.70), stick weight (1.23) and fibre yield (2.63) were recorded.

Conclusion

For productivity enhancement in jute, exploitation of non-additive (dominance and epistatic interaction) variance is very much needed which directly depends on estimation of combining ability and identification of superior crosses. Therefore the information generated under present work Vis a Vis identified superior crosses (OMU-19 x OMU-27 (16.73g) and OIN-255 x OEX-32 (15.72g) can

Table 3: Specific combining ability for fibre yield and yield attributing traits.

Cross	Plant height	Basal diameter	Green weight	Stick weight	Fibre yield
OMU-19 x OMU-07	4.33	-0.08	-15.90	13.48	1.82
OMU-19 x OIN-255	2.20	-0.50	-208.41	-25.67	-3.00
OMU-19 x OIJ-211	-11.34	0.68	-71.59	-24.62	-8.90
OMU-19 x OMU-27	9.44	1.66	461.95**	65.19**	19.53**
OMU-19 x OEX-29	-17.82	-1.51	-152.98	56.19**	11.44
OMU-19 x OEX-32	19.80	0.67	98.82	10.05	5.65
OMU-07 x OIN-255	39.23*	0.99	156.71	27.11	10.08
OMU-07 x OIJ-211	23.99	-0.03	120.50	29.03	9.65
OMU-07 x OMU-27	18.34	0.17	69.04	18.31	6.29
OMU-07 x OEX-29	19.00	0.04	215.84	28.65	8.20
OMU-07 x OEX-32	10.10	-1.36	-24.11	-35.15	-7.25
OIN-255 x OIJ-211	3.59	-2.15	-273.10*	-27.11	-4.18
OIN-255 x OMU-27	8.48	1.10	49.89	40.09	5.45
OIN-255 x OEX-29	-5.03	2.19	87.63	-3.57	6.30
OIN-255 x OEX-32	-23.09	0.27	317.64	51.22*	15.50*
OIJ-211 x OMU-27	-16.43	-0.36	11.96	-22.58	-3.63
OIJ-211 x OEX-29	25.29	2.13	385.15**	22.41	10.13
OIJ-211 x OEX-32	17.39	0.24	-52.96	12.60	6.14
OMU-27 x OEX-29	7.91	0.21	-2.53	6.69	10.24
OMU-27 x OEX-32	5.74	-0.03	-116.29	-25.77	6.11
OEX-29 x OEX-32	2.81	-0.66	-138.76	9.11	-9.30
Sij	41.60	3.00	369.98	55.29	15.78
Sij--Sik	61.81	4.46	549.64	82.14	23.44
Sij--Skl	57.81	4.17	514.14	76.83	21.93

Table 4: Mean performance, SCA and standard heterosis of fibre yield and yield attributing traits.

Cross	Plant height (cm)			Basal diameter (mm)			Green weight (g)			Stick weight (g)			Fibre yield (g)		
	Mean	SCA	Std. heterosis	Mean	SCA	Std. heterosis	Mean	SCA	Std. heterosis	Mean	SCA	Std. heterosis	Mean	SCA	Std. heterosis
OMU-19 x OMU-07	326.6	4.33	-1.74	14.9	-0.08	-6.62	230.51	-15.90	-19.97	36.07	13.48	25.87	11.53	1.82	-2.81
OMU-19 x OIN-255	358.13	2.20	7.74	14.84	-0.50	-7.04	239.31	-208.41	-16.91	35.93	-25.67	25.41	12.47	-3.00	5.06
OMU-19 x OIJ-211	335.97	-11.34	1.07	16.06	0.68	0.65	237.85	-71.59	-17.42	32.83	-24.62	14.56	9.97	-8.90	-15.96
OMU-19 x OMU-27	368.13	9.44	10.75	16.98	1.66	6.39	361.30	461.95**	25.44	54.47	65.19**	90.09**	16.73	19.53**	41.01**
OMU-19 x OEX-29	330.4	-17.82	-0.6	13.32	-1.51	-16.52	210.66	-152.98	-26.86	49.53	56.19**	72.87**	13.80	11.44	16.29
OMU-19 x OEX-32	361.33	19.80	8.7	15.35	0.67	-3.82	268.54	98.82	-6.76	40.33	10.05	40.76	13.33	5.65	12.36
OMU-07 x OIN-255	370.67	39.23*	11.51	16.18	0.99	1.36	292.67	156.71	1.61	40.53	27.11	41.46	13.60	10.08	14.61
OMU-07 x OIJ-211	346.8	23.99	4.33	15.19	-0.03	-4.80	256.60	120.50	-10.91	37.60	29.03	31.22	12.20	9.65	2.81
OMU-07 x OMU-27	352.53	18.34	6.06	15.33	0.17	-3.95	263.05	69.04	-8.67	39.13	18.31	36.58	12.60	6.29	6.18
OMU-07 x OEX-29	342.73	19.00	3.11	14.72	0.04	-7.75	264.76	215.84	-8.08	38.07	28.65	32.85	11.67	8.20	-1.69
OMU-07 x OEX-32	327.13	10.10	-1.58	13.26	-1.36	-16.94	224.29	-24.11	-22.13	25.33	-35.15	-11.59	9.27	-7.25	-21.91
OIN-255 x OIJ-211	360.07	3.59	8.32	13.43	-2.15	-15.85	225.18	-273.10*	-21.82	34.07	-27.11	18.89	11.33	-4.18	-4.49
OIN-255 x OMU-27	376.33	8.48	13.22	16.61	1.10	4.09	306.52	49.89	6.42	51.19	40.09	78.64**	14.33	5.45	20.79
OIN-255 x OEX-29	352.36	-5.03	6.00	17.22	2.19	7.92	286.42	87.63	-0.56	39.32	-3.57	37.23	13.19	6.30	11.12
OIN-255 x OEX-32	327.6	-23.09	-1.44	15.24	0.27	-4.49	339.94	317.64	18.03	50.31	51.22*	75.57**	15.72	15.50*	32.47*
OIJ-211 x OMU-27	342.8	-16.43	3.13	15.19	-0.36	-4.85	270.11	11.96	-6.22	35.33	-22.58	23.31	11.20	-3.63	-5.62
OIJ-211 x OEX-29	374.07	25.29	12.54	17.21	2.13	7.83	317.10	385.15**	10.10	41.20	22.41	43.79	12.64	10.13	6.52
OIJ-211 x OEX-32	359.47	17.39	8.14	15.26	0.24	-4.39	236.99	-52.96	-17.72	39.27	12.60	37.04	12.53	6.14	5.62
OMU-27 x OEX-29	368.07	7.91	10.73	15.22	0.21	-4.64	256.31	-2.53	-11.01	41.73	6.69	45.65*	13.73	10.24	15.73
OMU-27 x OEX-32	359.2	5.74	8.06	14.91	-0.03	-6.6	241.07	-116.29	-16.30	35.27	-25.77	23.08	13.60	6.11	14.61
OEX-29 x OEX-32	345.8	2.81	4.03	13.8	-0.66	-13.53	208.93	-138.76	-27.46	35.47	9.11	23.78	9.20	-9.30	-22.47
Check JRO 204	332.4			15.96			288.02			37.2			11.86		
CD value	46.31			3.00			82.31			12.31			3.51		

Table 5: Haymens Genetic parameters for fibre yield and yield attributing traits.

Genetic parameters	Plant height	Basal diameter	Green weight	Stick weight	Fibre yield
D (Additive effects)	1253.77*	-1.01	22549.88	667.31	26.59
F (mean Fr over array)	1224.26*	-1.21	4266.37	512.66	6.56
H1(Dominance effect)	1201.24*	1.94	111143.00*	4028.60*	398.66*
H2	911.83*	2.18	112124.50*	3544.17*	369.52*
E (environment component)	280.22*	1.46*	22755.31*	472.79*	38.30*
Mean degree of Dominance	0.98	1.39	2.22	2.46	3.87
Ratio of genes with +/- effects	0.19	0.28	0.25	0.22	0.23
H2/H1	0.78	1.12	1.01	0.88	0.93
Ratio of dominance & recessive effects	2.99	0.40	1.09	1.37	1.07
Va (Additive gene effects)	159.46	-0.02	8651.00	319.54	24.59
Vd (Dominance gene effects)	227.96	0.55	28031.13	886.04	92.38
h2/H2 (no. of gene groups)	2.27	0.30	0.70	1.23	2.63
h^2 (Heritability narrow sense)	0.24	-0.01	0.15	0.19	0.16

be of commercial importance after multi-location trials that will significantly contribute to the jute improvement programme.

Acknowledgement

Authors are thankful to director ICAR-CRIJAF, Barrack pore for providing research facilities. Valuable suggestions by anonymous reviewers are deeply acknowledged.

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Jyothsna and Satyanarayana, Vegetos 2016, 29:3
10.5958/2229-4473.2016.00084.7

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Genetic Divergence Studies for Fibre Yield and It's Contributing Traits in Roselle (*Hibiscus sabdariffa* L.)

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Abstract

Genetic divergence in thirty genotypes of roselle was assessed utilizing Mahalanobis D^2 statistic and grouped into six Clusters based on D^2 values. Cluster I was the largest comprising of 11 genotypes followed by Cluster IV comprised of ten genotypes, Cluster III & V accommodated three genotypes each whereas Cluster II comprised two genotypes and Cluster VI included solitary genotype. Maximum inter Cluster distance was observed between Cluster II and III, followed by Cluster III and IV and Cluster V and VI, while minimum inter Cluster distance was observed between Cluster I and Cluster II. Days to 50 % flowering was contributed maximum to genetic diversity followed by fibre yield, fibre wood ratio, plant height, fibre length, basal diameter, bark thickness, internodal length, green weight and number of nodes per plant.

Keywords

Roselle; Genetic divergence; Mesta; Mahalanobis D^2; Cluster distance

Introduction

Mesta is the second most important fibre crop after jute. Mesta crop which grown for its bast fibre in India consisting of two species *viz.*, Roselle (*Hibiscus sabdariffa* L.) and Kenaf (*Hibiscus cannabinus* L.) out of which roselle leads in area and production in India for bast fibre, while kenaf cultivated in developed countries for its pulp. To improve such important fibre crop through breeding, study on genetic variability of important traits responsible for fibre yield. In the outset, is a pre-requisite which gives an idea for improvement of the crop. The present study has been outlined to estimate genetic diversity present in a set of roselle germplasms on the basis of ten morpho-economic characters including fibre yield, so that desirable parents could be identified for utilization in crop improvement programme to obtain maximum fibre yield.

Materials and Methods

The experimental material comprising of thirty genotypes of roselle, obtained from the Agricultural Research station, Amadalavalasa, Andhra Pradesh and Central Jute and Allied Fibre Research Institute, Barrackpore, West Bengal. The design adopted was Randomised Block Design with three replications. Each plot consisted of three rows of 3 meters length with a spacing of 30 × 10 cm. The fertilizer dose of 40:20:20 kg NPK/ha (50% N in + Full P & K at the time of sowing) was applied at the time of sowing seed and seeds were sown by hand dibbling. The remaining 50% N was applied after three weeks of sowing. Standard pest management measures were taken during the crop growth period as and when required. Observations were recorded on five plants for ten quantitative characters *viz.*, days to 50% flowering, plant height, basal stem diameter, bark thickness, number of nodes per plant, internodal length per plant, green plant weight, fibre length per plant, fibre wood ratio and fibre yield per plant. Mahalanobis D^2 statistic [1] was used to assess the diversity among genotypes and they were grouped into different Clusters using the Tocher's method as described by Rao [2].

Results and Discussion

Analysis of variance indicated the existence of significant variation in the genotypes studied for all the characters. Thirty genotypes were grouped into six Clusters (Table 1) based on D^2 values. Cluster I was the largest comprising of 11 genotypes followed by Cluster IV comprised of ten genotypes, Cluster III & V accommodated three genotypes each whereas remaining Cluster II comprised two genotypes and Cluster VI included solitary genotype indicating the distinctness from other genotypes for most of the characters studied.

The average intercluster D^2 values ranged from 33.632 to 556.733 (Table 2). The inter cluster distance between Cluster II and III (556.733) was highest followed by Cluster III and VI (454.196) and Cluster II and V (412.045) which supports that genotype belonging to these clusters were highly diverse than the other genotypes. The minimum intercluster D^2 value (33.632) was observed between Cluster I and VI, indicating close genetic relationship between the genotypes of these two Clusters. Cluster V which consists of three genotypes showed highest intra cluster D^2 value (167.580) followed by Cluster IV (85.669) having ten genotypes and Cluster I (43.355) with eleven genotypes, which suggested that the genotypes within Cluster V (AHS-162, AHS-172 and AMV-5) were highly diverse. Therefore, based on inter cluster distances the genotypes belonging to Cluster II, III, V and VI could be used as parents in hybridization programme for obtaining transgressive seggregants with high fibre yield.

Cluster means (Table 3) for different characters indicated that the mean values for number of nodes per plant, green plant weight, fibre length per plant, and fibre yield per plant were highest in Cluster V

Table 1: Grouping of thirty genotypes of roselle (*Hibiscus sabdariffa* L.) in different Clusters.

Sr. No.	Cluster No.	Total No. of genotypes	Name of genotypes
1.	I	11	ER-1, ER-10, ER-38, ER-58, ER-63, AR-12, AR-71, AR-72, R-28, R-93, AS-80-31
2	II	2	JRR-9, JRRM-9-1
3.	III	3	R-78, AMV-4, HS-4288
4.	IV	10	R-134, R-200, R-83, AS-80-29, AS-80-31, AS-80-19, CRIJAFR-2, CRIJAFR-8, AHS-160, AHS-161, AHS-152
5.	V	3	AHS-162, AHS-172, AMV-5
6.	VI	1	AHS-179

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Received: November 03, 2015 **Accepted:** August 12, 2016 **Published:** August 18, 2016

Table 2: Average Intra-Cluster and inter-Cluster D^2 values among thirty genotypes of roselle.

Cluster	I	II	III	IV	V	VI
I	**43.355 (6.584)**	43.722 (6.612)	400.361 (20.009)	64.675 (8.042)	296.671 (17.224)	33.632 (5.799)
II		**4.959 (2.227)**	556.733 (23.595)	86.853 (9.319)	412.045 (20.299)	38.877 (6.235)
III			**12.528 (3.539)**	344.261 (18.554)	84.527 (9.194)	454.196 (21.312)
IV				**85.669 (9.256)**	258.297 (16.072)	66.707 (8.167)
V					**167.580 (12.945)**	339.979 (18.439)
VI						**0.000 (0.000)**

Table 3: Cluster mean values for ten characters in roselle.

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Days to 50% flowering	144.30	147.00	131.89	142.77	134.67	145.00
Plant height (cm)	306.84	339.62	338.29	319.76	336.76	240.12
Basal Diameter (cm)	19.88	20.58	21.68	20.35	22.27	15.68
Bark Thickness (cm)	3.81	4.51	3.53	3.55	3.87	2.83
No.of nodes per plant	62.63	67.20	68.91	66.86	70.39	45.47
Internodal length (cm)	5.35	5.37	5.01	5.31	4.90	5.62
Green plant weight (gm)	434.55	497.66	474.38	479.23	550.69	272.43
Fibre length (cm)	314.32	352.33	347.16	318.68	367.51	247.08
Fibre wood ratio	0.35	0.28	0.36	0.38	0.36	0.39
Fibre yield / plant (gm)	21.97	25.34	26.21	25.40	30.22	19.39

Table 4: Contribution of different characters towards divergence in roselle.

Sr. No.	Characters	Times ranked 1st	Per cent contribution
1.	Days to 50% flowering	232	53.33
2.	Plant height (cm)	16	3.68
3.	Basal Diameter (cm)	14	3.22
4.	Bark Thickness (cm)	10	2.30
5.	No.of nodes per plant	1	0.23
6.	Internodal length (cm)	10	2.30
7.	Green plant weight (gm)	3	0.69
8.	Fibre length (cm)	15	3.45
9.	Fibre wood ratio	16	3.68
10.	Fibre yield / plant (gm)	118	27.13
	TOTAL	435	100

(AHS-162, AHS-172, AMV-5); days to 50% flowering, plant height, bark thickness were highest in Cluster II (JRR-9, JRRM-9-1); Cluster VI (AHS-179) exhibited highest values for internodal length per plant, fibre wood ratio. Cluster III (R-78, AMV-4, HS-4288) had higher values for basal stem diameter.

Days to 50 % flowering (53.33%) contributed maximum towards total divergence (Table 4) followed by fibre yield (27.12%), fibre wood ratio (3.68%), plant height (3.68%), fibre length (3.45%), and basal diameter (3.22%). These results were in confirmation with the findings of Shobha and Dharmatti [3] in vegetable Mesta, Hariram Kumar [4] in Roselle and Dudhane [5] in tossa jute.

Conclusion

Based on inter cluster D^2 values and cluster mean values the genotypes *viz.*, JRR-9, JRRM-9-1, R-78, AMV-4, HS-4288, AHS-162, AHS-172, AMV-5 and AHS-179 may be utilized in future breeding programmes for quality high fibre yielding lines thereby increasing the productivity of the crop which gives higher returns to the farming community.

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Khanande et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00085.9

Vegetos- An International Journal of Plant Research

Research Article

A SCITECHNOL JOURNAL

Genetic Diversity in Vegetable and Grain Type Soybean Genotypes Identified using Morphological Descriptor and EST-SSR Markers

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Abstract

Thirty four soybean genotypes were evaluated for morphological, quality traits and genetic parameters. Correlation and path coefficients were studied for some traits. Analysis of variance and mean performance for various traits revealed significant differences diversity between all the genotypes for traits studied. The genetic diversity among the soybean genotypes was analyzed using ten expressed sequence tag derived simple sequence repeat (EST-SSR) markers. A total of 27 alleles were detected from 334 amplicons with an average of 2.7 alleles per locus. The polymorphism information content (PIC) values of EST-SSR markers ranged from 0.334 to 0.837 with an average of 0.559. These grain and vegetable soybean genotypes could be divided into 7 subgroups based on similarity matrix and arithmetic average (UPGMA) cluster with no correlation between genetic and morphological diversity. The analysis clearly indicated that even with the EST-SSR primers, reliable estimation of genetic diversity among the population could be obtained.

Keywords

Vegetable soybean; Aroma; Oil content; Correlation; Divergence; Multivariate analysis; EST-SSR

Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the legume crop species which is native to China with a history of more than 5,000 years. Since, it is one of the most important pulses cum oil seed crop and known for its useful nutrients including protein (36 to 42%) and oil content (19 to 20%), carbohydrate (35%, 17% of which dietary fiber), minerals (5%) and several other components including vitamins A, B and D and rich in lysine [1-3]. Vegetable soybean is also known by the Japanese term 'edamame', is a soybean harvested at approximately 80% maturity [4]. Soybean is popularly consumed after blanching in China, Korea, Japan, and other countries. It also has potential for cancer prevention and suppression owing to its high genistein content [4-6].

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Received: July 27, 2016 **Accepted:** August 17, 2016 **Published:** August 24, 2016

The productivity of soybean in India is less in comparison with the average world produce. The attributes identified for such a low productivity are 1) limited genetic diversity, 2) narrow genetic base [7] of Indian soybean varieties, 3) short growing period available in Indian latitude and 4) stagnant genetic potential for yield. Further, the simulation studies indicated, increasing temperature and CO_2 levels could pose a serious threat in decreasing the growth of soybean crop and hence the yield [8]. In India, narrowing down of the genetic potential is due to the repeated use of few parents in the breeding. Global awareness of the use and benefits of vegetable soybean has been increased through the efforts of Asian Vegetable Research and Development Centre (AVRDC) the World Vegetable Center. As a result, vegetable soybeans are now produced and marketed in various part of the globe like Zimbabwe, Mauritius, Uganda, Tanzania, Zambia, Sudan, and Mozambique [9,10].

Worldwide soybean cultivation was about 51.8 Mha in 2005, 83 Mha in 2010 [11] and estimated presently on more than 92.5 million ha (about 6% of the world's arable land) to produce 217.6 million tons of production each year [12]. However, it is estimated that the current area under vegetable soybean in the USA is about 2000 ha [13]. Over the past decade improved soybean varieties bred from lines developed at AVRDC have been introduced and distributed to farmers in North-East India [10].

As per Nair et al. [14] India is a developing nation and home to almost 1.2 billion people also, India hosts a significant part of the world's poverty and health problems, providing a clear target for global initiatives against hunger. Nair et al. [14] also discussed important approach of promoting more diverse, nutritional crops to the greater Indian population to facilitate a healthier and more balanced diets. Nair et al. [14] stated vegetable soybean as rich source of protein and other nutrients also accepted as a viable and promising option to improve nutrition in India.

Young et al. [15], represent that vegetable soybean generally harvested during R6 to R7 stage of crop growth when the pods are green and seeds fill at about 80-90% of the total weight. Larger and wider green pods, more dry weight, green seed coat, higher sugar content, smooth texture and better flavors than grain soybean are product features of vegetable soybeans [16,17] for better market values. Vegetable soybean can be either sold fresh as pots, shelled beans, or sold as frozen or canned products [18]. However, in some countries like Nepal, grain soybeans are harvested at the green pod stage and marketed as vegetable soybeans and grain soybean varieties have also been used as vegetable soybean in China, Taiwan and Thailand. Such beans are unpalatable and bias consumer attitude towards using soybean as vegetable. Vegetable soybean is slightly sweeter compared with the grain type, which is oily and slightly bitter [14]. It is rich in protein (13%), cholesterol free oil (5.7%), phosphorous (150 mg/100 g), calcium (78 mg/100 g), Vitamin B_1 (0.4 mg/100 g) and, B_2 (0.17 mg/100 g). They also contain isoflavon and vitamin E [19]. The trypsin inhib itors in vegetable soybean are lower than that in grain soybean [14]. Compared to vegetable pigeon pea (*C. cajan*) and green peas (*P. sativum*) the vegetable soybean provides more protein of higher quality and is considered as an excellent and complete protein source [20]. Harvesting pods at right time just after

the R6 growth stage is critical, as loss of nutritional quality occurs when the pod turns yellow.

Assessment of genetic variability for generation of information in the existing germplasm of a particular crop is a prerequisite [21]. Also, heritability is a basic for selection which implies the extent of transmissibility of traits to next generations [22]. Similarly, high genetic advance coupled with high heritability estimate offers the most effective condition for selection for a particular trait [23]. Increased seed yield is the ultimate goal of the breeders however; seed yield itself is an outcome of direct or indirect interactions of many component traits. Therefore, understanding the relationship between yield and its component traits is of great importance to breeders. Further, this helps in selecting desirable genotypes for yield improvement programs [24]. As correlation alone cannot explicate relationships among the traits, hence the path coefficient analysis has been used in different crop species for determination of the impact of the independent variables on the dependent one and to find direct and indirect effects [25].

Genetic diversity is normally assessed by common morphological traits which are affected by different environmental conditions, development stages of the crop, also the type of plant material and need several replications to establish the genotypic contributions. In modern plant breeding, tools based on molecular markers have proved their importance and found competent. Assessment of genetic diversity with molecular markers will overcome this hurdle through excluding environmental effects and provide a true representation of the entire genome.

Various molecular markers have been used to study the genetic diversity and population structure of plants such as restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs) etc. [26]. Among these markers, SSRs have stood out and are considered to be the most powerful tools because of its high abundance, co-dominant nature, resolving multiple alleles, reproducible behavior, wider coverage of genome, and easier detection procedure using polymerase chain reaction (PCR) [27-31]. However, due to extensive time requirement and high cost of their development, the wide use of SSRs is often limited. The recent development in studies of expressed sequence tags (ESTs) has produced a new foundation development of EST-SSRs. These EST-SSRs have some intrinsic advantages over genomic-SSR markers *viz*; (1) they are less costly (2) they are directly associated with transcribed genes; and (3) they have high transferability among related species [32]. As far as India is concerned, soybean is mainly cultivated as an oilseed crop. Considering the nutritional importance of vegetable soybean, efforts are being made to breed vegetable soybean varieties. As an initial step, present study was carried out to know variability and association among vegetable and grain type soybean genotypes for yield and quality attributing traits using morphological descriptor along with molecular markers.

Material and Methods

Experimental site

The experiment was carried out during *kharif* 2014 at the experimental field of Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra state, India located at 307.4 meters above mean sea level. The geographical situation is 20.42°N latitude and 77.02°E longitude. The soil was medium black, with clay, fairly leveled and uniform in topography with appropriate drainage.

Plant material

Thirty four soybean genotypes were used as the experimental material comprised of 7 vegetable types, 12 mutants and 15 released grain type soybean genotypes. The names of all 34 soybean genotypes included in the study are listed in Table 1 along with their source.

Experimental design and setting the experiment

The experiment was laid out in a randomized complete block design with three replicates. The seeds were sown maintaining distance of 45 × 10 cm. Seeds were sown with the help of hand drill during *Kharif* 2014. The basal fertilizer does were applied at the rate of 30 Kg N, 75 Kg P_2O_5 and 20 Kg K_2O per hectare at the time of sowing. Fertilizers were applied in the form of urea, single super phosphate and murrate of potash. Since the crop was grown during *Kharif* season, the irrigation was given at critical growth stages. As the crop is for vegetable purpose, 3-4 irrigations were provided after the initiation of flowering. Harvesting was done depending upon maturity of the genotypes.

Data collection

Data on five randomly selected plants from each replicate were recorded for various traits *viz*., days to 50% flowering, days to maturity, plant height, number of branches per plant, number of green pods per plant, pod length, pod width, 100 fresh pod weight, 100 beans weight, and test weight of beans, 100 seed weight, and photosynthetic efficiency (Table 2). Qualitative characters *viz*., protein content, total sugar content and oil content were estimated from all genotypes of each replication. Observations on flower color, seed shape, helium color, seed luster, seed coat color and pod color were recorded using color chart developed by RHS (The Royal Horticultural Society), London, United Kingdom (Royal Horticultural Society, 2001).

All soybean genotypes along with two checks, each of vegetable and grain type were evaluated for texture, aroma, taste and overall acceptability through organoleptic taste and generated data as per score card. The evaluation was done by a panel of six trained judges, including faculty members and students of the Department of Agricultural Botany, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

Determination of fragrance was done using the procedure given by Sood and Sidiq [33] with desirable modifications of the KOH concentration. The leaf and seed fragrance of each genotype was determined following the optimized procedure at R6-7 stage of the crop. About 2-3 g of green leaf and beans harvested from plants, sliced and immersed for 10 min in 10 ml of 3.0% KOH at room temperature, after which the fragrance was graded independently by six operators. Out of a random five samples of each genotype, if none were fragrant, the entry was deemed to be non-fragrant; if five successive samples were fragrant, the entry was considered to be fragrant.

Estimation of protein content (%)

Protein content of seeds harvested at R6-R7 stage was determined by Bradford method and expressed on per cent basis for each genotype. The estimation method is based on the protein dye binding method. The binding of Commassie Brilliant Blue (CBB) G-250 to protein in acidic condition shift the λ_{max} of dye from 465 nm to

Table 1: List of soybean genotypes and their source.

S No.	Genotype	Type	Source	SN	Genotypes	Type	Source
1	PK 1314	Grain	Dr. PDKV Akola, MS	18	MAUS-158	Grain	VNMKV, Parbhani, MS
2	AMS-6-1	Mutant	Dr. PDKV Akola, MS	19	IC 241949	Grain	Dr. PDKV Akola, MS
3	AMS-50(B)	Mutant	Dr. PDKV Akola, MS	20	IC-16815	Grain	Dr. PDKV Akola, MS
4	AMS-73	Mutant	Dr. PDKV Akola, MS	21	IC-118400	Grain	Dr. PDKV Akola, MS
5	AMS-247	Mutant	Dr. PDKV Akola, MS	22	IC-118452	Grain	Dr. PDKV Akola, MS
6	AMS-99-24	Mutant	Dr. PDKV Akola, MS	23	AGS-450	Vegetable	AVRDC, Hyderabad, AP
7	AMS-28(A)	Mutant	Dr. PDKV Akola, MS	24	AGS-457	Vegetable	AVRDC, Hyderabad, AP
8	AMS-353	Mutant	Dr. PDKV Akola, MS	25	Swarna Vasundhara	Vegetable	AVRDC, Hyderabad, AP
9	AMS-5-18	Mutant	Dr. PDKV Akola, MS	26	AGS-459	Vegetable	AVRDC, Hyderabad, AP
10	AMS-37	Mutant	Dr. PDKV Akola, MS	27	GC-84501-32-1	Vegetable	AVRDC, Hyderabad, AP
11	AMS-90-1	Mutant	Dr. PDKV Akola, MS	28	AGS-339	Vegetable	AVRDC, Hyderabad, AP
12	AMS-93	Mutant	Dr. PDKV Akola, MS	29	MACS-450	Grain	ARI, Pune, MS
13	AMS-65	Mutant	Dr. PDKV Akola, MS	30	MACS-1508	Grain	ARI, Pune, MS
14	EC-251411	Grain	Dr. PDKV Akola, MS	31	MACS-1188	Grain	ARI, Pune, MS
15	NRC-40	Grain	Dr. PDKV Akola, MS	32	TAMS 98-21	Grain	Dr. PDKV Akola, MS
16	NRC-2	Grain	Dr. PDKV Akola, MS	33	HIMSO 1685(C)	Vegetable	RRC Amravati, MS
17	TAMS-38	Grain	RRC Amravati, MS	34	JS-335(C)	Grain	JNKV, Jabalpur, MP

Table 2: List of traits and their description of measurement.

S No.	Traits	Method of measurement
1	Days to 50% flowering	The number of days from sowing to flowering of 50% plants
2	Days to maturity	The number of days from sowing until approximately 90% pod turned into brownish color
3	Plant height (cm)	The height from the base of the plant to the tip of last leaf
4	Primary branches per plant (number)	Total number of pod bearing primary branches in a plant
5	Pods per plant (number)	Total number of pods with seed in a plant at R6
6	Seeds per pod (number)	Total number of seeds in a pod at R6
7	Pod length (cm)	Average length of five pods from each genotype was randomly selected was measured in millimeters using vernier calliper (Tricle brand-name) and average length recorded
8	Pod width (cm)	Average width of five pods from each genotype was randomly selected was measured in millimeters using vernier caliper (Tricle brand-name) and average length recorded
9	100-seed wt. (g)	One hundred beans randomly counted and then weighed at R6
10	100- fresh pod wt. (g)	One hundred pods randomly counted and then weighed at R6
11	100- mature seed wt. (g)	One hundred seeds randomly counted and then weighed at R8
12	Test weight of seeds (g)	One thousand seeds randomly counted and then weighed at R6
13	Photosynthetic efficiency	This was recorded with the help of Chlorofluro meter at R6 stage
14	Sugar content (%)	Total sugars were estimated in edible portion using the Phenol sulfuric acid method
15	Protein content (%)	Protein content of seeds harvested at R6-R7 stage was determined by Bradford method and expressed in per cent
16	Oil content (%)	Oil content was determined by using NMR (nuclear magnetic resonance) at R8
17	Seed yield per plant (g)	Weighing the total number of seeds produced in a plant
18	Flower color	Flower colour was scored using the descriptor*
19	Seed shape	General shape of seed was scored using descriptor*
20	Helium color	Hilum colour using descriptor*
21	Seed luster	Shape of seed was scored using descriptor*
22	Seed coat color	Seed coat colour was recorded using the colour using Royal Colors Chart**
23	Pod color and appearance	Pod colour was recorded at R6 and scored using Royal Colors Chart**
24	Texture	Force required compressing the grain between one's teeth
25	Taste	Organoleptic test was carried out using 0-5 scale at R6
26	Aroma	About 0.8 to 1 g of seeds at R6 were cut into pieces and placed in a 15 ml tube. Ten ml of 1.7% KOH was added, and the tube was capped and kept for 10 min at 37°C.

Note: *Descriptor for soybean, IBPGR/84/183, Rome (1984) (http://www.bioversityinternational.org/uploads/tx_news/Descriptors_for_soyabean_252.pdf)
**The Royal Horticultural Society, London, United Kingdom (2001)

595 nm. Absorption of the blue colored protein dye complex at 595 nm is directly related to concentration of protein present in sample (Sengar and Chaudhary, 2014).

Estimation of sugar content (%)

Phenol sulfuric acid method is the most widely used colorimetric method to date for determination of total sugar concentration in aqueous solutions. The basic principle of this method is that carbohydrates, when dehydrated by the reaction using concentrated sulfuric acid, produce furfural derivatives. Further, the reaction between furfural derivatives and phenol develops the detectible color [34].

Estimation of oil content (%)

The oil composition of soybean seeds was determined using the NMR spectrometry (Nuclear Magnetic Resonance) at the

Instrumental cell, Oilseed Research Unit, Dr. PDKV, Akola. For this purpose, 25-30 gm of seeds per soybean genotype was measured with two replications. The oil content of soybean seeds was determined by calibrating the NMR signal against a suitable reference using MQC Benchtop NMR Analyzer, Oxford instrument.

Statistical analysis

The data were analyzed using MSTAT program for Analysis of variance (ANOVA). Phenotypic, genotypic and error variances were estimated following the procedure described by Johnson et al. [35]. Genotypic and phenotypic variation was estimated according to Burton [36]. Broad sense heritability and genetic advance in percent of means were estimated using the formula suggested by Johnson et al. [35].

Genotypic and phenotypic correlation coefficients for different characters were calculated in all possible combinations using the formula given by Miller et al. [37]. The path coefficient analysis was made following the procedure of Dewey and Lu [38]. Mahalanobis's generalized distance (D^2) statistics was used for clustering of genotypes by estimating the divergence among genotypes for the traits measured as per the Joshi and Vashi [39].

Molecular diversity studies

DNA isolation and PCR amplification: DNA was extracted from 15-day old seedlings of each genotype. A total of 0.3 g of fresh leaves was used for each genotype and DNA was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) method. The relative purity and concentration of the extracted DNA were estimated with spectrophotometer. The final concentration of each DNA sample was adjusted to 20 ng/µl. Ten informative EST-SSR primers based earlier reports of Zhang et al. [40] were selected, and used in this study.

The polymerase chain reaction (PCR) was performed in a final volume of 20 µl, containing 10 mM Tris-Cl, 50 mM KCl, 2 mM $MgCl_2$, 100 mM of each dNTP, 0.4 mM of each primer, 20 ng genomic DNA, and 1 U of *Taq* DNA polymerase. Each of the 40 PCR cycles consisted of 30 s at 94°C for template denaturation, 30s at 47°C for primer annealing, and 30s at 72°C for primer extension. The PCR reaction was completed with 5 min incubation at 72°C. The PCR products were separated on 2.0% Agarose and visualized under AlfaImager.

Statistical analysis: For the statistical analysis, the patterns of all SSR loci were scored for each polymorphic amplicon as '1' for presence and '0' for absence. This allowed estimating at each locus of the number of alleles present (NA) and the polymorphic information content (PIC) value. The PIC value of each primer was calculated by the formula:

$$PIC = 1 - \sum_{i=1}^{n} (Pi)^2$$

Where, Pi is the frequency of the i^{th} allele. Similarity coefficients based on EST-SSR profiles were calculated according to procedure described by Nei and Li [41], and a dendrogram based on the similarity matrix and UPGMA clustering was produced using the online software.

Results and Discussion

Mean performances

The mean performances of all soybean genotypes for different traits are shown in Figure 1. The shortest time required to flowering and maturity was observed in vegetables genotype AGS-450 (20 and 77.67 days) closely followed by AGS-457 (20.67 and 77.33 days) and AGS-339 (20 and 81.67 days). The longest duration was required in the grain type soybean genotype TAMS-98-21 (46.70 and 89.33 days) followed by MACS-1188 (42.33 and 104.67 days) and MACS-1508 (41.00 and 100.00 days). Results suggested that some of vegetable genotypes required lower flowering and maturity period than the grain type and mutant genotypes. The wide range was observed for plant height (27.88 to 76.20 cm) and number of primary branches (1.43-5.50). Most of the mutants showed significant variation in plant height but no any genotype has found significant for containing sugar, protein, oil or green pod yield.

Among seven vegetable type genotypes, four (Swarna-Vasundhara, AGS-450, AGS-457 and AGS-459) produced significantly higher green pod yield per plant than mutants and other grain type genotypes. However, most of the genotype produced significantly higher number of green pod per plant except the GC-84501-32-1, but not found superior in green pod yield per plant. Vegetable genotypes showed significantly high pod length, pod width, 100-seedweight, 100-fresh pod weight, 100-mature seed weight, test weight and photosynthetic efficiency as compared to rest of the genotypes under study. The genotype AGS-450 had significant highest level of sugar and protein content (92.01 mg/g and 45.07%, respectively) followed by AGS-459 (91.07 mg/g and 42.91%). The highest oil content was recorded in mutant genotype AMS-353 (20.84%) followed by TAMS 98-21 (20.77%) and AMS-93 (20.65%).

Genetic variability, Heritability and Genetic Advance

Analysis of variance revealed that mean square due to genotypes were highly significant ($P<0.01$) for all the 17 quantitative traits (Table 3). These results revealed highly significant genotypic variation among genotypes for all the traits. Phenotypic and genotypic coefficient of variation (PCV and GCV), broad sense heritability and genetic advance was calculated for all seventeen traits (Table 4). The estimates of PCV were higher than corresponding estimates of GCV for all characters under study. The highest PCV and GCV with low environmental variance for all the traits indicate that the expressions of genes controlling these characters are not marked by influence of the environmental conditions. The highest PCV and GCV were observed for test weight (61.17 and 60.57%, respectively) and the lowest PCV and GCV were recorded for oil content (4.12 and 2.66%, respectively). Similarly, significant variations have also been reported earlier by several researches for various traits [42-44].

The PCV and GCV of days to 50% flowering (16.19 and 15.6%), days to maturity (6.01 and 5.62%), plant height (26.04 and 24.79%), number of primary branch (38.39 and 37.26%), number of green pod per plant (16.25 and 15.06%), number of seed per plant (9.83 and 9.20%), pod length (14.20 and 13.28%), pod width (35.21 and 33.80%), 100 beans weight (49.28 and 48.63%), 100 fresh pod weight (48.89 and 48.80%), 100 mature seed weight (59.78 and 59.53%), photosynthetic efficiency (8.47 and 7.74%), sugar content (25.05 and 24.84 %), protein content (11.44 and 10.55%) and green pod yield per plant (33.50 and 32.61%) results showed narrow difference between PCV and GCV for most of traits. All the characters exhibited high heritability which varied from 41.8% in oil content to 99.6% in fresh pod weight. Among the traits, oil content had relatively low heritability. The genetic advance as present of mean (GA%) ranged from 4.54% in oil content to 158.38% test weight of beans. Among the traits, test weight of bean, 100-mature seed weight, 100-fresh

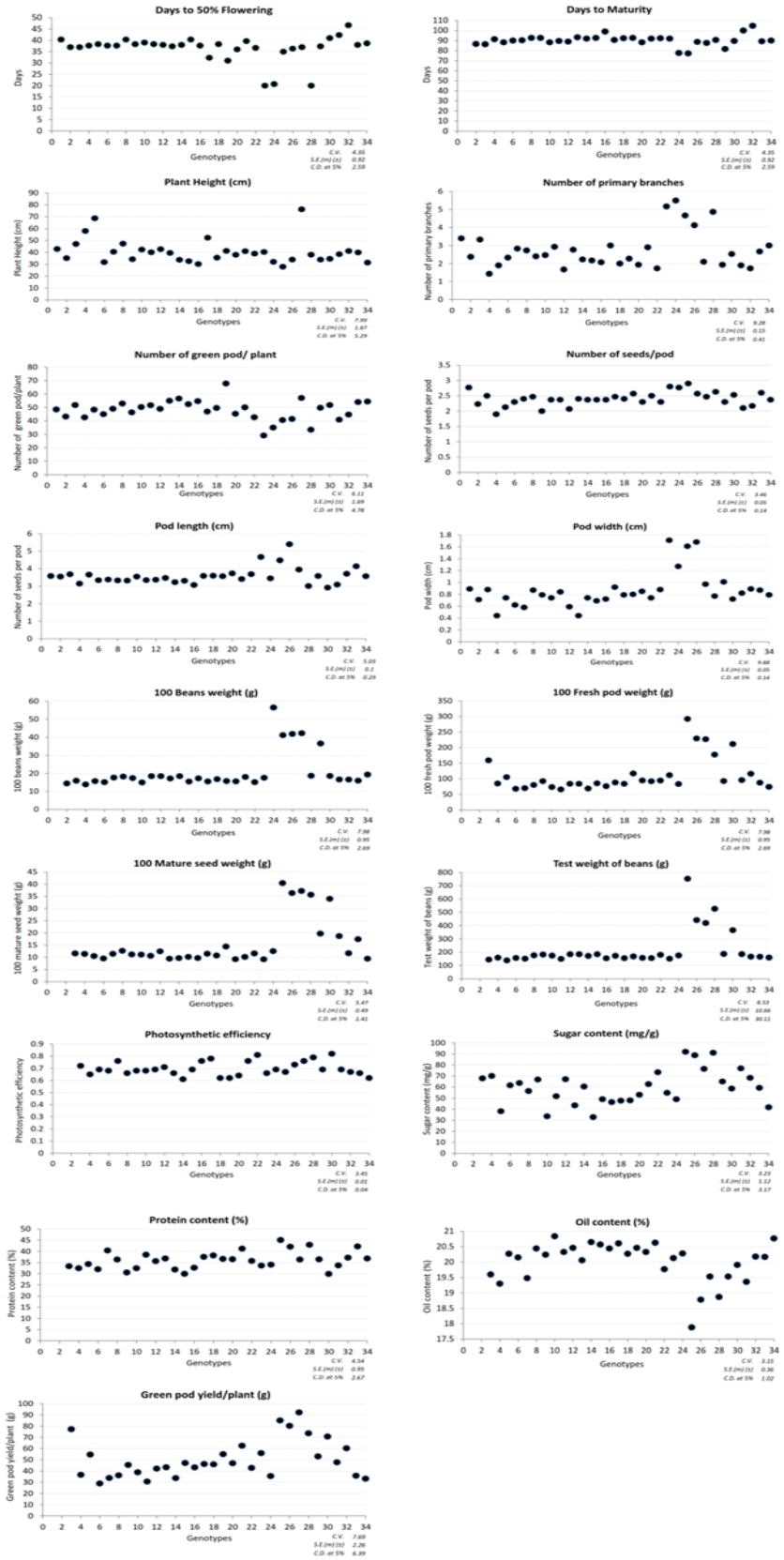


Figure 1: Mean performance of 34 genotypes for various characters in vegetable and grain type soybean.

Table 3: Analysis of variance for mean sum of squares for various characters in vegetable and grain type soybean.

Source	df	Days to 50%flowe-ring	Days to maturity	Plant height (cm)	Number of primary branches	Number of green Pod/ Plant	Number of seed /pod	Pod length (cm)	Pod width (cm)	100 Beans weight (g)	100 fresh pod weight (g)	100 Mature seed weight (g)	Test weight of beans (g)	Photosynthetic efficiency	Sugar Content (mg/g)	Protein content (%)	Oil content (%)	Green pod yield/ plant (g)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Replication	2	6.970	4.320	27.810	0.013	5.800	0.004	0.006	0.002	5.510	31.090	0.690	911.860	0.001	11.530	7.150	1.100	44.380
Genotypes	33	99.62**	81.39**	314.61**	3.19**	165.44**	0.15**	0.71**	0.26**	307.19**	8873.80**	260.89**	51946.10**	0.01**	675.64**	46.35**	1.25**	844.06**
Error	66	2.52	3.69	10.53	0.06	8.61	0.001	0.03	0.01	2.73	11.11	0.73	341.14	0.001	3.79	2.69	0.39	15.35

Note: **Significance at 1% level

Table 4: Estimation of genetic parameters in seventeen characters of 34 genotypes in vegetable and grain type soybean.

Characters	Range		Mean	Mean sum of squares	CV%	Genotypic variance	Phenotypic variance	GCV%	PCV%	Heritability (h^2) (BS) (%)	Genetic Advance (GA)
	Lowest	Highest									
Days to 50% flowering	20.00	46.70	36.47	99.62**	4.35	32.37	34.88	15.6	16.19	92.8	39.67
Days to maturity	77.33	104.67	90.41	81.39**	2.13	25.90	29.60	5.62	6.02	87.5	13.90
Plant height (cm)	27.88	76.20	40.61	314.61**	7.99	101.36	111.89	24.79	26.05	90.6	62.29
Number of primary branch	1.43	5.50	2.74	3.19**	9.28	1.04	1.11	37.26	38.40	94.2	95.46
Number of green pod/plan	29.10	67.80	47.99	165.44**	6.11	52.28	60.89	15.06	16.26	85.9	36.86
Number of seed per pod	1.90	2.90	2.40	0.15**	3.46	0.05	0.06	9.20	9.83	87.6	22.73
Pod length (cm)	2.90	5.40	3.59	0.71**	5.03	0.23	0.26	13.28	14.20	87.5	32.82
Pod width (cm)	0.40	1.70	0.86	0.26**	9.88	0.09	0.093	33.80	35.21	92.1	85.66
100 seed weight (g)	13.85	56.51	20.71	307.19**	7.98	101.49	104.22	48.63	49.28	97.4	126.71
100 fresh pod weight (g)	65.96	291.90	111.38	8873.80**	2.99	2954.26	2965.37	48.80	48.89	99.6	128.60
100 mature seed weight (g)	9.20	40.47	15.64	260.89**	5.47	86.72	87.45	59.53	59.78	99.2	156.50
Test weight of seed (g)	138.53	751.47	216.52	51946.10**	8.53	17201.65	17542.79	60.57	61.17	98.1	158.35
Photosynthesis efficiency	0.61	0.82	0.70	0.01**	3.45	0.003	0.003	7.74	8.47	83.5	18.68
Sugar content (mg/g)	32.82	92.01	60.22	675.64**	3.23	223.96	227.74	24.84	25.05	98.3	65.05
Protein content %	29.89	45.07	36.13	46.35**	4.54	14.56	17.24	10.55	11.49	84.4	25.61
Oil content %	17.88	20.84	20.03	1.25**	3.15	0.29	0.68	2.66	4.12	41.8	4.54
Green pod yield/plant (g)	28.84	92.18	50.96	844.06**	7.69	276.24	291.59	32.61	33.50	94.7	83.80

Note: **Significance at 1% level

pod weight, 100-seed weight, number of primary branch and green pod yield exhibited higher percentage of genetic advance. Narrow difference between PCV and GCV for all the characters tested indicates less influence of environmental factor on their expression and the chance of high selection gain. The heritability estimation helps breeders in selection based on the basis of phenotypic performance. Heritability and genetic advance together with high GCV could provide the best image of the amount of advancement to be expected thought phenotypic selection [35,45].

Therefore, high value of heritability and genetic advance (%) along with high GCV for the traits like green pod yield (g), 100 seed weight (g), 100 fresh pod weight (g), protein content (%) and the sugar content (mg/g) can be considered as favorable traits for improvement of vegetable soybean through effective phenotypic selection of these traits and high expected genetic gain from the selection of these traits can be achieved. This suggests that these characters are under control of additive gene action and would respond very well to continuous selection [46]. Consequently, high estimate of heritability and genetic advance (%) along with low GCV of the rest of traits like days to 50% flowering number of seed per pod, oil content (%) green pod yield per plant (g) indicated the expression of these traits are under involvement of non- additive gene action and phenotypic selection of these traits might not be effective.

Creation of new plant type with high yield is the main objective

in plant breeding. In the present investigation, it was observed that amongst 34 genotypes; five genotypes performed superiorly in respect to green pod yield per plant along with other morphological and quality like early days to 50% flowering, number of primary branches, number of seed per pod, pod length and width, 100 seed weight, 100 fresh pod weight, sugar content, oil content and green pod yield per plant. These results are in accordance of the result of Kundi et al. [47], Hussain et al. [48] and Malek et al. [49].

Character association and path coefficient analysis

Genotypic and phenotypic correlations were calculated followed by path coefficient analysis to partition the correlation coefficients of traits with yield per plant into direct and indirect effects (Table 5). The estimation of genotypic correlation coefficient was found to be higher than their respective phenotypic correlation coefficient. There are in the agreement with the result of Malek et al. [49] however, Weber and Morrthy [50] observed low phenotypic correlation due to the modifying effect of environment on the genetic association among the traits. The characters exhibited significant positive correlation with green pod yield per plant were found to be number of primary branches, number of seed/pod, pod width, pod length, 100 fresh pod weight, 100 beans weight, 100 mature seed weight, test weight of beans, photosynthetic efficiency and protein. These characters were also, positively interlinked among themselves which indicated the importance of these characters while selection.

Further the results indicated that the increase in one character will increase in the correlated character. For example, number of primary branches was positively one significantly correlated with green pod yield per plant; hence the plants having more number of primary branches are more likely to produce greater number of pods, per plant. Thus selection for higher green pod yield on the basis of above characters would be reliable.

Among the yield contributing characters themselves, number of seeds per pod was positively and significantly correlated with green pod yield per plant followed by 100 fresh pod weight, number of primary branches and while, significant and positive association with test weight of beans, photosynthetic efficiency, sugar content and protein content. This indicates the importance of the character number of seeds per pod in increasing 100 fresh pods weight, with test weight beans, photosynthetic efficiency, sugar content and protein content. Nagarjuna et al. [51] reported similar results and showed positive and significant correlation of seed yield with number of seeds per pod, number of preliminary branches, number of pod per plant and 100 seed weight. Therefore, to improve yield of soybean, emphasis should be given on the correlated traits based on strength of their correlation.

The plant height was negatively and significantly correlated with green pod yield per plant followed by number of primary branches per plant, number of seed per plant, pod length, pod width, test weight of beans, photosynthetic efficiency, sugar content, oil content and protein content. The remaining association was less important due to their positive or negative non-significant correlation coefficient values. It means that increase of plant height decreases the green pod yield per plant. Arshad et al. [52] and Rajanna et al. [53] reported similar findings for different parameters.

The genotypic correlation of green pod yield per plant (g) was positive correlated with number of primary branches, number of seed per pod, pod width (cm), 100 seed weight, 100 fresh pod weight, 100 mature seed weight, test weight of beans, photosynthetic efficiency and protein content (%). The genotypic association of 100 seed weight with 100 fresh pod weight, 100 mature seed weight, sugar content and green pod yield per plant was highly significant in positive discussion.

The significant positive correlation of number of seed per pod, 100 fresh pod weight, 100 mature seed weight, pod width, pod length, number of seed per pod, protein content and sugar content with green pod yield per plant indicated that in selecting high yielding vegetable type genotype, these characters should be more emphasis as the best detection criteria. These results are also in agreement with the results of Vijayalakshmi et al. [54].

The path coefficient analysis showed the importance of yield contributing characters *viz.* number of primary branches, number

Table 5: Estimates of Genotypic correlation coefficient (r) for different characters.

S No.	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17
X1	1	0.755**	0.072	-0.756**	0.532**	-0.536**	-0.198	-0.446**	-0.749**	-0.763**	-0.730**	-0.720**	-0.344*	-0.464**	-0.223	0.699**	-0.595**
X2		1	-0.003	-0.660**	0.486**	-0.444**	-0.371*	-0.481**	-0.651**	-0.645**	-0.591**	-0.640**	-0.208	-0.455**	-0.160	0.803**	-0.494**
X3			1	-0.271	0.169	-0.229	0.017	-0.175	-0.234	-0.229	-0.174	-0.203	-0.052	-0.120	-0.043	-0.030	-0.222
X4				1	-0.562**	0.817**	0.420**	0.701**	0.855**	0.920**	0.839**	0.811**	0.340*	0.463**	0.243	-0.705**	0.849**
X5					1	-0.169	-0.269	-0.504**	-0.703**	-0.654**	-0.687**	-0.686**	-0.117	-0.470**	-0.186	0.795**	-0.303*
X6						1	0.451**	0.649**	0.630**	0.813**	0.642**	0.592**	0.324*	0.384*	0.237	-0.516**	0.947**
X7							1	0.835**	0.628**	0.527**	0.601**	0.672**	0.261	0.549**	0.499**	-0.694**	0.543**
X8								1	0.835**	0.791**	0.840**	0.839**	0.265	0.632**	0.609**	-0.896**	0.731**
X9									1	0.919**	0.960**	0.986**	0.337*	0.647**	0.43**	-0.89**	0.739**
X10										1	0.908**	0.893**	0.337*	0.631**	0.389*	-0.893**	0.902**
X11											1	0.918**	0.402**	0.681**	0.441**	-0.920**	0.769**
X12												1	0.279	0.651**	0.484**	-0.927**	0.702**
X13													1	0.338*	0.116	-0.329*	0.384*
X14														1	0.468**	-0.959**	0.565**
X15															1	-0.552**	0.365*
X16																1	-0.681**
X17																	1

Note: * Significant at 5% probability level; ** Significant at 1% probability level

X1- Days to 50% flowering; X2- Days to maturity; X3 – Plant height (cm); X4-Number of primary branches; X5-Number of green pod /plant; X6-Number of seed per pod; X7-Pod length (cm); X8-Pod width (cm); X9-100 fresh seed weight (g); X10-100 fresh pod weight (g); X11-100 mature seed weight; X12-Test weight of beans (g); X13-Photosynthetic efficiency; X14-Sugar content (mg/g); X15-Protein content (%); X16-Oil content (%); X17-Green pod yield/plant (g)

of green pod per plant, pod width (cm), 100 fresh pod weight (g), test weight of beans, 100 mature seed weight (g), pod length (cm), number of seed per pod, protein content (%), and sugar content (mg/g) which showed high positive direct effect as the major yield contributing traits, for enhancing the yield of soybean (Figure 2).

These results are in accordance with Abady et al. [55]; Sarutayophat [56] and Malik et al. [57]. The highest direct positive effect of pod length and 100 fresh pod weight on green pod yield per plant, the residual effect is low (0.0289) which indicate selected characters are desirable to study the contribution for green pod yield per plant at R6 stage.

Both the correlation and path analysis revealed that the number of primary branches, number of seed per pod, pod length and width, 100 fresh pod weight, appeared to be the first order yield components and priority should be given to these characters during selection.

Morphological descriptor

The genetic diversity in the grain and vegetable type soybean was determined by analyzing variation in ten morphological traits. The seed coat color had the highest variation among all ten traits followed by helium color, seed luster, seed shape, flower color, texture, and aroma (Table 6).

The percentages of genotypes with white, intermediate purple and purple flower color were 35.29, 20.58 and 44.11%, respectively (Figure 3). Figure 4 showed most of the genotypes had spherical seed shape (61.76%) and few of them were oval (35.29%) and spherical flatten in shape (2.94%).

The helium color was black and brown (29.45%, respectively) and rest of the genotypes had grey (23.52%), imperfect black (14.70%) and yellow (2.94%). Four different kind of seed luster could be classified as shiny (50%), intermediate (23.52%), dull (14.70%) and shine (11.764%). Only 8.82% of genotypes were aromatic amongst 34 genotypes. The genotypes with nuttiness and beany taste were 44.11%, respectively and only 11.76% genotypes had sweet taste. Based on organoleptic test, only five genotypes viz., AGS-450 AGS-457, Swarna Vasundhara, AGS-459 and MACS-1508 have found with good acceptability.

Genetic divergence studies

Cluster analysis using twelve morphological and few quality traits grouped the 34 genotypes in to three main clusters at the genetic distance of 12230.93. It was also found that among the three clusters, cluster I was the largest consisting of 29 genotypes (all 12 mutants, 10 released varieties, 6 germplasm and 1 vegetable genotype) and the second largest group was cluster II consisted four vegetable genotypes. However, a single vegetable genotype AGS-450 was felt in cluster III (Table 7 and Figure 5). The mean values of 12 different traits for three clusters among 34 genotypes are depicted in the Table 8. Results showed that among three clusters, III had the highest average mean for all the traits except days to 50% flowering, days to maturity, plant height and number of green pod per plant. On the contrary, cluster I revealed the lowest mean for various traits like pod length, pod width, 100-seed weight, 100-fresh seed weight, sugar and protein content and green pod yield per plant.

Cluster analysis based on twelve morphological and quality traits grouped 34 soybean genotypes in to three different clusters and indicated that 34 genotypes exhibited notable genetic divergence in terms of these traits (Table 7 and Figure 5). Therefore, classifications in the study based on these twelve traits are in agreement with previous report. Formation of different number of clusters using morphological and quality traits in diverse soybean genotypes was also reported earlier [57-60]. The dendogram tends to group some of the grain type genotypes including mutant and other genotypes

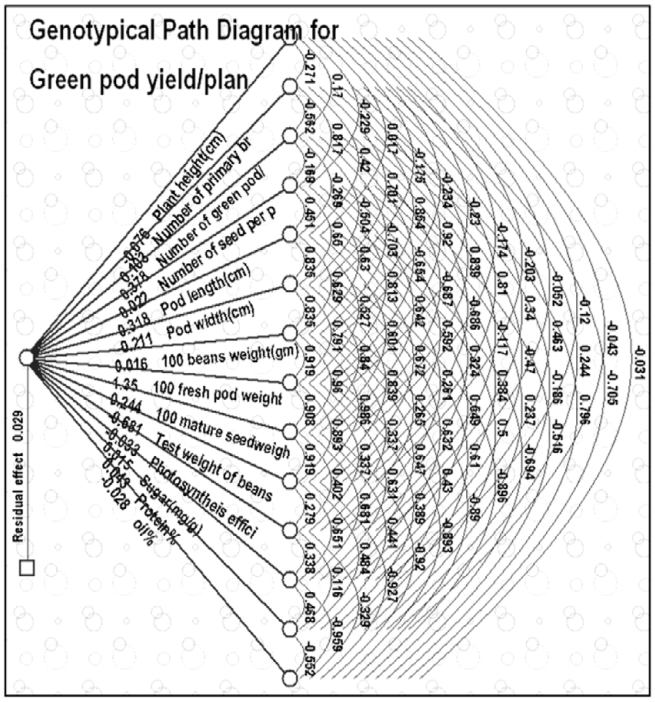


Figure 2: Genotypic path analysis diagram showing effect of different characters on green pod yield per plant.

Table 6: Qualitative characteristics of soybean genotypes.

Genotype	Flower color	Seed Shape	Helium color	Seed luster	Seed coat color at R8	Pod Color at R6	Texture	Taste	Aroma	Over all acceptability
PK-1314	Purple	Spherical	Imperfect black	Dull	Greyed-orange	Dark green	Extremely resistant	Nuttiness	Absent	Poor
AMS-6-1	Purple	Spherical	Grey	Shiny	Greyed-yellow	Dark green	Extremely resistant	Nuttiness	Absent	Poor
AMS-50	White	Spherical	Grey	Shiny	Greyed-yellow	Dark green	Extremely resistant	Nuttiness	Absent	Poor
AMS-73	Intermediate Purple	Spherical	Black	Shiny	Greyed-yellow	Dark green	Extremely resistant	Nuttiness	Absent	Poor
AMS-247	Intermediate Purple	Spherical	Imperfect black	Shiny	Greyed-yellow	Light green	Extremely resistant	Beaniness	Absent	Poor
AMS-9924	Purple	Spherical	Black	Shiny	Greyed-yellow	Light green	Extremely resistant	Beaniness	Absent	Poor
AMS-28	Purple	Oval	Grey	Shiny	Greyed-yellow	Dark green	Extremely resistant	Beaniness	Absent	Poor
AMS-353	Purple	Oval	Black	Shiny	Pale-yellow	Dark green	Extremely resistant	Beaniness	Absent	Poor
AMS-518	White	Oval	Brown	Shiny	Pale-yellow	Dark green	Extremely resistant	Beaniness	Absent	Poor
AMS-37	Purple	Spherical	Grey	Intermediate	Greyed-yellow	Dark green	Extremely resistant	Nuttiness	Absent	Poor
AMS-90-1	Purple	Spherical	Grey	Shiny	Greyed-orange	Yellow green	Extremely resistant	Beaniness	Absent	Poor
AMS-93	White	Spherical	Brown	Intermediate	Greyed-yellow	Yellow green	Extremely resistant	Beaniness	Absent	Poor
AMS-65	Purple	Spherical	Grey	Shiny	Greyed-yellow	Yellow green	Extremely resistant	Nuttiness	Absent	Poor
EC-251411	White	Oval	Brown	Intermediate	Greyed-yellow	Yellow green	Extremely resistant	Nuttiness	Absent	Poor
NRC-40	White	Oval	Black	Shiny	Greyed-orange	Yellow green	Extremely resistant	Nuttiness	Absent	Poor
NRC-2	Purple	Spherical	Black	Shiny	Pale-yellow	Dark green	Extremely resistant	Nuttiness	Absent	Poor
TAMS-38	White	Spherical	Brown	Shiny	Greyed-yellow	Yellow green	Extremely resistant	Beaniness	Absent	Poor
MAUS-158	White	Spherical	Brown	Intermediate	Greyed-orange	Yellow green	Extremely resistant	Beaniness	Absent	Poor
IC-241949	White	Oval	Imperfect black	Intermediate	Greyed-yellow	Yellow green	Extremely resistant	Nuttiness	Absent	Poor
IC-16815	Purple	Spherical	Imperfect black	Shiny	Greyed-yellow	Light green	Extremely resistant	Nuttiness	Absent	Poor
IC-118400	White	Spherical	Brown	Dull	Greyed-yellow	Light green	Extremely resistant	Nuttiness	Absent	Poor
IC-118452	Purple	Spherical	Grey	Shiny	Greyed-yellow	Light green	Extremely resistant	Beaniness	Absent	Poor
AGS-450	Intermediate Purple	Spherical flatten	Brown	Shiny	Moderate- brown	Yellow green	Not resistant	Sweetness	Absent	Good
AGS-457	White	Oval		Shiny	Moderate- brown	Light green	Not resistant	Sweetness	Present	Good
Swarna Vasundhara	Intermediate Purple	Oval	Brown	Intermediate	Greyed-yellow	Yellow green	Not resistant	Sweetness	Present	Good
AGS-459	White	Oval	Black	Shiny	Blackish	Light green	Not resistant	Sweetness	Present	Good
GC-84501-32-1	Intermediate Purple	Oval	Black	Intermediate	Light- grayish green	Dark green	Extremely resistant	Nuttiness	Absent	Moderately good
AGS-339	Intermediate Purple	Oval	Yellow	Dull	Moderate yellow	Light green	Not resistant	Beaniness	Absent	Poor
MACS-450	Intermediate Purple	Spherical	Black	Intermediate	Grayish- yellow green	Yellow green	Extremely resistant	Beaniness	Absent	Poor
MACS-1508	White	Spherical	Black	Dull	Greyed-orange	Dark green	Not resistant	Nuttiness	Absent	Good
MACS-1188	White	Spherical	Black	Shiny	Greyed-orange	Yellow green	Extremely resistant	Beaniness	Absent	Poor
TAMS-98-21	Purple	Spherical	Brown	Shiny	Greyed-yellow	Light green	Extremely resistant	Beaniness	Absent	Poor
HIMSO-1685	Purple	Oval	Brown	Dull	Grayish- yellow green	Dark green	Extremely resistant	Beaniness	Absent	Poor
JS-335	Purple	Spherical	Grey	Shiny	Greyed-yellow	Light green	Extremely resistant	Nuttiness	Absent	Poor

Note: *Descriptor for soybean, IBPGR/84/183, Rome (1984) (http://www.bioversityinternational.org/uploads/tx_news/Descriptors_for_soyabean_252.pdf)

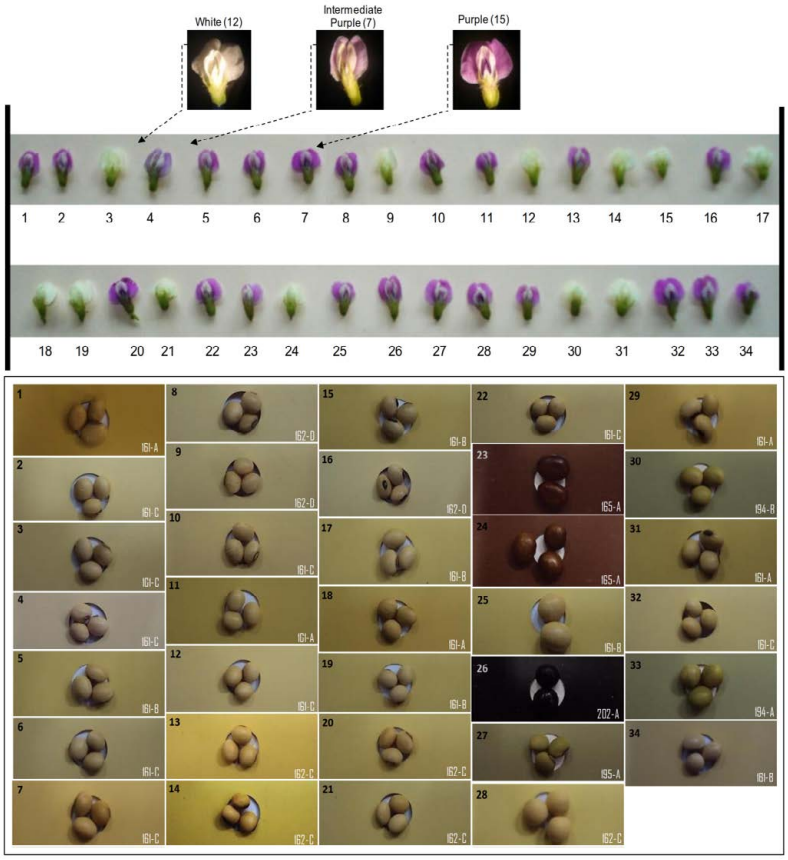
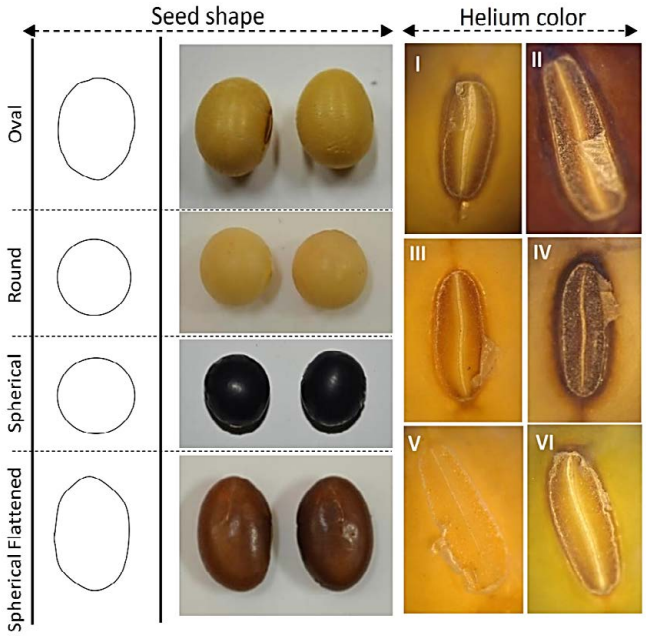


Figure 3: Variation in flower and mature seed coat color soybean genotypes under study as per The Royal Horticultural Society catalog, London.



Note: I-Grey, genotypeNo. 13; II-Dark brown, genotype no. 24; III-Medium Brown, genotype no. 25; IV-Black, genotype no. 28-Black, genotype no. 29; V-Yellow; VI-Imperfect black, genotype no. 33.

Figure 4: Variation in seed shape and helium color amongst the genotypes under study.

Table 7: Grouping of soybean genotypes in to three clusters based on various morphological traits.

Cluster	No. of genotypes	Percent of population	Name of genotypes
I	29	85.29	EC-251411, NRC-2, AMS-90-1, NRC-40, MAUS-158, AMS-99-24, IC-118452, AMS-5-18, TAMS98-21, JS-335, AMS-93, AMS-37, AMS-28(A), IC-118400, IC241949, AMS-6-1, MACS-1188, IC-16815, AMS-73, AMS-247, GC-84501-32-1, MACS-450, AMS-50(B), TAMS-38, MACS-1508, AMS-353, AMS-65, HIMSO(C), PK1314
II	4	11.76	AGS-457, Swarna Vasundhara, AGS-339, AGS-459
III	1	2.90	AGS-450

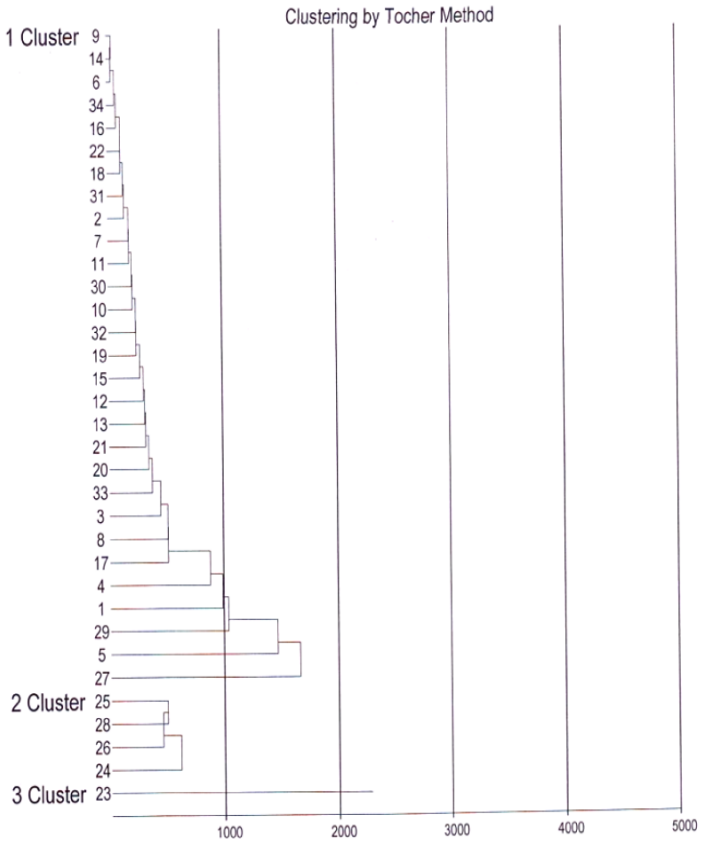


Figure 5: Dendrogram showing relationship among the soybean genotypes using morphological and quality traits.

Table 8: Mean values of different traits for three groups revealed by cluster analysis among 34 soybean genotypes.

Character	Group1	Group2	Group3	Contribution (%)
Days to 50% flowering	38.21	28.00	20.00	1.60
Days to maturity	91.76	83.83	77.67	2.50
Plant Height (cm)	41.68	32.97	40.27	7.84
No. of green pods per plant	50.07	37.62	29.10	2.14
Pod length (cm)	3.48	4.08	4.67	0.18
Pod width (cm)	0.77	1.33	1.71	0.18
100 seed weight (g)	16.75	40.47	56.51	0.18
100 fresh pod weight (g)	91.36	211.32	291.90	53.12
Sugar (mg/g)	56.57	78.78	92.01	30.66
Protein content (%)	35.60	37.80	45.07	1.25
Oil content (%)	20.21	19.27	17.88	0.18
Grain pod yield per plant (g)	45.89	79.19	85.04	0.18

with similar morphological traits in to the same cluster. However, the vegetable type soybean genotypes comprised in the cluster II separately. Similar results were also reported in soybean and other crops by Cui et al. [61], Abdullah et al. [62] and Kumar et al. [60].

Results also revealed that, the cluster III comprised of a single genotype AGS-450 on the basis of its performance of having high 100 seed weight, fresh pod weight, mature seed weight, sugar and protein content and lowest oil content at R6 stage. Therefore, the genotype

from cluster II and III could be used for hybridization programme with these genotypes in order to develop superior vegetable type soybean varieties.

Molecular diversity

Characteristics of EST-SSR markers studied: Based on the earlier studies and their polymorphic nature, ten EST-SSR primer pairs were selected for molecular characterization of 34 soybean genotypes. Among these primers, 8 were found polymorphic and two were found monomorphic. The number of repeats ranged from 8 to 18 with an average of 11.3. All the motifs of EST-SSR were ranged from 24 to 36 bp with an average of 29.1 bp (Table 9). Among 8 polymorphic SSR loci, six (75%) were trinucleotide repeats and two (25%) were dinucleotide repeats (Figure 6).

Number of alleles: Amplification of ten EST-SSR (microsatellite) markers using 34 soybean genotypes produced 334 amplicons. A total of 30 alleles were detected and distributed in the population studied with the range from 1 (CSSR391 and CSSR400) to 5 (SSR472) for respective primers. With an average of 3.0 alleles across 10 loci, with single allele for two marker, 2 alleles for one marker, 3 alleles for three markers, 4 alleles for the three markers, and 5 alleles for one marker. The overall size of the amplified products ranged from 82 bp (CSSR472) to 520 bp (GMES0709). The number of alleles, range of allele size and the PIC values of different soybean genotypes for ten EST-SSR markers are depicted in Table 9.

Monomorphic, frequent, and rare alleles: Very frequent alleles were considered to be those occurring in more than 10% of the varieties in the collection, whereas those occurring between 2% and 10% of the varieties in the collection were classified as rare alleles [63]. In this experiment amongst total of 30 alleles, 11 were monomorphic, 18 were frequent and one rare allele was indentified at 10 microsatellite loci with an average of 3.0 alleles per locus. The frequency of most common allele at each locus ranged from 36.2 (GMES4774) to 54.0 (GMES0709). Since, eighteen frequent alleles were found across the population with an average of 0.6 alleles per locus and 1 rare allele was found across the population with an average of 0.03 alleles per locus (Table 9). The rare allele was found in the genotype AMS-5-18, NRC-40 and Himso(C). There was no specific allele amplified discriminating vegetable type genotype(s).

Polymorphism in EST-SSR: All the ten EST-SSR markers used in this study generated polymorphic bands among the soybean genotypes. Similar analysis were reported by Dong (2014); Zhang [40]. The PIC values of EST-SSR loci were ranged from 0.38 (CSSR472) to 0.66 (GMES4774) with an average of 0.43. The highest PIC value (0.66) was obtained for GMES4774, followed by GMSE0709 (0.60), CSSR405 (0.58), CSSR385 (0.56), CG819919.1 (0.54), GMES0644 (0.50), and CSSR540 (0.48) (Table 4). The lowest PIC value (0.38) was obtained for CSSR472.

Genetic distance-based analysis

An un-rooted neighbor-joining tree (Figure 7) showed the genetic relationships among the soybean varieties. There were four clusters and one out-group member (NRC2) observed in NJ-Tree, interestingly; three varieties (TAMS38, IC241949, and IC118400) were grouped far from other three clusters containing remaining varieties. Vegetable type genotypes (AGS-457, Swarna Vasundhara, AGS-459, GC-84501-32-1, and AGS-339) were found grouped in a single cluster along with other genotypes however; HIMSO 1685(C) was grouped with TAMS9821. The UPGMA-based dendrogram obtained from the binary data of the samples analyzed. This pooled data analysis grouped the 34 soybean genotypes into seven clusters (Figure 8 and Table 10).

The dice similarity among the accessions ranged from 0.167 to 0.944 similarity coefficient with an average of 0.675. About 35.30% of the population among the soybean genotype showed similarity greater than 0.98 while about 64.70% showing similarity lower than

Table 9: Characteristics of EST-SSR markers used in the study.

S No.	Locus name	Corresponding ID	Forward/ Reverse	Primer sequence (5'–3')	Motif	Annealing (°C)	Range (bp)	Total alleles	Monomorphic alleles	Frequent alleles	Rare alleles	Highest freq. allele (%)	PIC values
1.	Gmp-017	CG819919.1	Forward:	ACCTCTTCCCCCATTCAGTT	$(AT)_{12}$	55	198-236	3	2	0	1	47.9	0.54
			Reverse:	ACCTCTTCCCCCATTCAGTT									
2.	Gmp-048	CSSR391	Forward:	CCGCCGAAGTACGAAGTAGA	$(GTC)_9$	54	247-263	1	1	0	0	-	0
			Reverse:	CCGCCGAAGTACGAAGTAGA									
3.	Gmp-049	CSSR400	Forward:	CTTCTCTCAGCACCCTCCAC	$(TC)_{18}$	54	250-283	1	1	0	0	-	0
			Reverse:	AACCCTTCTTCCACTTCCGT									
4.	Gmp-050	CSSR405	Forward:	AACAACAACAGCCACCACAA	$(CAA)_8$	54	197-238	4	0	4	0	47.1	0.58
			Reverse:	CTGGCATTGACACTGTTGCT									
5.	Gmp-066	CSSR472	Forward:	GGTTACGGCACTTCCTACCA	$(AAC)_9$	55	202-235	5	1	4	0	40.0	0.38
			Reverse:	AATTTTTGCGTTGTTGAGGG									
6.	Gmp-088	CSSR540	Forward:	GAGGTTGGTGCCTGGAGATA	$(GAT)_9$	56	197-235	4	1	3	0	42.0	0.48
			Reverse:	TGGCGAGTTACGAGGCTATT									
7.	Gmp-122	GMES0644	Forward:	AGATTGGAAGAGCCATCCCT	$(AGA)_{12}$	54	294-308	2	1	1	0	51.5	0.50
			Reverse:	ACTTCTCGCCCTCGTTCTTT									
8.	Gmp-133	GMES0709	Forward:	ACAGGTTGTGGGACGGTAAA	$(ACA)_9$	55	197-221	3	1	2	0	54.0	0.60
			Reverse:	ACCAAATAGCTGGAATCCCC									
9.	Gmp-197	GMES4774	Forward:	AGGATCACATACCAGGCACC	$(TA)_{18}$	56	253-285	3	1	2	0	36.2	0.66
			Reverse:	AGGATCACATACCAGGCACC									
10.	Gmp-046	CSSR385	Forward:	AACCCTTCTTCCACTTCCGT	$(CAA)_9$	55	197-211	4	2	2	0	37.8	0.56
			Reverse:	AACCCTTCTTCCACTTCCGT									
	Total							**30**	**11**	**18**	**1**	**-**	**-**
	Average							**3**	**1.1**	**1.8**	**0.1**	**35.65**	**0.43**

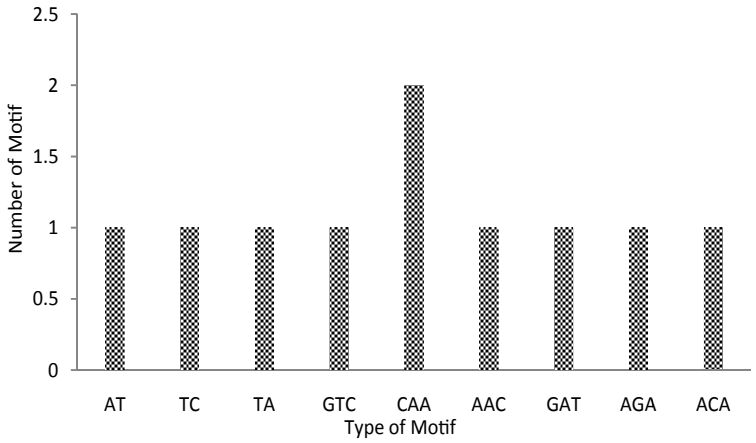


Figure 6: Figure showing distribution of different EST-SSR motifs.

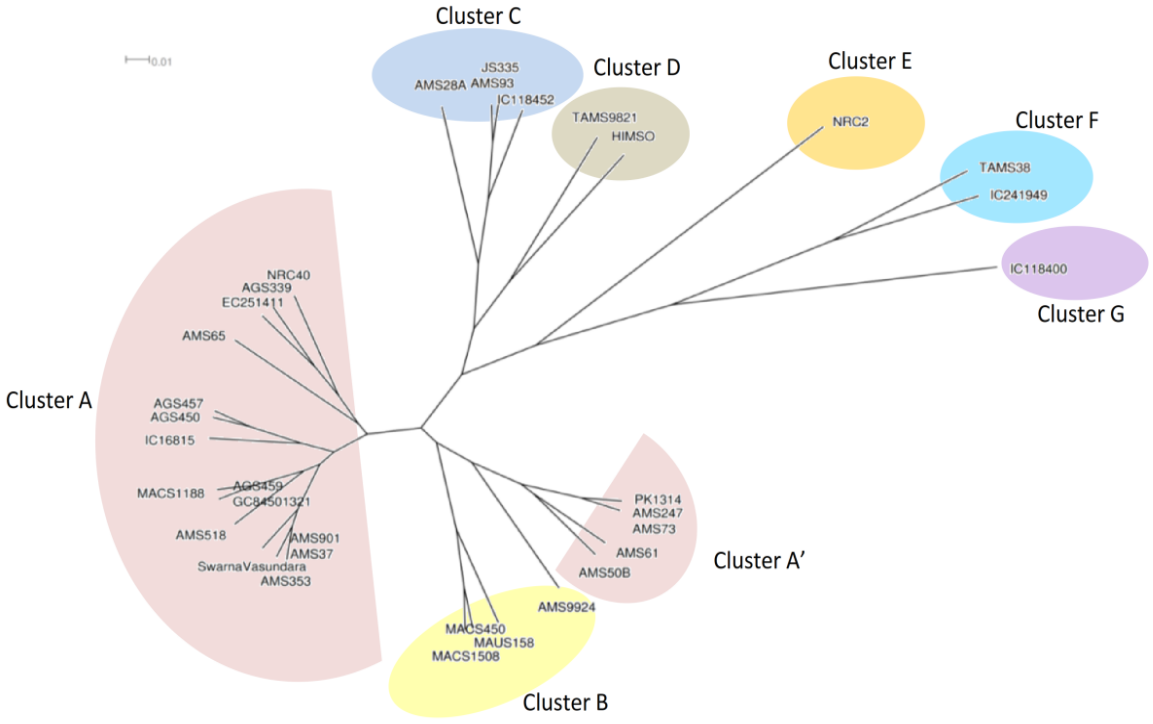


Figure 7: Neighbor joining tree showing the genetic relationship among soybean genotypes generated using molecular diversity studies.

0.675. There was strong similarity between the accessions in the clusters A, B and C. Lowest similarities were found between single individuals in the clusters E and G (Figure 8).

The results of the analysis of genetic diversity provide estimates on the level of genetic variation among the accessions that can be used in management and improvement. In this study, morphological data analysis was coupled with molecular analyses (EST-SSR markers) to investigate the genetic relationships among the soybean genotypes including vegetable-types.

The range of genetic distance based on the morphological traits was on average lower than EST-SSR markers which might be a reflection of the environmental influence on the performance of the materials. Therefore, the DNA markers and morpho-physiological traits will not necessarily gain closely matching results [64]. Mertinez et al. [65] believed that the correspondence between different methods might be improved by analyzing multiple morphological and DNA based markers. Two reasons for low or no correlations between molecular and morphological markers as well as biochemical data have been suggested by Semagn [66]. One is, DNA markers cover a larger proportion of the genome, including coding and non-coding regions, than the morphological markers and second are, DNA markers are less subjected to artificial selection compared to the morphological markers.

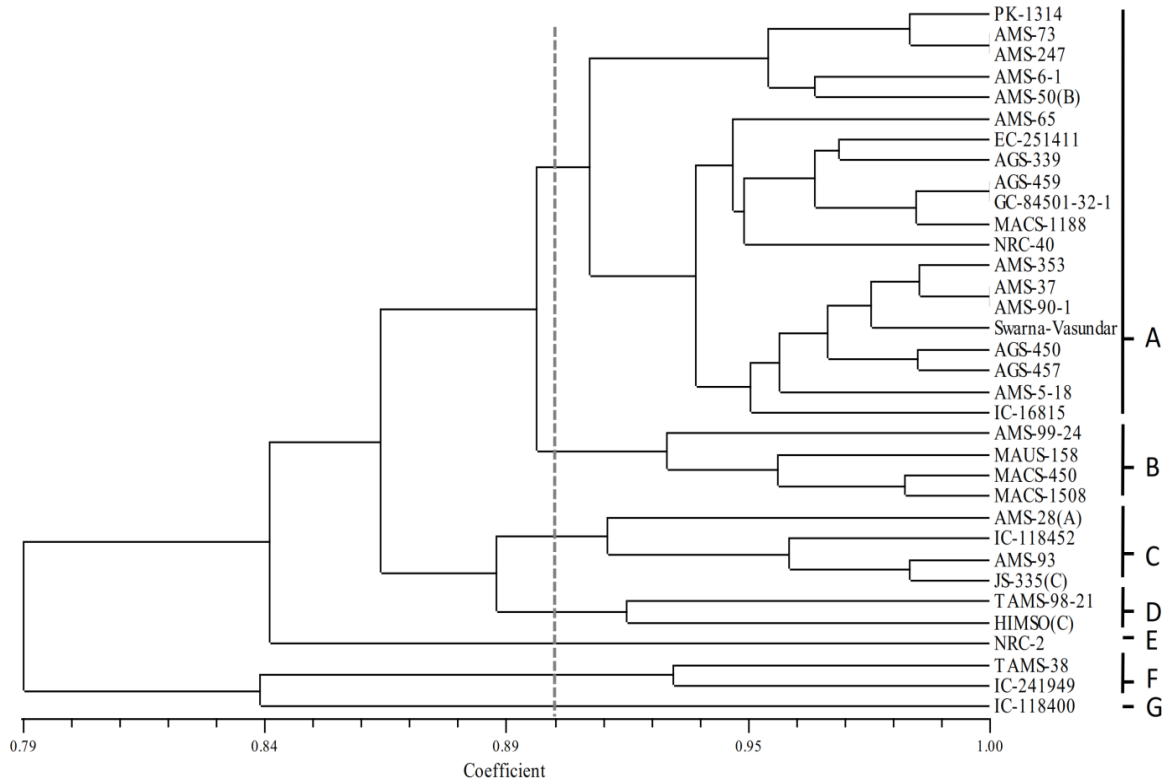


Figure 8: Dendrogram of genetic relationship among soybean genotypes based on ten EST-SSR markers. Clusters were defined at Jaccard's Coefficient. Alphabets on the right side of the dendrogram are indicating clusters which are listed in Table 10.

Table 10: Grouping of soybean genotypes in to seven clusters using SSR based molecular characterization.

Cluster	No. of genotypes	Percent of population	Name of genotypes
A	20	58.82	EC-251411, AMS-90-1, NRC-40, AMS-5-18, AMS-37, AMS-6-1, MACS-1188, IC-16815, AMS-73, AMS-247, GC-84501-32-1, AMS-50(B), AMS-353, AMS-65, PK1314, AGS-339, AGS-459, Swarna Vasundhara, AGS-457, AGS-450
B	4	11.76	MAUS-158, AMS-99-24, MACS-450, MACS-1508
C	4	11.76	IC-118452, JS-335, AMS-93, AMS-28(A)
D	2	5.88	TAMS-98-21, HIMSO(C)
E	1	2.94	NRC-2
F	2	5.88	TAMS-38, IC-241949
G	1	2.94	IC-118400

In review, the data shows significant variation among the soybean accessions their mutants and vegetable type genotypes. The information generated can be used in selecting diverse parents in breeding programme and in maintaining genetic variation in germplasm.

Acknowledgements

The authors are thankful to Dr. Ramakrishnan M. Nair, Vegetable Breeder Legumes, AVRDC-Regional center for South Asia, ICRISAT Campus, Patancheru-502 324, Hyderabad (Andhra Pradesh), India for providing seed material of vegetable type soybean genotypes to undertake present investigation.

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Yaashikaa et al., Vegetos 2016, 29:4
10.5958/2229-4473.2016.00092.6

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Enhanced Agroproductivity by Phosphate Solubilising Bacteria

Yaashikaa PR, Tamilselvi S*, Suji S and Murugesan GS

Abstract

The demand in agricultural field can be fulfilled by providing supplements in the form of biofertilizers and biopesticides. Biofertilizers are a consortium of microorganisms that helps for the growth of plants by providing necessary nutrients either by fixing atmospheric nitrogen or by solubilising the phosphorous present in the soil. In this present study, *Bacillus coagulans* acts as phosphate solubiliser in degrading rock phosphate by providing suitable carrier material. *Bacillus coagulans* was grown in Nutrient Medium and bioprocess conditions for maximum growth of the organism was optimized. It was found that *Bacillus coagulans* showed maximum cell concentration at pH 7.5, temperature 37°C and incubation period of 24 hrs. The culture is then mixed with suitable powder and liquid carriers. Powder carrier such as water hyacinth and groundnut shell are mixed with bio inoculum. Liquid carriers such as herbal mixture solution, archaea bacterial solution and treated waste water are mixed with culture at equal proportion. Life span of the organism along with the carrier material was scrutinized for a period of 120 days. The field response of the formulated biofertilizer was checked by applying on shallots. Among the powder carriers, water hyacinth showed highest cell count of 74×10^9 cfu/ml. Comparitively, among the liquid carriers archaea bacterial solution showed highest cell count of 77×10^9 cfu/ml. In order to determine the efficiency, morphological characters of the shallots were examined.

Keywords

Bacillus coagulans; Biofertilizers; Liquid carriers; Powder carriers; Shelf-life study

Introduction

Biotechnology is gaining more attention in vast areas most importantly in the field of agriculture. It is said that agriculture is the backbone of India and also to the people in India. Agriculture is facing destructive activities because of incorporation of chemical fertilizers. The use of chemical fertilizers prevents agriculture practicing naturally. Plants require nutrients for their optimum growth and maximum yield [1]. Chemical fertilizers cause various effects to soil during application and also to humans who consume the products from it. Mainly the soil loses all the natural nutrients it possess finally leading to infertility. Soil infertility makes the land unfit for practicing agriculture thus converting the agricultural lands to industrial areas [2]. In future there is a fear of losing lands for agriculture and agriculture may become extinct. Integrated Soil Management System can be implemented in which methods for fixation of biological nitrogen and implementation of nutrients to the soil in natural method is proposed [3].

Biofertilizer is not a new term to be introduced since the natural fertilizers has been used in olden days before chemical fertilizers were introduced. These natural fertilizers have been used without the scientific knowledge of its benefits [4]. The advent of chemical fertilizers has made a drastic change in agricultural field. Chemical fertilizers help in fast growth of plants, ripening of fruits quickly and fast flowering in plants by supplying chemically synthesised NPK (Nitrogen, Phosphorous and Potassium [5]. The ill-effects caused by these chemical fertilizers are dangerous leading to extreme stages of cancer also. On spraying, these fertilizers cause air pollution by travelling along with the air to the areas distant from application.

Biofertilizers are a universally accepted mode of supply of nutrients for plant growth and development. Microorganisms rule the whole world invisibly. They are an in-depth part of soil and influence plant growth [6]. Microbes play a major role in solubilisation of insoluble phosphorous to soluble phosphorous, fixing atmospheric nitrogen, mobilization of phosphorous and production of hormones required for plant growth [7]. Though microbes are present in soil, inoculating specific microorganism along with suitable vehicle provides additional food for plants for optimum growth [8]. Different microorganism contributes in providing numerous nutrients for growth of plants.

Microorganism belonging to genus *Rhizobium* [9], *Azospirillum, Azobacter*, etc., helps in fixing atmospheric nitrogen in the soil. Nitrogen is the major nutrient required by plants mainly for their growth, development and production of fruits and grains. Crops like rice require about 18 kg of N for every tone of effective production [10-12]. Nitrogen is effectively transferred from mature leaves immediately to the young leaves. So this mobilization of nitrogen is indicated by the deficiency symptoms in the mature leaves [13]. *Bacillus, Pseudomonas, Aspergillus*, etc., are best known for their phosphate solubilising properties. Phosphorous is second major nutrient next to nitrogen required by plants for their growth. Generally P content is low in soil. Increase in P in soil is influenced by plant development and productivity. Phosphorous supplied through chemical fertilizers is available in insoluble forms which are not easily taken by the plants. Microorganisms help in the conversion of insoluble phosphate into soluble form easily made available for plant growth [14]. Excess application of insoluble phosphorous may lead to overloading of insoluble P in soil [15]. This may lead to excessive pollution indirectly affecting runoff water [16]. Plant Growth Promoting Rhizobacteria (PGPR) is group of bacteria present on the Rhizosphere near the root surface in association with them for optimum growth and development of plants [17]. PGPR through its motility reaches actively on the surface of roots and easily adapts to the changing environment [18]. Bacterial species of *Pseudomonas* [19], *Bacillus, Klebiella, Enterobacter*, etc., are important in possessing these characteristics [20].

The present study deals with *Bacillus coagulans* plant growth promoting Rhizobacteria in acting as biocontrol agent for plant growth. Carrier material serves as vehicle for the microorganisms to provide nutrients. Carrier material must possess good moisture

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Received: September 09, 2016 **Accepted:** October 25, 2016 **Published:** October 31, 2016

absorption property, non-toxic, inexpensive, easily available, etc. In this study carrier material is formulated in such a way that they provide additional nutrients to the plant for their growth.

Material and Methods

Procurement of organism

The microorganism was procured from Bannari Amman Bio labs in Sathyamangalam. The organism was transferred to laboratory for reviving and was refrigerated for further use. *Bacillus coagulans* was revived in Nutrient Medium providing suitable growth conditions.

Optimization of growth conditions

Conditions for optimum growth of *Bacillus coagulans* were optimized by varying pH, temperature and incubation period. Optimization was performed using simple method. The organism was grown at different pH (6,6.5,7,7.5,8), temperature (27,30,33,37,40) and incubation period (12,24,36,48,60).

Selection of carrier materials

Powder carriers: Water hyacinth and Groundnut shell were selected for this study. The collected samples were transferred to laboratory. The carriers were dried in shade for about 2-3 days to remove moisture content fully. Then the carrier materials were grinded to fine powders. After grinding, the carrier materials were sieved such that the size of the carriers ranges between 10-40 µm. The grinded carrier materials were stored in sterile containers for future use.

Liquid carriers: Domestic and agricultural wastes are chosen as liquid carriers. Three liquid carriers were formulated namely i) Herbal Mixture Solution ii) Archaea Bacterial Solution iii) Waste water. Herbal Mixture Solution formulation contains butter milk + *Terminalia chebula* (sweet wood (adhimadhuram)) + *Glycyrrhiza glabra* (kadukai). Archaea Bacterial Solution is formulation using cow dung, jaggery, *Terminalia chebula* and *Glycyrrhiza glabra* and water. Waste water was collected from Bannari Amman Institute of Technology, Sathyamangalam. These liquid carriers were stored in sterile bottles after formulation (Figure 1).

Pretreatment of carrier materials

The powder carriers were subjected to pre-treatment process before they are mixed with the organisms. To neutralize pH, the carrier materials are mixed with calcium carbonate. Sterilization of the carrier material is essential to keep high number of inoculants bacteria on carrier for long storage period. Gamma radiation is the best way of sterilizing carrier materials. In lab scale, sterilization is usually done by autoclaving. Carrier materials are packed into thin-walled polythene bags and autoclaved at 121°C for 1 hour at 15 lbs pressure.

Carrier-based inoculum preparation

After sterilization of the carrier materials, it is mixed with the inoculums in aseptic condition. The quantity of powder carrier and culture was optimized using response surface methodology. Broth was added in such a way that no excess broth remains during blending. Then the mixture is air-dried to remove moisture content. Drying was continued till the moisture content reaches below 20%. The air-dried formulation is then packed into sterile polythene bags and tightly sealed. Liquid carriers formulations were also optimized using software Design Expert 7.0.0. The response surface methodology method reduces the total number of experiments to be performed providing optimized condition. After formulating with the organisms, liquid carriers were kept for incubation for shelf-life studies.

Shelf-life analysis

After formulating both the powder and liquid carriers, it is subjected to shelf-life analysis. Shelf-life was checked regularly once in 30 days (Figure 2). The life span of microorganism in powder carriers is determined by serial dilution method followed by plating in Nutrient medium. Then the total number of cells in various dilutions were counted using hemocytometer. For liquid carriers, the shelf-life is determined by measuring the optical density at 600nm. Serial dilution followed by plating is done and finally total viable cells are calculated using cell counter.

In vivo studies

In order to determine the efficiency of the biofertilizer produced, *in vivo* studies was performed. To about 150 grams of soil taken in beaker, one-tenth of biofertilizer was added. Powder and liquid carriers were added in separate individual beakers containing soil. One beaker without biofertilizer added was taken as control. *Allium cepa* (onion) was grown in all the beakers. Then the results were interpreted.

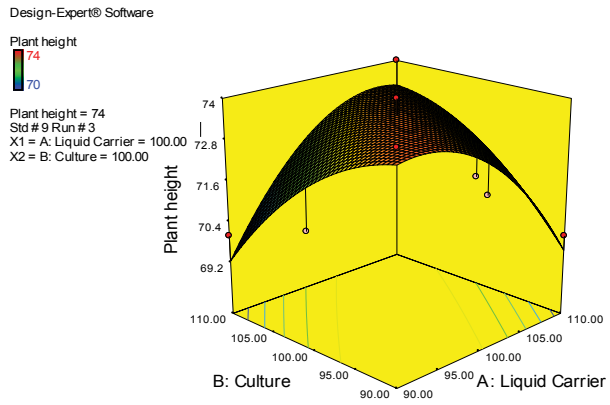


Figure 1: Optimization of liquid carrier and Bacillus coagulans culture in response surface methodology method using Design Expert 7.0.0.

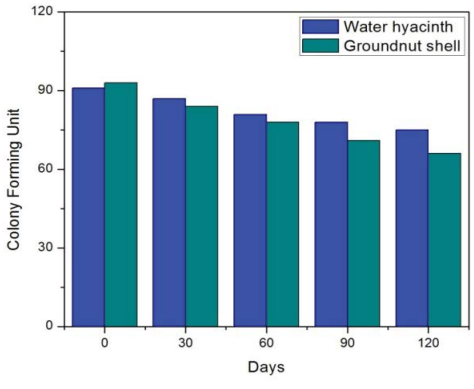


Figure 2: Graphical representation of shelf-life analysis of powder carriers.

Results and Discussion

Optimization of growth conditions

Bacillus coagulans was grown in Nutrient Medium. After optimization, it was found that *Bacillus coagulans* showed maximum growth at pH 7.5, temperature 37°C and incubation period of 24 hours (Figure 3).

Optimization of carrier and culture

The composition of blending of powder carrier and culture for maximum yield of plants were optimized using response surface methodology. On interpretation it was found that on mixing 150 g of carrier with 75 ml of broth total plant height of 73 cm is obtained. A total of 20 runs of experiments were conducted out of which ratio of 75:150 of culture and carrier resulted in production of maximum yield of plant height with increase in leaf number, leaf height, root length, bulb diameter and bulb weight.

A Normal plot of residuals was obtained which represents the yield of plant height by indication of colours. The reddish-orange colour in the (Figure 4) represents the maximum height of the plant nearly 74 cm. This indicates that the proportion of blending of powder carrier and culture was optimum to be 75 g and 150 ml respectively.

Liquid carriers

The quantity of blending of liquid carriers was also optimized using Design Expert 7.0.0 software (Figure 5). A total of 13 runs of experiments were performed. It was found that on mixing equal ratio of liquid carrier and culture, maximum yield was obtained. The total plant height was found to be 74 cm. This also resulted in increase in morphological characters of the plant such as root (root height, bulb diameter and bulb weight) and shoot (leaf number, leaf length and plant height).

The Normal Probability curve also depicts the optimised ratio of liquid carrier and culture for blending. The colour indicates the maximum growth of the plant when supplement is provided at optimized condition. The reddish-orange colour indicates the yield of plant height to be 74 cm. The 1:1 ratio of liquid carrier and culture proves to be efficient mixing for obtaining maximum yield.

Shelf-life study

The lifespan of the organism *Bacillus coagulans* was inspected for a period of 120 days. Among the powder carriers, water hyacinth showed a maximum cell count of 74×10^9 cfu/ml. The shelf-life study of *Bacillus coagulans* was analysed using Minitab 16.1.1.

Life Span in liquid carriers

The shelf-life study of *Bacillus coagulans* in liquid carriers is shown in (Figure 6). Among the three liquid carriers, Archaea bacterial solution showed maximum cell count of 77×10^9 cfu. The organism has the capacity to survive in archaea bacterial solution compared to herbal mixture solution and treated waste water.

Morphological comparison of shallots

The morphological characters such as leaf length, leaf numbers, plant height, bulb diameter, bulb weight and root length were analysed and compared among the powder and liquid carriers to determine the suitable carrier material for *Bacillus coagulans.* The shoot and root characters were analysed using Minitab 16.1.1 (Figures 7, 8, 9 and 10).

Liquid carriers

The three liquid carriers showed significant results compared to control. The morphological characters in control do not show similar results when compared with the liquid carriers. Of the three liquid carriers, archaea bacterial solution proves to be better on interpretation. The leaf length was similar and more in case of archaea

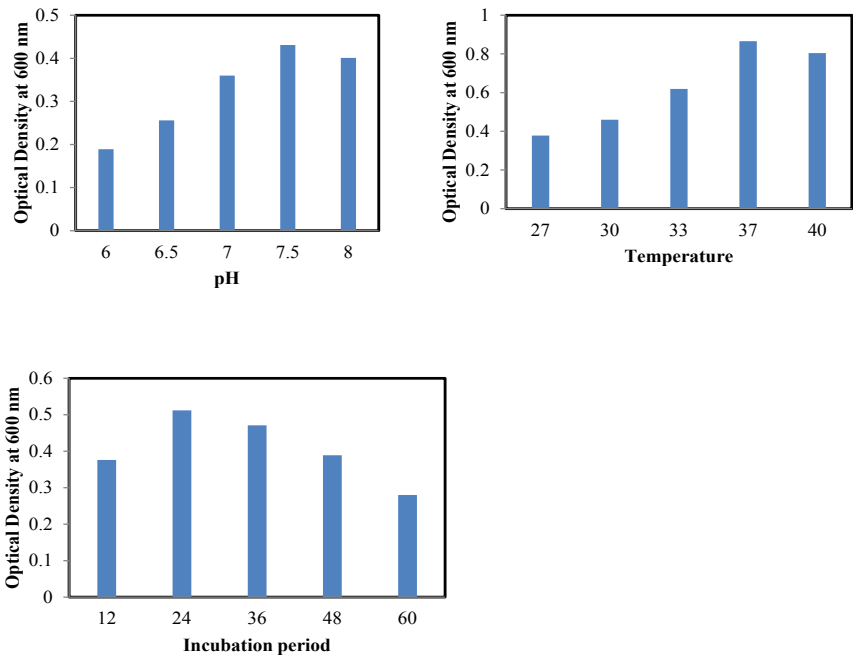


Figure 3: Optimization of growth conditions for *Bacillus coagulans*.

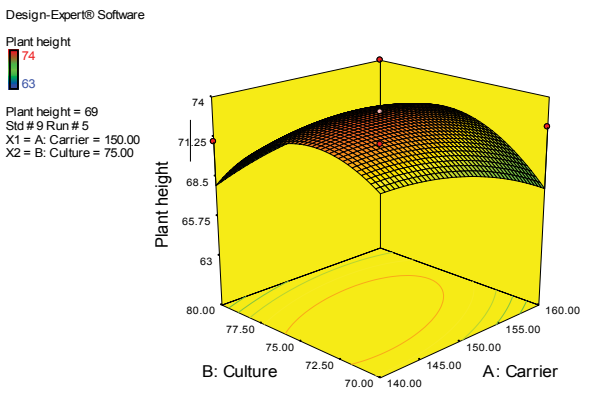


Figure 4: Optimization of quantity of powder carrier and culture of Bacillus coagulans using response surface methodology method in Design Expert – 7.0.0.

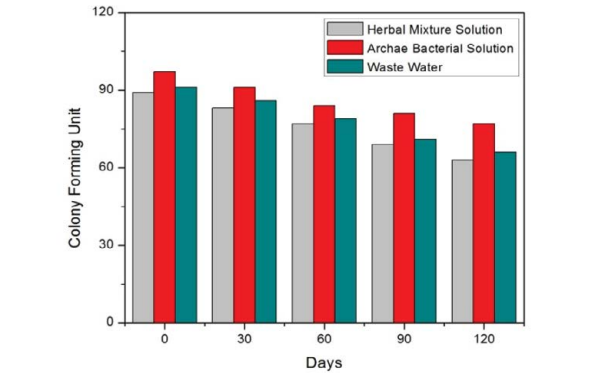


Figure 5: Graphical representation of shelf-life analysis of liquid carriers.

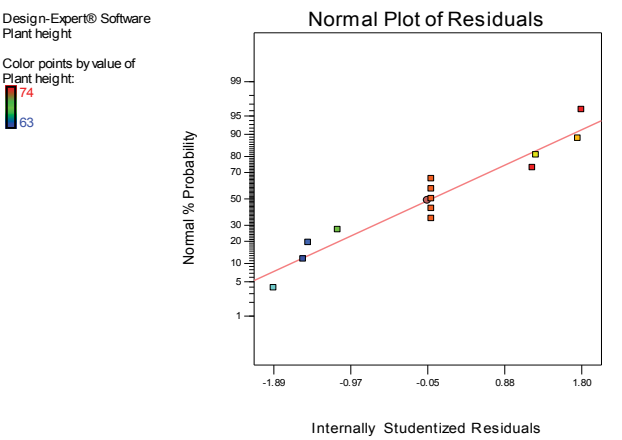
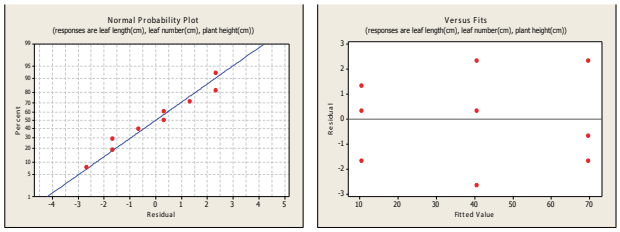


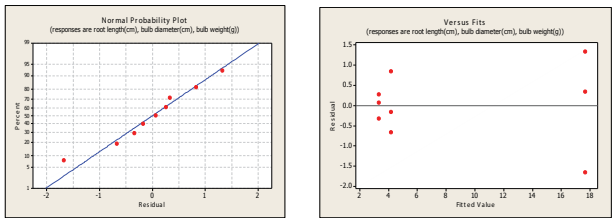
Figure 6: Normal Residual Plot of Response Surface Methodology using Design Expert 7.

bacterial solution and herbal mixture solution compared to treat waste water. The leaf number was more in archaea bacterial solution and treated waste and water. All the liquid carriers showed significant plant height but archaea bacterial solution showed increased plant height. The root length was more in treated waste water but the number of root hairs increased in archaea bacterial solution. The diameter of the



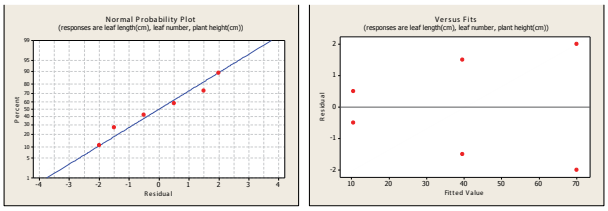
Standard deviation=2.082 R-Sq=99.50% R-Sq(adj)=99.34%
Pooled StDev=2.082

Figure 7: Analysis of shoot characters (leaf length, leaf number and plant height) using Minitab 16.1.1.



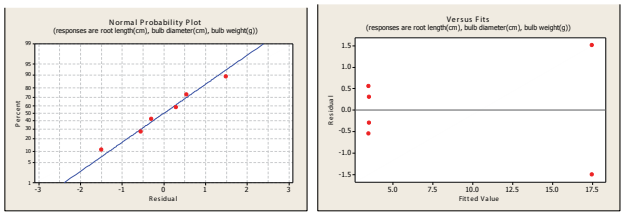
Standard deviation=1.327 R-Sq=98.02% R-Sq(adj)=96.71%
Pooled StDev=1.327

Figure 8: Analysis of root characters (root length, bulb diameter and bulb weight) using Minitab 16.1.1.



Standard deviation=2.082 R-Sq=99.63% R-Sq(adj)=99.39% ; Pooled StDev=2.082

Figure 9: Analysis of shoot characters (leaf length, leaf number and plant height) using Minitab 16.1.1.



Standard deviation=1.327 R-Sq=98.02% R-Sq(adj)=96.71%
Pooled StDev=1.327

Figure 10: Analysis of root characters (plant height, bulb diameter and bulb weight) using Minitab 16.1.1.

bulb was high in archaea bacterial solution and treated waste water. Herbal Mixture Solution showed insignificant bulb diameter. Archaea bacterial solution showed increased bulb weight compared to other carriers. Overall, Archaea bacterial solution proves to be effective and suitable carrier on scrutinising the results.

Powder carriers

Among the powder carriers, water hyacinth showed increased leaf length compared to groundnut shell. This also increased the plant height. The number of leaves for both water hyacinth and groundnut

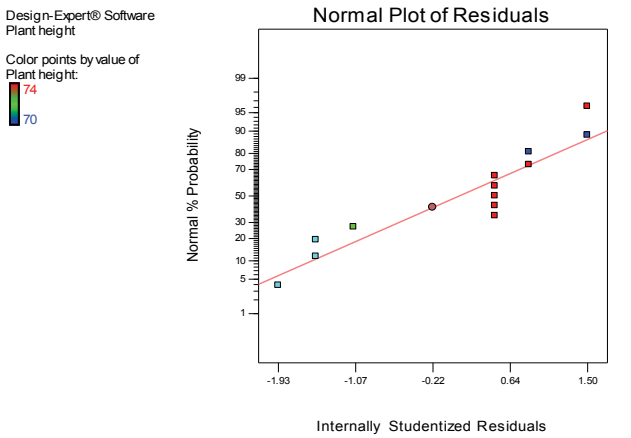


Figure 11: Normal residual plot of response surface methodology using Design Expert 7.0.0.

shell are significant. The root length was more in water hyacinth than groundnut shell. Also the number of root hairs showed high in water hyacinth only. The bulb weight was similar in case of both the powder carriers. The water hyacinth showed large bulb diameter than groundnut shell. The control showed poor results compared to both water hyacinth and groundnut shell (Figure 11).

The Normal Probability Plot for the shoot and root characters for both powder and liquid carriers depicts that the statistical data obtained from the analysis of morphological characteristics of shallots are significant. The points obtained are near and around the linear line indicating that the carriers are significant to be used as biofertilizer. Also the standard deviation obtained for powder and liquid carriers are less than 5 indicating the carrier suitable through the statistical analysis. The regression square value additionally supports the capability of the powder and liquid carrier to be best (Figure 9).

Conclusion

From this study, it was found that on culturing *Bacillus coagulans* in Nutrient Medium, the optimum conditions for effective growth were found to be at pH 7.5, temperature 37°C and incubation period of 24 hours. The highest cell concentration was found in water hyacinth with cell count of 74×10^9 cfu/ml and 77×10^9 cfu/ml in liquid carrier archaea bacterial solution. The morphological characteristics of shallots such as leaf length, leaf numbers, plant height, root length and hairs, bulb diameter and weight were found to be increased in water hyacinth powder carrier whereas in liquid carrier archaea bacterial solution leaf length, leaf number, root length and bulb diameter showed better results. Thus formulated biofertilizer with water hyacinth as powder carrier and archaea bacterial solution as liquid carrier proved to be effective than chemically synthesised fertilizers to fulfil the agricultural demand.

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Machenahalli et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00087.2

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Integrated Management of Die-Back and Fruit Rot of Chilli (*Capsicum annuum* L.)

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Abstract

Fruit rot and die-back diseases are major yield limiting factor in all chilli growing areas of India. Integrated management of chilli fruit rot disease during *kharif* 2012 and 2013 at Main Agricultural Research Station, University of Agricultural Sciences Dharwad, Karnataka, indicated that adoptive module including seed treatment with carboxin + thiram at 2 g/kg, seedling dip in *P. fluorescens* (10 g/l), spray with neem oil (10 ml/l), hexaconazole, propicoanzole (0.1%) and carbendazim + mancozeb (0.2%) showed least seedling infection (7.06%), die-back incidence (1.21%) and severity (9.30 PDI), fruit rot incidence (4.47%), severity (2.68 PDI) with high dry chilli yield (8.92 q/ha) and C:B ratio (2.44).

Keywords

Chilli; Fruit rot; Die-back; Integrated disease management

Introduction

Chilli (*Capsicum annuum* L.) is one of the very popular spice and vegetable crop grown worldwide. It is also known for its medicinal and health benefiting properties. India is the largest producer of chilli, grown over an area of 0.79 m. ha with an annual production of 0.13 m. tons with the productivity of 1.6 m tons/ha [1]. Chilli is suffering from several economically important diseases like damping off, die back, fruit rot, leaf spots, leaf curl, wilt etc. which are posing a serious threat to the successful large-scale cultivation. The fruit rot disease is more severe in India because of its complex nature, caused by fungi *Colletotrichum* spp. (*C. capsici*, *C. gloeosporioides* and *C. acutatum*), also *Alternaria alternata* and *Fusarium* spp. is major yield limiting factor. These pathogens also cause death of vegetative branch from tip to downwards, reduces yield from 10% to 80% of the crop production [2]. Fruit rot causes extensive damage and considerably reduce the market value of the produce [3]. It is essential to manage the disease in an integrated manner in which fungicides, botanicals and bio-agents play an integral part and becoming more relevant in the present day disease management scenario. Therefore in this present paper bio-intensive, adoptive (IDM) and chemical modules evaluation results were presented which are of utmost concern to identify best module for management of disease with maximum cost benefit ratio which will help the farming community to a greater extent.

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Received: July 21, 2016 **Accepted:** August 19, 2016 **Published:** August 26, 2016

Material and Methods

Field experiment was conducted to develop integrated diseases management strategies. Experiments were laid out in four modules namely bio intensive module for fruit rot disease and insect pests (M_1), bio-intensive module for disease with chemical pesticides for insect pests (M_2), Adoptive module (M_3) chemical intensive module (M_4). The study was conducted during *kharif* 2012 and 2013 at Main Agricultural Research Station, University of Agricultural Sciences Dharwad, Karnataka state, India. The fruit rot and die-back severity was assessed following the score chart mentioned by Mayee and Datar. The Byadgi Dabbi variety seedlings were planted in plot size of 12.0 × 9.0 m with spacing 60.0 × 60.0 cm on 25th June 2012 and 2013. There were four modules with five replications were laid out in a randomized complete block design, details of the modules mentioned in Table 1. Seedling rot incidence, die-back and fruit rot Incidence and severity was recorded, statistical analysis was carried out as per the procedures given by Panse and Sukhatme [3]. Details of soil properties and environmental conditions at experimental site are given in Appendices I, II and III.

Fruit rot and die-back score chart:

Grade	Per cent fruit area infection / Branches infected per plant	Reaction
0	0	Immune
1	1-10	Resistant
3	11 – 25	Moderately resistant
5	26 – 50	Moderately susceptible
7	51 – 75	Susceptible
9	> 75	Highly susceptible

Per cent disease incidence of fruit rot was calculated by

$$\text{Percent diseases incidence} = \frac{\text{Numberof fruits infected}}{\text{Totalnumber of fruits examined}} \times 100$$

Per cent Disease Index was calculated to estimate the disease severity of fruit rot disease as per the formula given by Wheeler [4].

$$\text{Fruitrot PDI} = \frac{\text{Sumof numerical disease rating}}{\text{Totalno. of samples x Maximum of disease rating scale}} \times 100$$

Per cent disease incidence of die-back was calculated by

$$\text{Percent diseases incidence} = \frac{\text{Numberof plants infected}}{\text{Totalnumber of plants examined}} \times 100$$

Die-back severity was estimated as per the formula

$$\text{Die}-\text{backPDI} = \frac{\text{Sumof numerical disease rating}}{\text{Totalno. of plants x Maximum of disease rating scale}} \times 100$$

Results and Discussion

No single specific management program could eliminate chilli fruit rot complex. Effective management of such complex diseases usually involves the integrated management strategies to reduce the use of fungicides, to enforce eco-friendly low cost and effective management by combination of cultural, biological, chemical management with intrinsic resistance strategies. Soil solarization and application of bio agents with organic matter manages the

Table 1: Details of the management modules.

Stage/Application method	Bio intensive module for both disease and insects. M1	Bio intensive module for disease with chemical pesticides for insects. M2	Adoptive module M3	Chemical intensive module M4
Seed treatment	*Trichoderma harzianum* 6 g/kg	*Trichoderma harzianum* 6g/kg	Carboxin 37.5 + thiram 37.5 WS (2.5 g/kg)	Carboxin 37.5 + thiram 37.5 WS (2.5 g/kg)
15 DAS spray	*Pseudomonas fluorescens* (10 g/l)	*Pseudomonas fluorescens* (10 g/l)	*Pseudomonas fluorescens* (10 g/l)	Hexaconazole 5 EC (0.1%)
Seedling dip	*Pseudomonas fluorescens* (10 g/l)	*Pseudomonas fluorescens* (10 g/l)	*Pseudomonas fluorescens* (10 g/l)	Carbendazim 50 WP (0.1%)
94 DAT spray	Neem oil (10 ml/l)	Neem oil (10 ml/l)	Neem oil (10ml/l)	Carbendazim 12% + Mancozeb 63% WP (0.25%)
108 DAT spray	*Bacillus subtilis* (10 g/l)	*Bacillus subtilis* (10 g/l)	Hexaconazole 5 EC (0.1%)	Difenconazole 25 EC (0.1%)
122 DAT spray	Neem oil (10 ml/l)	Neem oil (10 ml/l)	Propiconazole 25 EC (0.1%)	Pyraclostrobin 20% WG (0.1%)
130 DAT, spray	*Pseudomonas fluorescens* (10 g/l)	*Pseudomonas fluorescens* (10 g/l)	Carbendazim 12% + Mancozeb 63% WP (0.25%)	Mancozeb 75WP (2.5%)
Insect pest management				
15 DAS spray	*Verticillium lecanii* (4 g/l)	Imidachloprid 17.80 SL, (0.5 ml/l) + Fenazaquin 10%EC (2.0 ml/l)	Imidachloprid 17.80 SL, (0.5 ml/l) + Fenazaquin 10%EC (2.0 ml/l)	Imidachloprid 17.80 SL, (0.5ml/l) + Fenazaquin 10%EC (2.0 ml/l)
30 DAT spray	*Verticillium lecanii* (4 g/l)	Imidachloprid 17.80 SL, (0.5 ml/l) + Fenazaquin 10%EC (2.0 ml/l)	Imidachloprid 17.80 SL, (0.5 ml/l) + Fenazaquin 10%EC (2.0 ml/l)	Imidachloprid 17.80 SL, (0.5 ml/l) + Fenazaquin 10%EC (2.0 ml/l)
60 DAT spray	Neem oil (10 ml/l)	Neem oil (10 ml/l)	Neem oil (10 ml/l)	Imidachloprid 17.80 SL, (0.5 ml/l)
90 DAT spray	*Verticillium lecanii* (4 g/l)	Imidachloprid 17.80 SL, (0.5 ml/l) + Spiromesifen 22.9% (0.75 ml/l)	Imidachloprid 17.80 SL, (0.5 ml/l) + Spiromesifen 22.9% (0.75 ml/l)	Imidachloprid 17.80 SL, (0.5 ml/l)+ Spiromesifen 22.9% (0.75 ml/l)
125DAT, spray	*Nomuraea rileyi* (4 g/l)	Indoxacarb 14.5 SC (0.5ml/l)	Indoxacarb 14.5 SC (0.5 ml/l)	Indoxacarb14.5 SC (0.5 ml/l)

Table 2: Management modules for chilli fruit rot disease during kharif 2012-13.

Module	Fruit rot incidence (%) DAT								Fruit rot severity (%) DAT								Yield
	93	100	107	114	121	128	135	Mean	93	100	107	114	121	128	135	Mean	q/ha
Biointensive module for both disease and insects. (M_1)	2.31	9.26	13.03	13.32	9.4	10.07	12.67	9.93	1.8	6.72	7.45	7.93	6.28	6.12	6.93	6.14	5.53
	(8.73)*	(17.71)	(21.14)	(21.3)	(17.84)	(18.48)	(20.81)	(17.96)	(7.54)	(14.98)	(15.79)	(16.33)	(14.49)	(14.29)	(15.13)	(14.08)	
Bio intensive module for disease (M_2)	2.04	8.3	12.65	12.83	8.59	9.57	10.22	9.24	1.6	6.39	6.51	6.83	5.77	5.98	7.15	5.71	6.1
	(8.1)	(16.74)	(20.79)	(20.97)	(16.97)	(17.96)	(18.6)	(17.28)	(7.17)	(14.55)	(14.77)	(15.08)	(13.8)	(14.11)	(15.46)	(13.56)	
Adoptive module (M_3)	1.6	4.84	5.05	7.38	3.52	4.21	4.58	4.45	1.2	2.64	2.76	3.24	2.83	3.25	3.69	2.8	9.02
	(7.21)	(12.62)	(12.82)	(15.73)	(10.79)	(11.68)	(12.29)	(11.96)	(6.21)	(9.260)	(9.54)	(10.35)	(9.61)	(10.31)	(10.95)	(9.53)	
Chemical intensive module (M_4)	1.72	6.06	8.36	10.04	5.24	5.5	6.42	6.19	1.4	3.07	3.24	3.57	3.26	3.13	4.23	3.18	8.8
	(7.46)	(14.22)	(16.77)	(18.45)	(13.11)	(13.54)	(14.48)	(14.07)	(6.69)	(10.01)	(10.32)	(10.82)	(10.36)	(10.1)	(11.81)	(10.21)	
S.Em. ±	0.73	0.52	0.4	0.67	0.65	1.13	0.39	2.12	0.96	0.87	0.65	0.6	0.84	0.85	1.16	2.75	0.61
CD @ 0.05	1.55	1.6	1.23	2.06	2	3.48	1.2	3.7	2.04	1.85	1.38	1.28	1.79	1.81	2.47	3.47	1.82

* Figures in parentheses are arc sine values DAT: Days after transplanting q/ha : quintal per hectare

nursery diseases of solanaceous vegetables effectively [5]. Hence, in the present investigation emphasis was given on seed treatment by chemical and bio fungicides to eliminate primary inoculum and evaluated four management modules which include bio-intensive, chemical and adoptive by combination of both biological and chemical management.

Kharif 2012

The results of *kharif 2012* indicated that (Table 2 and 3) least seedling rot infection (7.31%) was recorded in chemical module which was on par with adoptive module (7.33%). At 93 DAT (days after transplanting) M_3 (Adoptive module) recorded the least fruit rot incidence (1.60%) which is on par with M_4 (Chemical intensive module) (1.72%), M_2 (Bio intensive module for disease) (2.04%) and M_1 (Bio intensive module for both disease and insects) (2.31%). The least fruit rot severity (1.20 PDI) was recorded in M_3 (Adoptive module) which is also on par with M_4 (Chemical intensive module), M_2 (Bio intensive module for disease) and M_1 (Biointensive module for both disease and insects). At 100 DAT M_3 (Adoptive module) recorded the least fruit rot incidence (4.84%) which is on par with M_4 (Chemical intensive module) (6.06%) followed by M_2 (Bio intensive module for disease) (8.30 per cent. The least fruit rot severity (2.64 PDI) recorded in M_3 (Adoptive module) which is also on par with M_4 (Chemical intensive module) (3.07 PDI). At 135 DAT M_3 (Adoptive module) recorded the least fruit rot incidence (4.58%) followed by M_4 (Chemical intensive module) (6.42%). The least fruit rot severity (3.69 PDI) recorded in M_3 (Adoptive module) which is on par with M_4 (Chemical intensive module) (4.23 PDI). The least die-back incidence (1.20%) was recorded in M_3 (Adoptive module) which was on par with M_4 (Chemical intensive module) (1.40%). The least die-back severity (10.40 PDI) was recorded in M_3 (Adoptive module) followed by M_4 (Chemical intensive module) (12.80 PDI).

The yield was significantly superior in M_3 (Adoptive module)

Table 3: Economics of disease management modules against chilli fruit rot and dieback disease during 2012-13.

Module	Seedling rot Incidence (%)	Fruit rot Incidence (%)	Fruit rot Severity (%)	Dieback incidence	Dieback severity	Yield q/ha	Treatment cost (Rs/ha)	Treatment cost + cost of cultivation (Rs/ha)	Net income* (Rs/ha)	C: B ratio
Biointensive module for both disease and insects. (M_1)	16.16 (23.68)*	9.93 (17.96)	6.14 (14.08)	2.00 (8.13)	19.0 (25.82)	5.53	3028	33028	55300	1.67
Bio intensive module for disease (M_2)	16.12 (23.62)	9.24 (17.28)	5.71 (13.56)	2.00 (8.13)	18.60 (25.54)	6.10	6559	36559	61000	1.66
Adoptive module (M_3)	7.33 (15.67)	4.45 (11.96)	2.80 (9.53)	1.20 (6.21)	10.40 (18.79)	9.02	6514	36514	90200	2.47
Chemical intensive module (M_4)	7.31 (15.67)	6.19 (14.07)	3.18 (10.21)	1.40 (6.69)	12.80 (20.95)	8.80	7503	37503	88000	2.34
S.Em. ±	0.77	2.12	2.75	0.79	0.63	0.61				
CD @ 0.05	1.65	3.70	3.47	1.69	1.35	1.82				

*Figures in parentheses are arc sine values
Rs. 10000/q., fixed cost: Rs. 30,000/ha.
Rs. – Rupees, C:B ratio – Cost Benefit ratio q/ha: quintal per hectare

Table 4: Management modules for chilli fruit rot disease during *kharif* 2013-14.

Module	Fruit rot incidence (%) DAT								Fruit rot severity (%) DAT								Yield q/ha
	93	100	107	114	121	128	135	Mean	93	100	107	114	121	128	135	Mean	
Biointensive module for both disease and insects. (M_1)	2.37 (8.83)*	9.42 (17.85)	9.47 (17.92)	9.65 (17.84)	8.07 (16.41)	9.29 (17.68)	9.36 (17.80)	8.09 (16.25)	1.60 (7.17)	5.90 (14.01)	7.08 (15.38)	7.48 (15.85)	5.80 (13.88)	6.12 (14.25)	7.00 (15.30)	5.83 (13.72)	5.20
Bio intensive module for disease (M_2)	1.98 (7.99)	9.00 (17.45)	8.92 (17.28)	9.23 (17.61)	7.91 (16.33)	8.48 (16.85)	8.87 (17.27)	7.41 (15.50)	1.40 (6.69)	5.81 (13.84)	6.02 (14.15)	6.36 (14.56)	5.46 (13.44)	5.66 (13.69)	6.86 (15.12)	5.38 (13.16)	5.90
Adoptive module (M_3)	1.48 (6.90)	5.05 (12.88)	5.41 (12.90)	5.57 (13.46)	5.43 (13.35)	4.12 11.70	4.40 (11.97)	4.49 (12.05)	1.00 (5.73)	2.28 (8.50)	2.60 (9.25)	2.68 (9.34)	3.00 (9.86)	3.06 (9.98)	3.40 (10.41)	2.57 (9.11)	8.80
Chemical intensive module (M_4)	1.60 (7.20)	5.87 (14.00)	6.85 (14.54)	7.67 (15.95)	6.57 (14.71)	6.13 14.33	6.39 (14.45)	5.86 (13.76)	1.20 (6.21)	2.90 (9.76)	2.76 (9.37)	2.90 (9.72)	2.80 (9.54)	3.08 (10.03)	3.90 (11.32)	2.79 (9.51)	8.60
S.Em. ±	0.64	1.82	1.45	1.08	1.32	1.35	0.80	0.77	0.78	0.92	0.88	0.61	1.42	1.09	1.10	2.28	0.65
CD @ 0.05	1.37	3.86	3.09	2.29	2.81	2.88	1.71	1.34	1.67	1.97	1.87	1.30	3.03	2.32	2.35	3.98	1.94

* Figures in parentheses are arc sine values DAT: Days after transplanting q/ha : quintal per hectare

(9.02 q/ha) with 2.47 cost benefit ratio, which was on par with M_4 (Chemical intensive module) (8.80 q/ha, 2.34 C: B ratio), whereas the least yield (5.53 q/ha, 1.67 C:B ratio) was recorded in M_1 (Bio intensive module for both disease and insects).

Kharif 2013

The results of *kharif 2013* indicated (Table 4 and 5) that least seedling rot infection (6.78%) was recorded in adoptive module which was on par with chemical module (6.93%). At 93 DAT M_3 (Adoptive module) recorded the least fruit rot incidence (1.48%) which was on par with M_4 (Chemical intensive module) (1.60%) and M_2 (Bio intensive module for disease) (1.98%). The least fruit rot severity (1.00 PDI) was recorded in M_3 (Adoptive module) which was on par with M_4 (Chemical intensive module), M_2 (Bio intensive module for disease) and M_3 (Biointensive module for both disease and insects). At 100 DAT M_3 (Adoptive module) recorded the least fruit rot incidence (5.05%) which was on par with M_4 (Chemical intensive module) (5.87%) followed by M_2 (Bio intensive module for disease) (9.00%). The least fruit rot severity (2.28 PDI) recorded in M_3 (Adoptive module) which was on par with M_4 (Chemical intensive module) (2.90 PDI). At 135 DAT M_3 (Adoptive module) recorded the least fruit rot incidence (4.40%) which was on par with M_4 (Chemical intensive module) (6.39%) followed by M_2 (Bio intensive module for disease) (8.87%). The least fruit rot severity (3.40 PDI) recorded in M_3 (Adoptive module) which was on par with M_2 (Chemical intensive module) (3.90 PDI) followed by M_2 (Bio intensive module for disease) (6.86 PDI).

The least die-back incidence (1.00%) was recorded in M_4 (Chemical intensive module) which was on par with M_3 (Adoptive module) (1.20%) followed by M_2 (Bio intensive module for disease) (2.10%). The least die-back severity (8.20 PDI) was recorded in M_3 (Adoptive module) which was on par with M_4 (Chemical intensive module) (9.40 PDI). The yield was significantly superior in M_3 (Adoptive module) (8.80 q/ha) with 2.41 cost benefit ratio, which was on par with M_4 (Chemical intensive module) (8.60 q/ha, 2.29 C: B ratio) followed by M_2 (Bio intensive module for disease) (5.90 q/ha, 1.61 C:B ratio).

The pooled results of *kharif* 2012 and 2013 indicated (Table 6) that least seedling rot incidence (7.06%) was recorded in M_3 (Adoptive module) which was on par with M_4 (Chemical intensive module) (7.12%) followed by M_2 (Bio intensive module for disease) (16.06%). Combination of *T. harzianum* 5.0 g + *P. fluorescens* 5.0 g was most effective in management of seedling rot causing seed borne fungal pathogens [6]. In M_3 (Adoptive module) least fruit rot incidence (4.47%) was recorded which was on par with M_4 (Chemical intensive

Table 5: Economics of disease management modules against chilli fruit rot and dieback disease during kharif 2013-14.

Module	Seedling rot Incidence (%)	Fruit rot Incidence (%)	Fruit rot Severity (%)	Dieback incidence	Dieback severity	Yield q/ha	Treatment cost (Rs/ha)	Treatment cost + cost of cultivation (Rs/ha)	Net income* (Rs/ha)	C: B ratio
Biointensive module for both disease and insects. (M_1)	16.38 (23.85)*	8.09 (16.25)	5.83 (13.72)	2.20 (8.38)	2.20 (8.38)	5.20	3028	33028	52000	1.57
Bio intensive module for disease (M_2)	16.00 (23.51)	7.41 (15.50)	5.38 (13.16)	2.10 (8.33)	17.60 (24.77)	5.90	6559	36559	59000	1.61
Adoptive module (M_3)	6.78 (15.07)	4.49 (12.05)	2.57 (9.11)	2.57 (9.11)	8.20 (16.51)	8.80	6514	36514	88000	2.41
Chemical intensive module (M_4)	6.93 (15.24)	5.86 (13.76)	2.79 (9.51)	1.00 (5.43)	1.00 (5.43)	8.60	7503	37503	86000	2.29
S.Em. ±	1.11	0.77	2.28	1.19	0.98	0.65				
CD @ 0.05	2.36	1.34	3.98	2.53	2.87	1.94				

*Figures in parentheses are arc sine values
Rs. 10000/q., fixed cost: Rs. 30,000/ha.
Rs. – Rupees, C:B ratio – Cost Benefit ratio q/ha : quintal per hectare

Table 6: Pooled analysis management modules for chilli fruit rot disease during *kharif* 2012-13 and 2013-14.

Module	Seedling rot Incidence (%)			Fruit rot Incidence (%)			Fruit rot Severity (%)			Dieback incidence			Dieback severity			Yield q/ha			C: B ratio
	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled	Pooled
Biointensive module for both disease and insects. (M_1)	16.16 (23.68)*	16.38 (23.85)	16.27 (23.77)	9.93 (17.96)	8.09 (16.25)	9.01 (17.43)	6.14 14.08	5.83 (13.72)	5.98 (14.07)	2.00 (8.13)	2.20 (8.38)	2.10 (8.25)	19.0 (25.82)	18.00 (25.06)	18.54 (25.48)	5.53	5.20	5.36	1.62
Bio intensive module for disease (M_2)	16.12 (23.62)	16.00 (23.51)	16.06 (23.59)	9.24 (17.28)	7.41 (15.50)	8.33 (16.70)	5.71 13.56	5.38 (13.16)	5.54 (13.50)	2.00 (8.13)	2.10 (8.33)	2.05 8.23	18.60 (25.54)	17.60 (24.77)	18.10 (25.16)	6.10	5.90	6.00	1.64
Adoptive module (M_3)	7.33 (15.67)	6.78 (15.07)	7.06 (15.33)	4.45 (11.96)	4.49 (12.05)	4.47 (14.73)	2.80 9.538	2.57 (9.11)	2.68 (9.35)	1.20 (6.21)	1.20 (6.21)	1.20 6.21	10.40 (18.79)	8.20 (16.51)	9.30 (17.71)	9.02	8.80	8.92	2.44
Chemical intensive module (M_4)	7.31 (15.67)	6.93 (15.24)	7.12 (16.10)	6.19 (14.07)	5.86 (13.76)	6.02 (12.87)	3.18 10.21	2.79 (9.51)	2.98 (9.90)	1.40 (6.69)	1.00 (5.43)	1.20 6.21	12.80 (20.95)	9.40 (17.82)	11.11 (19.45)	8.80	8.60	8.71	2.32
S.Em. ±	0.77	1.11	0.76	2.12	0.77	0.84	2.75	2.28	0.86	0.79	1.19	0.79	0.63	0.98	0.75	0.61	0.65	0.94	
CD @ 0.05	1.65	2.36	1.63	3.70	1.34	1.80	3.47	3.98	1.84	1.69	2.53	1.68	1.35	2.87	1.61	1.82	1.94	2.89	

* Figures in parentheses are arc sine values
C:B ratio – Cost Benefit ratio q/ha : quintal per hectare

module) (6.02%) followed by M_2 (Bio intensive module for disease) (8.33%). The least fruit rot severity (2.68 PDI) was recorded in M_3 (Adoptive module) which was on par with M_4 (Chemical module) (2.98 PDI) followed by M_2 (Bio intensive module for disease) (5.54 PDI). Among management modules, biological, chemical and Adoptive (Integrated disease management) module. Adoptive module was superior to biological and chemical modules in chilli fruit rot management [7-9]. The least die-back incidence (1.21%) was recorded in both M_3 (Adoptive module) and M_4 (Chemical intensive module) followed by M_2 (Bio intensive module for disease) (2.05%). The least die-back severity (9.30 PDI) was recorded in M_3 (Adoptive module) which was on par with M_4 (Chemical module) (11.11 PDI) followed by M_2 (Bio intensive module for disease) (18.10 PDI). Difenconazole 25 EC and pyraclostrobin 20 WG at 0.1% concentration and among five combiproduct fungicides, tricyclazole 18% + mancozeb 62% WP and pyraclostrobin 5% + metiram 55% were found effective against fruit rot and dieback disease [10].

The yield was significantly superior in M_3 (Adoptive module) (8.92 q/ha) with 1.25 cost benefit ratio, which was on par with M_4 (Chemical intensive module) (8.71 q/ha, 2.32 C:B ratio) followed by M_2 (Bio intensive module for disease) (6.0 q/ha, 1.64 C:B ratio), whereas least yield (5.36 q/ha, 1.62 C:B ratio) was recorded in M_1(Bio intensive module for disease and insects). As no single specific management practice could eliminate chilli fruit rot complex here adoptive module involves the integrated management strategies at right time application reduces the use of fungicides, and it enforces eco-friendly low cost and effective management of chilli fruit rot.

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Baliyan et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00088.4

Vegetos- An International Journal of Plant Research

Research Article

A SCITECHNOL JOURNAL

Evaluation of Pyramided Rice Genotypes Derived from Cross Between CSR-30 and IRBB-60 Basmati Variety against Bacterial Leaf Blight

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Abstract

Bacterial leaf blight (BB) disease caused by *Xanthomonas oryzae pv. oryzae* is one of the most severe diseases effecting Basmati rice production in India. CSR-30 is widely grown in Haryana and is extremely popular amongst rice farmers and consumers because of its salt tolerance, aroma, high yield, medium slender grains and excellent cooking and eating qualities. CSR-30 lacks tolerance to BB. The present investigation was therefore planned to introgress BB resistance genes (*xa5, xa13* & *Xa21*) from IRBB-60 to CSR-30. The pyramided BC_3F_1 genotypes were evaluated for BB incidence under artificial field inoculation. Scoring of inoculated plant was done 14 days after inoculation. The triple and double resistance genes pyramided genotypes provided enhanced resistance as expressed by smaller mean lesion length in comparison to genotypes with individual genes.

Keywords

Basmati rice; Bacterial leaf blight; Gene pyramiding; Resistance genes; *Xanthomonas oryzae* pv. *oryzae*

Introduction

BB is one of the most devastating diseases of rice worldwide [1,2]. It affects rate of photosynthesis and lead to yield losses of up to 80-100% in severe cases [3,4]. The main symptoms of the disease are water soaked stripes along the margin of leaf blades, which enlarges later on and turn yellow. These lesions may cover the entire blade, may extend to the lower end of leaf sheath. Several rice resistance genes are expressed at the highest level only in the adult stage [5,6]. *Xa21* mediated resistance gene was shown to be expressed since the seedling stage but the plants were found susceptible, and *Xa7* gene showed broad resistance only in adult plants [7]. On the contrary, *xa5* gene could confer resistance at all growth stages and exhibit a broad spectrum of resistance to *Xoo* isolates (India and Nepal) [8].

The development of resistant cultivars is the best approach of protecting the rice from BB disease [9]. Gene to gene interaction of BB with rice makes it an effective model to study plant pathogen interaction [10]. Long-term cultivation of rice varieties carrying single resistance gene has resulted in a significant shift in pathogen-race frequency and consequent breakdown of resistance [11]. Pyramiding of multiple resistance genes in the background of modern high yielding varieties is a tangible solution to resistance breakdown. The probability of simultaneous pathogen mutations for virulence to defeat two or more effective genes is much lower than with a single gene [12]. In view of the importance of genetic resistance for disease control, studies were undertaken to evaluate the pyramided rice genotypes against BB disease.

Materials and Methods

The experimental material for the present study consisted of BB resistance genes pyramided BC_3F_1 genotypes derived from cross between BB susceptible Basmati rice variety CSR-30 (recurrent parent) and donor BB resistant IRBB-60 (having genes, *xa5*, *xa13* and *Xa21*). The pyramided BC_3F_1 genotypes were evaluated for disease reaction and compared with the donor parent, IRBB-60.

Isolation of bacteria (*Xanthomonas oryzae* pv. *oryzae*)

Infected rice leaves showing BB symptoms were collected from the BB infected fields of RRS, Kaul. These leaves were surface-sterilized with 2% sodium hypochlorite for 1 min and washed twice with sterile distilled water. The leaves were then cut into 0.5 cm pieces and placed in 10 ml of sterile distilled water. The cells were allowed to ooze from leaves into sterile water and streaked for single-colony isolation on PSA plates. The *Xoo* isolate was multiplied and maintained on peptone sucrose agar (PSA) at 28°C. These isolates were preserved in glycerol at -70°C (Table 1).

Artificial inoculation

Plants selected on the basis of molecular marker analysis from the BC_3F_1 generation (CSR-30 x IRBB-60) carrying resistance genes (*Xa21*, *xa13* and *xa5*) individually and in combinations, along with the control, were inoculated with the predominant *Xoo* isolate prevalent in Haryana State using a bacterial suspension of 10^9cells/ml [13]. The plants were clip inoculated at maximum tillering stage. The leaf blades were inoculated by clipping with scissors at 3 cm below the leaf tips. On an average five leaves per plant were inoculated and the disease incidence (DI) using 0-5 scale (Table 2) was measured 14 days after inoculation.

Results

The *Xa21*, *xa13* and *xa5* pyramided lines in different combinations (Table 3) were evaluated for their resistance to BB in field and net house

Table 1: Composition of Peptone Sucrose Agar (PSA) media.

Composition of Peptone Sucrose Agar (PSA) media	
Sucrose	5.0g
Sodium glutamate	1.0g
Ferrous sulphate	0.25g
Yeast extract	2.5g
Peptone	10.0g
Agar	15.0g
pH	6.0

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Received: July 07, 2016 **Accepted:** August 22, 2016 **Published:** August 29, 2016

using the *Xanthomonas. oryzae* strain isolated from the BB infected fields of RRS, Kaul, CCSHAU, Hisar. Isolation of bacteria was done by adapting streak plate method. On PSA plates, *Xanthomonas oryzae* pv. *oryzae* having circular, entire, smooth, convex, opaque, whitish yellow at first and straw yellow later was identified. Well separated colonies of the isolate were picked up and streaked on PSA media and incubated at 28°C for 72 hours. The pure colonies obtained were again streaked on PSA slants and kept for incubation at 28°C for 72 hours. The cultures so obtained were stored in the refrigerator at 5°C, which served as a stock culture for further studies. The pyramided lines along with the control were inoculated using a bacterial suspension of 10^9cells/ml. The leaf blades were inoculated by clipping with scissors at 3 cm below the leaf tips. On an average five leaves per plant were inoculated and the disease incidence using 0-5 scale was measured 14 days after inoculation.

The three-gene pyramided BC_3F_1 plants derived in this study from the cross CSR-30 x IRBB-60, were found to be equally effective against the virulent *Xoo* strain (mean lesion length of 0.4 cm) as compared to the donor line IRBB-60 (mean lesion length of 0.5 cm). Also the pyramided lines having either *Xa21* (mean lesion length of 1.2 cm) or *xa5* (mean lesion length of 1.1 cm) resistance genes alone were found to be resistant or moderately resistant to the BB disease. However, pyramided lines with *xa13* gene (mean lesion length of 4.8 cm) alone were found to be susceptible to BLB disease. The pyramided lines (two gene or three gene combinations) had a higher level of resistance and broader spectrum of resistance than parental lines or lines with a single gene. The *Xa21* and *xa5* pyramided lines exhibited a mean lesion length of 0.8 cm whereas those having *Xa21* and *xa13* showed a mean lesion length of 1.5 cm. The pyramided lines having both *xa13* and *xa5* showed a mean lesion length of 2.8 cm (Table 4). This might be due to interaction and/or complementation between the resistance genes (Figure 1).

Discussion

As compared to the recurrent parent CSR-30, the pyramided lines with two to three gene (*Xa21, xa13* and *xa5*) combinations exhibited high level of resistance to BB disease. The lines containing *Xa21*or *xa5* alone exhibited moderate BB resistance. However, lines with *xa-13* gene alone were found to be susceptible to BB disease. The results indicated that the genes in combinations were more effective against the pathogen than a single gene (Figure 2).

Based on the above results, we infer that, individually, *xa5* and *Xa21* were more effective resistance genes than *xa13*. Li et al. [14] reported that a high level of durable resistance to *Xoo* can be achieved by the cumulative effects of multiple QTLs, including the residual effects of defeated major resistance genes. They revealed a complex genetic network of epistatic effects between resistance genes and QTLs for resistance in rice. They reported that resistance to specific *Xoo* strains is governed by both major resistance genes with a qualitative effect that condition complete resistance and polygenes with a quantitative effect (QTL) that condition partial resistance. In a similar study, Singh et al. [15] reported that the genes in combinations were more effective against the pathogen than a single gene, by inoculating the selected BC_2F_3 PR106 lines homozygous for each of the individual genes and with different combinations with the 17 isolates of *Xoo* prevalent in Punjab. They found that *Xa21* was the most effective gene, followed by *xa5* and that gene *xa13* was the least effective. These results were in accordance with those reported in our study. Huang et al. (2012), in another study, introgressed four bacterial leaf blight (BB) resistance genes, *Xa7, Xa21, Xa22* and *Xa23*, into an elite hybrid rice restorer line Huahui 1035, using MAS and found that restorer lines (HBQ809 and HBQ810) with *Xa23* gene were resistant to all eleven Chinese representative *Xoo* races, showing broad spectrum resistance to BB. Dokku et al. [16] through marker assisted backcrossing transferred three BB resistance genes *i.e. xa5*, *xa13* and *Xa21* from IRBB60 to Tapaswini having *Xa4* gene. The four gene combination expressed higher levels of resistance in comparison to all other gene combinations and genes in combination were more effective than a single gene. *Xa21* was most effective with shorter lesions lengths followed by *xa13* while lines with *xa5* were susceptible to all isolates except *xd-1*. The findings of this work is contrary to that concluded by our study where *Xa21* was found most effective against BB followed by *xa5* and *xa13* gene.

Table 2: Disease rating using 0-5 scale.

Infection (%)	Score	Host response
0	0	Highly resistant (HR)
1-10	1	Resistant (R)
10-30	2	Moderately resistant (MR)
30-50	3	Moderately susceptible (MS)
50-75	4	Susceptible (S)
75-100	5	Highly susceptible (HS)

Table 3: Number of BC_3F_1 plants with multiple resistance gene combinations.

S. No	Gene combinations	(CSR-30 x IRBB 60) BC_3F_1 plants	Line numbers
1	*Xa21/Xa21Xa13/Xa13 Xa5/Xa5*	17	R-1, R-2, R-7, R-9, R-11, R-12, R-21, R-23, R-29, R-36, R-37, R-49, R-52, R-66, R-78, R-92, R-101
2	*xa21/xa21xa13/xa13 Xa5/Xa5*	14	R-3, R-5, R-10, R-13, R-17, R-24, R-31, R-35, R-62, R-72, R-86,R-97, R-102, R-108
3	*xa21/xa21Xa13/Xa13 xa5/xa5*	8	R-22, R-47, R-76, R-82, R-98, R-105, R-106, R-112,
4	*Xa21/Xa21xa13/xa13 Xa5/Xa5*	36	R-4, R-6, R-8, R-15, R-18, R-19, R-20, R-25, R-27, R-30, R-33, R-34, R-45, R-46, R-50, R-54, R-57, R- R-60, R-61, R-65, R-67, R-71, R-73, R-75, R-77, R-79, R-80, R-85, R-88, R-91, R-96, R-99, R-100,R-103, R-107, R-110
5	*Xa21/Xa21Xa13/Xa13 xa5/xa5*	5	R-14, R-39, R-43, R-44, R-93
6	*xa21/xa21xa13/xa13 xa5/xa5*	3	R-26, R-70, R-87
7	*Xa21/Xa21xa13/xa13 xa5/xa5*	15	R-28, R-32, R-38, R-42, R-58, R-59, R-63, R-68, R-69, R-74, R-81, R-90, R-94, R-95, R-111

Table 4: Disease reaction of BC_3F_1 rice genotypes (containing one to three BB resistance genes) to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Five point rating scale for scoring/screening of BB disease).

S. No	Parents and pyramid lines	*Xa21*	*xa13*	*xa5*	Disease rating	Reaction category	BB mean lesion length (cm)
1	IRBB-60	+	+	+	1	HR	0.5
2	CSR-30	-	-	-	5	HS	18.8
3	R-1	+	-	-	2	MR	0.9
4	R-2	+	-	-	2	MR	1.6
5	R-3	-	+	-	3	MS	3.7
6	R-4**	+	+	-	2	MR	1.4
7	R-5	-	+	-	4	S	4.2
8	R-6**	+	+	-	2	MR	1.6
9	R-7	+	-	-	2	MR	0.7
10	R-8**	+	+	-	2	MR	1.5
11	R-9	+	-	-	2	MR	0.8
12	R-10	-	+	-	4	S	5.1
13	R-11	+	-	-	3	MS	3.1
14	R-12	+	-	-	2	MR	1.6
15	R-13	-	+	-	4	S	5.7
16	R-14**	+	-	+	2	MR	1.0
17	R-15**	+	+	-	2	MR	1.6
18	R-17	-	+	-	3	MS	4.4
19	R-18**	+	+	-	2	MR	1.4
20	R-19**	+	+	-	2	MR	1.5
21	R-20**	+	+	-	2	MR	1.6
22	R-21	+	-	-	2	MR	0.6
23	R-22	-	-	+	2	MR	0.9
24	R-23	+	-	-	2	MR	0.4
25	R-24	-	+	-	3	MS	4.7
26	R-25**	+	+	-	2	MR	1.5
27	R-26**	-	+	+	2	MR	1.9
28	R-27**	+	+	-	0	MR	1.1
29	R-28*	+	+	+	0	HR	0.3
30	R-29	+	-	-	2	MR	1.2
31	R-30**	+	+	-	2	MR	1.1
32	R-31	-	+	-	4	S	4.3
33	R-32*	+	+	+	0	HR	0.3
34	R-33**	+	+	-	2	MR	1.6
35	R-34**	+	+	-	2	MR	1.5
36	R-35	-	+	-	3	MS	4.2
37	R-36	+	-	-	2	MR	1.9
38	R-37	+	-	-	2	MR	1.7
39	R-38*	+	+	+	1	R	0.2
40	R-39**	+	-	+	3	MS	0.8
41	R-42*	+	+	+	0	HR	0.4
42	R-43**	+	-	+	1	R	0.6
43	R-44	+	-	+	1	R	0.8
44	R-45**	+	+	-	2	MR	1.7
45	R-46**	+	+	-	2	MR	1.4
46	R-47	-	-	+	1	R	1.6
47	R-49	+	-	-	2	MR	0.5
48	R-50**	+	+	-	1	R	1.0
49	R-52	+	-	-	2	MR	0.4
50	R-54**	+	+	-	2	MR	1.9

51	R-57**	+	+	-	2	MR	1.7
52	R-58*	+	+	+	1	R	0.4
53	R-59*	+	+	+	0	HR	0.2
54	R-60**	+	+	-	2	MR	1.2
55	R-61**	+	+	-	2	MR	1.7
56	R-62	-	+	-	4	S	5.5
57	R-63*	+	+	+	0	HR	0.3
58	R-65**	+	+	-	2	MR	1.5
59	R-66	+	-	-	2	MR	1.9
60	R-67**	+	+	-	1	R	1.0
61	R-68*	+	+	+	0	HR	0.3
62	R-69*	+	+	+	1	R	0.4
63	R-70**	-	+	+	2	MR	3.4
64	R-71**	+	+	-	2	MR	1.5
65	R-72	-	+	-	4	S	5.5
66	R-73**	+	+	-	2	MR	1.2
67	R-74*	+	+	+	2	MR	0.2
68	R-75**	+	+	-	2	MR	1.8
69	R-76	-	-	+	2	MR	0.9
70	R-77**	+	+	-	2	MR	1.9
71	R-78	+	-	-	2	MR	0.6
72	R-79**	+	+	-	2	MR	1.5
73	R-80**	+	+	-	2	MR	1.1
74	R-81*	+	+	+	0	HR	0.3
75	R-82	-	-	+	2	MR	1.4
76	R-85**	+	+	-	2	MR	1.7
77	R-86	-	+	-	4	S	5.1
78	R-87**	-	+	+	2	MR	2.9
79	R-88**	+	+	-	2	MR	1.4
80	R-90*	+	+	+	0	HR	0.3
81	R-91**	+	+	-	2	MR	1.5
82	R-92	+	-	-	2	MR	0.7
83	R-93**	+	-	+	1	R	0.5
84	R-94*	+	+	+	0	HR	0.3
85	R-95*	+	+	+	1	R	0.4
86	R-96**	+	+	-	2	MR	1.3
87	R-97	-	+	-	3	MS	4.1
88	R-98	-	-	+	2	MR	0.8
89	R-99**	+	+	-	2	MR	1.6
90	R-100**	+	+	-	2	MR	1.9
91	R-101	+	-	-	2	MR	1.3
92	R-102	-	+	-	3	MS	5.1
93	R-103**	+	+	-	2	MR	1.3
94	R-105	-	-	+	2	MR	0.9
95	R-106	-	-	+	2	MR	1.0
96	R-107**	+	+	-	2	MR	2.0
97	R-108	-	+	-	4	S	4.8
98	R-110**	+	+	-	2	MR	1.4
99	R-111*	+	+	+	0	HR	0.4
100	R-112	-	-	+	1	R	0.7

Note: *indicates three-gene pyramided genotypes

**indicates two-gene pyramided genotypes

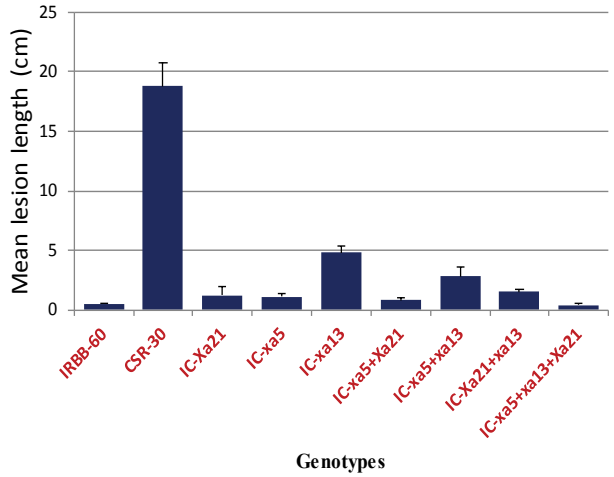

Figure 1: Disease reaction of the donor parent (IRBB-60), recurrent parent (CSR-30) and pyramided BC_3F_1 genotypes (carrying one to three BB resistance genes).

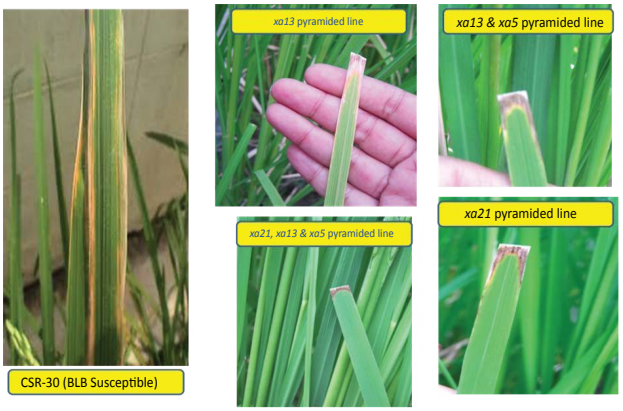

Figure 2: Disease scoring after fourteen days of inoculation in the lines carrying multiple gene combinations (one to three BB resistance gene combinations).

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Sadrai et al., Vegetos 2016, 29:3
10.5958/2229-4473.2016.00089.6

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

A Brief Comparative Effectiveness Research (CER) about Conventional and Homeopathic Treatment of Type 2 Diabetic Patients

Sima Sadrai*, Ali Ghezelsefloo, Mojgan Asadi, Parisa Osanlo and Arash Jafaripoor

Abstract

Background: The purpose of this study was to compare the effectiveness of homeopathy in type 2 diabetes to see if advising to integrate it with conventional therapy, popularizing its use, or limiting it due ineffectiveness.

Methods: In a retrospective study the data of thirty-one type 2 diabetic samples from Shariati Hospital and thirty-one type 2 diabetic from two homeopath MDs offices were collected and analyzed by SPSS.

Results: The average values for hemoglobinA1c (HbA1c), fasting blood glucose(FBS) and 2-hour postprandial blood glucose (2hpp) in homeopathic group were at the beginning of treatment 8.6%, 172.8 and 226.5 mg/dL, and after treatment, 7.8%, 149.4 and 204.8 mg/dL and in conventional medicine group, at the beginning of treatment 10.5%, 216 and 302.4 mg/dL and after treatment, 8.3%, 147 and 215.5 mg/dL and all treatment outcomes better at the end with p-values<0.001 and after treatment the results in both groups was not significantly different (p values=0.22, 0.86, 0.48, respectively), but the HbA1c mean was 0.5% less in homeopathic group.

Conclusion: Regarding the values of HbA1c and FBS and 2hpp before and after homeopathic treatment suggests the effectiveness of homeopathic treatment in type 2 diabetes. This study certainly cannot conclude that homeopathic treatment alone can be more effective than or as effective as conventional medicine. However, according to reducing in the number and dose of anti-diabetic drugs and better conditions of patients, integrating homeopathy in their treatment could be recommended.

Keywords

Type 2 Diabetes; Complementary and alternative medicine (CAM); Homeopathy; Comparative Effectiveness Research (CER)

Introduction

Alongside conventional medicine CAM (Complementary and Alternative Medicine) treats and restores health to the patients and is widely used preventing the diseases in healthy individuals. Studies in other countries show significant use of complementary methods. Three reports (2002, 2007 and 2012) of National Institutes of Health (NIH) referring to CAM reached to about 40% [1,2]. In a study by Chang from Australia, the approach to CAM for the treatment of diabetes has been reported as 72.8% [3]. Our method was a brief comparative effectiveness research (CER). CER is designed to inform health-care decisions by providing evidence on the effectiveness, benefits, and harms of different treatment options. In CER we can look at the available evidence and records. The goal of CER helps physicians and medical providers and health policy makers to make informed decisions to improve health care in both the individual and social level [4].

Diabetes is a metabolic disorder and relatively common chronic diseases which its prevalence and incidence in many communities, especially in developing countries is increasing. Overall, half of all people with diabetes are unaware of their disease. Lack of control of disease complications in the future will increase [5]. The screening tests used to detect diabetes are blood glucose levels and HbA1c [6]. The World Health Organization declared that homeopathy is the second largest medical system that is used worldwide. One major difference between homeopathy and conventional medicine is that in homeopathy trying to restore balance to the body [7]. In Pomposelli study the following result was concluded: "It was possible to treat patients with homeopathy, monitored by the conventional diabetes specialist, without any major problem of compatibility between the two forms of therapy" [8]. *Lycopodium clavatum* (n=132), Phosphorus (n=27) and Sulphur (n=26) were the medicines most frequently prescribed in Chaturbhuja study and was concluded homeopathic medicines may be effective in managing the symptoms of diabetic polyneuropathy patients [9].

The purpose of this study was to compare the effectiveness of homeopathy in type 2 diabetes. It is important for health stakeholders to see if advising to integrate homeopathy with conventional therapy, popularizing its use or limiting it due ineffectiveness.

Methods

The study was a retrospective study. From February 2014 till august 2015 the records of type 2 diabetic patients at the Shariati hospital of Tehran Medical Sciences and two Medical doctor offices with homeopathy method by a random sampling method and the data were collected by means of a questionnaire.

Inclusion criteria

Patients with type 2 diabetes, with complete records data and who have had more than one subsequent visit to the doctor.

Exclusion criteria

Patients with incomplete laboratory results, who are addicted to drugs and alcohol, who have died during treatment, children and the elderly over 80 years old, diseases such as transplant patients and cancer, more than 1 year from the last visit to the doctor and if other interventions is used to treat diabetes. By sample size calculation at least 26 patients per group is required and due to the possible omit

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Received: July 19, 2016 **Accepted:** August 22, 2016 **Published:** August 29, 2016

of some patients in each study arm 31 patients with type 2 diabetes in each group is selected. After data collection, data were analyzed by SPSS version 22.0 software and the means were compared by t-test and qui-square, with significant level of 0.05.

Results and Discussion

Data of sixty-two patients with type 2 diabetes was collected. The average duration of diabetes, diet and exercise levels, BMI value (27.92 ± 4.26 in conv. and 27.41 ± 3.74 in hom.), the number of people with high blood pressure, lipid profile, triglyceride and LDL and HDL are not significant (Table 1).

In both group FBS, 2-hour postprandial blood glucose and HbA1c before treatment are not significantly different but because of four outliers in the range of data in conventional group (there are inpatients) the range differs. In Figure 1 we can see this difference for FBS. In Table 2 the p-values are reported and due this different range we analyzed the study by unequal variance t-test.

In Table 3 in two groups the values of HbA1c, FBS and 2-hour postprandial blood glucose after treatment are compared and there is no significant difference. Only it is to mention the physicians with homeopathic treatment claim a lower HbA1c in their patients. There is a 0.5 difference in HbA1c between two groups (the homeopathic group has lower values) but it is not statistically significant and for a conclusive decision a larger sample size in future studies is recommended.

Conclusion

Regarding the values of HbA1c and FBS and 2hpp before and after homeopathic treatment suggests the effectiveness of homeopathic treatment in type 2 diabetes. According to the results, we can say that homeopathy is effective in treatment of type 2 diabetes, but it is not clear whether the results are better than conventional medicine. In homeopathy due its holistic look the patient's life style and diet has improved and this could be considered as an advantage. In patients who have chosen homeopathic remedies it can be seen that the frequency and the dose of anti-diabetic medicine are decreased. In some patients after some time no medication is needed. Also a lower HbA1c which is not statistical significant (larger sample size is needed for proving this hypothesis) (Tables 4 and 5).

One of the disadvantages in homeopathic group was the higher number of visits to the homeopath. However, homeopathic medicines are less expensive and with regard to reducing the use of conventional drugs it is in terms of cost savings. Also less complications such as hypoglycemia (common side effects is another advantage of homeopathic treatment.

Table 1: The study of education, exercise, blood pressure, diseases blood fat, drugs, diet.

		Conventional medicine		Homeopathy	
		Frequency	**Percent**	**Frequency**	**Percent**
Sex	Female	17	55	21	68
	Male	14	45	10	32
Education	Under Diploma	22	71	15	48.4
	Diploma Holder	9	29	12	38.7
	Bachelor	0	0	4	12.9
Exercise	Daily	6	19.4	8	25.8
	Weekly	9	29	8	25.8
	No Exercise	16	51.6	15	48.4
Blood pressure		17	54.8	13	41.9
Diseases blood fat		23	74.2	22	71
Drugs	**Metformin** **Glibenclamide**	28 11	90.3 35.5	26 13	83.9 41.9
Diet		8	25.8	14	45.1

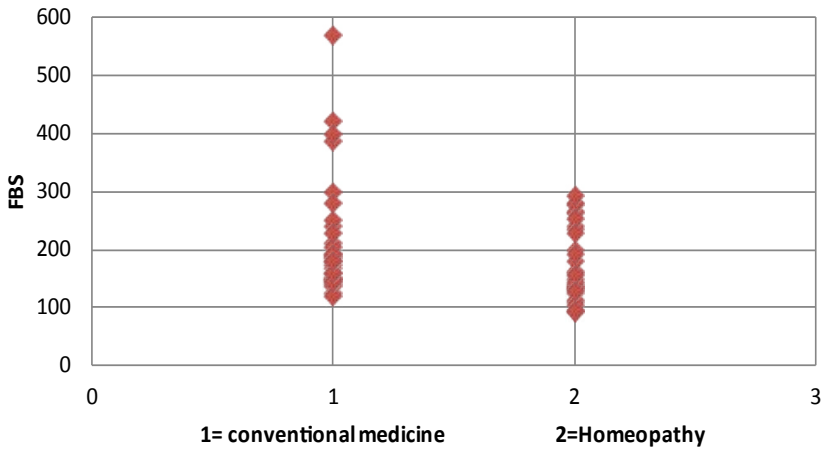

Figure 1: Distribution of FBS in the two treatment groups.

Table 2: HbA1c values and FBS and 2hpp before treatment in both groups.

	Group	N	Mean	Standard Deviation	P Value
HbA1c before treatment	**Conventional medicine**	31	10.5	2.28	
	Homeopathy	31	8.6	1.95	0.53
FBS before treatment	**Conventional medicine**	31	216	102.4	
	Homeopathy	31	172.8	62.75	0.01
2hpp before treatment	**Conventional medicine**	31	302.5	99	
	Homeopathy	31	226.5	83.08	0.02
Fasting blood sugar (FBS) and hemoglobin A1c (HbA1c) and 2-hour postprandial blood glucose (2hpp) at the begin					

Table 3: HbA1c values and FBS and 2hpp after treatment in both groups.

	Group	N	Mean	Standard Deviation	P Value
HbA1c after treatment	**Conventional medicine**	31	8.3	1.47	0.22
	Homeopathy	31	7.8	1.80	
FBS after treatment	**Conventional medicine**	31	147.06	51.64	0.86
	Homeopathy	31	149.42	51.36	
2hpp after treatment	**Conventional medicine**	31	215.58	59.89	0.48
	Homeopathy	31	204.81	60.36	
Fasting blood sugar (FBS) and hemoglobin A1c (HbA1c) and 2-hour postprandial blood glucose (2hpp) after treatment					

Table 4: Values FBS and HbA1c and 2hpp, before and after treatment in homeopathy.

	Mean	N	Standard Deviation	P Value
HbA1c before homeopathic treatment	8.6	31	1.95	<0.001
HbA1c after homeopathic treatment	7.8	31	1.80	
FBS before homeopathic treatment	172.81	31	62.75	<0.001
FBS after homeopathic treatment	149.42	31	51.36	
2hpp before homeopathic treatment	226.55	31	83.08	<0.001
2hpp after homeopathic treatment	204.81	31	60.36	
Hemoglobin A1c (HbA1c), fasting blood sugar (FBS) and 2-hour postprandial blood glucose (2hpp) after homeopathic treatment				

Table 5: Values HbA1c, FBS and 2hpp, before and after treatment in conventional medicine.

	Mean	N	Standard Deviation	P Value
HbA1c before conventional treatment	10.5	31	2.28	<0.001
HbA1c after conventional treatment	8.3	31	1.47	
FBS before conventional treatment	216	31	102.4	<0.001
FBS after conventional treatment	147.06	31	51.6	
2hpp before conventional treatment	302.45	31	99.1	<0.001
2hpp after conventional treatment	215.58	31	59.9	
Hemoglobin A1c (HbA1c), fasting blood sugar (FBS) and 2-hour postprandial blood glucose (2hpp) after homeopathic treatment				

Another limitation of this study was that the patients who were treated in homeopathic group continue conventional medicine and homeopathic physicians recommend the conventional treatment tapped step by step. So, this study certainly cannot conclude that homeopathic treatment alone can be more effective than or as effective as conventional medicine. However, according to reducing in the number and dose of anti-diabetic drugs and better conditions of patients, integrating homeopathy in their treatment could be recommended.

Acknowledgement

Here with we thank Mrs Shahla Nabiloo due her help in patient's data collection and Mr Hossain Zadi for his help in statistical analysis. This work is a research grant from Tehran University of Medical Sciences.

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