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## Evaluation of pea (*Pisum sativum* L.) genotypes against *Ascochyta pisi*

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**Abstract**

One of the most important disease of pea is Ascochyta blight. Since the disease is complex and caused by more than one pathogen *Viz. Ascochyta pisi, Ascochyta pinodes* and *Ascochyta pinodella*. However, under Kashmir conditions there is more prevalence of *Ascochyta pisi* thus the genotypes were elevated for this particular specie and its was found that none of the genotypes were resistant or moderately resistance due to lack of diversity in resistant genes. The majority of genotypes, including most cultivated cultivar Rachna were found Susceptible to the incident of Ascochyta blight and 40 genotypes were found susceptible while, thirteen genotypes were moderately susceptible and ten genotypes were highly susceptible The highest disease intensity was recorded in genotype SHM – 59 (53.99%) and lowest was in KDP – 47 (13.23%).

**Keywords:** Genotypes, pea, resistance, susceptible

**Introduction**

Pea (*Pisum sativum* L.) possesses a position of utmost significance as edible leguminous crop grown throughout the world. It is a local crop of northwest and southwest Asia. Globally, it is the third most important pulse crop, after dry bean and chickpea and third most popular rabi pulse of India after chick pea and lentil (Anonymous, 2014) <sup>[1]</sup>. This is due to the datum that diseases have an adverse impact upon the rate of production. More than 25 fungi, bacteria, nematodes, and viruses affects the pea (Kraft and Pflieger, 2001) <sup>[6]</sup>. It is susceptible to a number of diseases *Viz. root rot (Fusarium oxysporum f.sp pisi), powdery mildew (Erysiphe pisi), rust (Uromyces pisi), white rot (Sclerotinia sclerotium), downy mildew (Peronospora pisi), stem rot (Sclerotium rolfsii), leaf spot (Alternaria alternate), grey mould (Botrytis cinerea) seed rot and damping off (Pythium spp., Rhizoctonia solani), collar rot (Fusarium solani f.sp. pisi), foot rot (Phoma medicaginis var. pinodella), ascochyta blight (Ascochyta spp.), bacterial blight (Pseudomonas syringae pv. syringae) and pea seed borne mosaic virus.* Among these, Ascochyta blight caused by *Ascochyta sp.* is a highly destructive disease of pea throughout the major pea growing areas of the world (Khan *et al.*, 2013) <sup>[5]</sup>. In 1830, it was first defined by Libert in Europe who named the pathogen *Ascochyta pisi* (Skoglund *et al.*, 2011) <sup>[13]</sup>.

Ascochyta blight is a polycyclic disease that can progress rapidly during periods of wet weather and moderate temperatures. The temperature between 20-25 °C with high relative humidity is ideal for disease development. Major source of initial inoculum in the field is the ascospore that are released from matured pseudothecia that develops on infected stubble from the previous season (Salam *et al.*, 2011) <sup>[10]</sup>. Secondary inoculum comprises of pycnidiospores that develop in pycnidia molded in lesions on leaves, stems and pods. According to Peever *et al.* (2007) <sup>[9]</sup> *Ascochyta pisi* causes tan coloured lesions with distinct margins similar to those of Ascochyta blight of chickpea and lentil. Since both *Ascochyta Pinodes* and *Ascochyta Pinodella* causes similar symptoms and cause foot rot but sever foot rot is caused by *Ascochyta Pinodella* with less aerial damage. In adversely impacted pea crops, a drop in seed number per plant can total 18 and 25 percent and seed yield decline 13.5 and 16.7 percent (Tivoli *et al.*, 1996) <sup>[14]</sup>.

**Material and methods****a) Isolation**

The isolations of the pathogen from the diseased samples collected from different pea fields

was carried out by tissue bit transfer method (Padder *et al.*, 2012) [8]. The leaf area diseased along with some healthy portion was cut into small bits with a sterilized sharp blade and then these bits were surface sterilized in 0.1% mercuric chloride for 30 seconds followed by three washing in sterilized distilled water to remove the traces of mercuric chloride. After blotting dry with sterilized filter paper, these bits were transferred to sterilized potato dextrose agar medium on sterilized plates and incubated for seven days at  $24 \pm 1$  °C.

#### b) Purification and maintenance of the pathogen isolates

The pure cultures of the fungal isolates isolated from diseased samples collected across different locations were made by single spore isolation technique.

#### c) Pathogenicity

The pathogenicity of all the isolates of *Ascochyta pisi* were proved by confirming Koch's postulates. Pathogenicity test of pathogen isolates was performed on five week old pea seedling variety rachna grown individually in pot containing sterilized potting mixture. Conidial suspension of  $1 \times 10^4$  spores  $\text{ml}^{-1}$  of each isolate of *Ascochyta pisi* was individually sprayed to run off on to five week old pea plants. After inoculation, plants were kept moist by regularly misting them, in order to ensure high humidity, plants were covered with plastic covers for 72 hrs. Similarly, an uninoculated pea plant are maintained under same condition that served as check. After 72 hrs, cover was removed plants were transferred to greenhouse. Disease development was recorded after 7 days of inoculation.

#### Evaluation of germplasm lines

A pot experiment was conducted to evaluate and identify resistant genotypes against *Ascochyta* blight with sixty four pea genotypes procured from DARS Budgam and Division of vegetable sciences FoH, SKUAST- Kashmir, Shalimar were screened under greenhouse condition. The following is the list of genotypes that were screened

#### Preparation of inoculum

The fungus was grown on the potato dextrose agar for 15 days in order to prepare inoculum and by scrapping conidia were washed into the sterilized distilled water and then suspension was filtered. With help of hemocytometer concentration of conidia in the suspension was determined and adjusted to  $1 \times 10^4$  conidia  $\text{ml}^{-1}$  of water.

#### Inoculation procedure

The experiment consist sixty four genotypes and was conducted in polyhouse. Five week old plant were artificially inoculated by using spray inoculation technique. Inoculated plants were covered with clear polyethene for 72 hrs

immediately after inoculation to allow infection. The disease intensity was recorded after 15 days by using 0-5 scale given by Tivoli *et al.*, (1996) [14] and host plant reaction was categorized into five reaction categories given by Sindhan *et al.*, (1999) [11]. The severity of the *Ascochyta* blight of pea was calculated as per the following formula:

$$\text{Disease intensity} = \frac{\sum n v \times 100}{N \times G}$$

Where,

$\sum$  = Summation

$V$  = Disease score

$n$  = Number of plants showing a particular score.

$N$  = Total number of plants examined.

$G$  = Maximum Score.

The disease intensity was calculated using 0-5 scale suggested by Tivoli *et al.*, (1996) [14] and rating was done as under:

0 = No disease.

1 = A few scattered flecks.

2 = Numerous flecks.

3 = 25-50% plant parts covered by small coalesced lesions.

4 = 50- 75% plant parts covered with lesions.

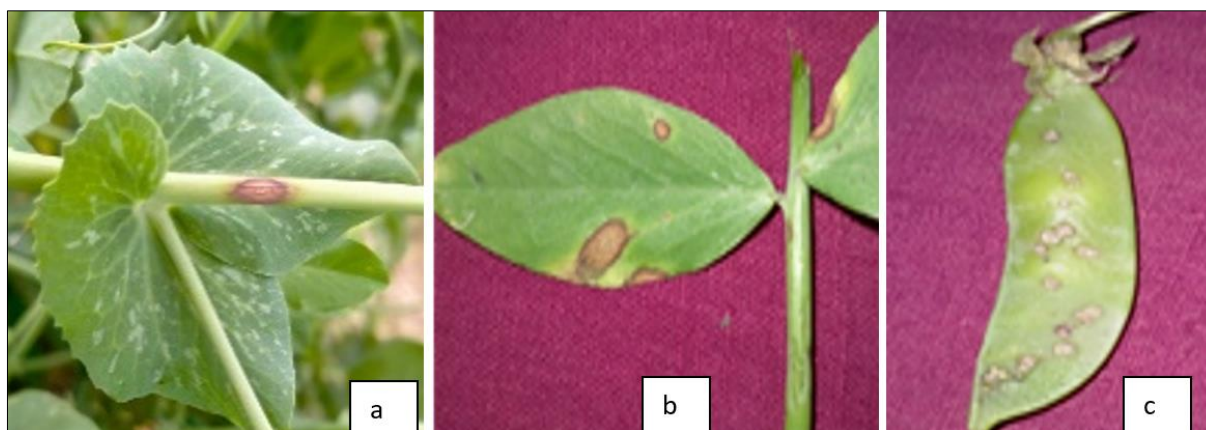
5 = 75-100% plant parts covered with extensive coalesced lesions.

The germplasm was categorized on the basis of an established scale (Sindhan *et al.*, 1999) [11] with some modifications:

S. No.	Reaction	Disease (%)
1.	Resistant	0-5.0
2.	Moderately Resistant	5.1-10.0
3.	Moderately Susceptible	10.1-25.0
4.	Susceptible	25.1-50.0
5.	Highly Susceptible	>50

#### Results

The leaf spots caused by *Ascochyta pisi* were 2 – 5 mm in diameter, circular, slightly sunken and tan coloured and appeared on upper surface of leaf with distinct dark brown margins (Plate 1). The pycnidia were of pin head size black in colour scattered throughout the spots. The spots usually appeared on leaves, stem and pods but more frequently were observed on lower leaves of the canopy. Similar spots were observed on stem and pods. However, base of stem showed no symptoms of foot rot but spots got elongated along the axis of the stem and hence did not result in mortality of plants. The Scale used for the evaluation of the disease intensity is given in Plate 2.



**Plate 1:** Symptoms of *Ascochyta pisi* on different parts of pea plant a) on Stem, b) on Leaves and c) on Pods



**Plate 2:** Scale used for scoring of disease intensity *Ascochyta* blight caused by *Ascochyta pisi* on Pea leaves.

The Screen-house results showed that the highest disease intensity in genotype SHM – 59 (53.99%) and lowest was in KDP – 47 (13.23%). Under the controlled condition, none of the screened material was found resistant or moderately resistant to *Ascochyta* blight. The results of the germplasm screening are given in table 1. The majority of germplasm lines, including most cultivated cultivar Rachna were found Susceptible to the incident of *Ascochyta* blight and other 40 germplasm lines were found susceptible namely KDP – 43, KDP – 7, KDP – 12, , KDP – 24, KDP – 2, Shalimar pea – 1, HFP – 715, VRPM – 11, VRPM – 10, VRP – 29, KDP – 5, KDP – 8, KDP – 33, Prakash, Arkal, PB -18, DHVP – K, NO -11, KDP – 22, HUDP – 15, Shalimar matter, SR – 71, PB –

18, Early gaint, VRP – 361, KDP – 58, KDP – 59, KDP – 65, KDP – 35, KDP – 1, KDP – 32, KDP – 9, VL – 45, Bonville, KSS – 288, VRP – 287, KDP – 252, VRP – 233, PB – 12 and KDP – 287. While as, thirteen genotypes namely KDP – 13 KDP – 25, KDP – 3, KDP – 60 KDP – 64, KDP – 47, KDP – 46, KDP – 61, KDP – 62, KDP – 55, KDP – 48 and VRP – 293 were categorized as moderately susceptible and ten genotypes VRP – 1166, VRP – 256, VRP – 284, VRP – 216, VRP – 270, VRPP -2, VRPM – 12 and SHM – 59 were highly susceptible. During the study, the genotypes were grouped on the basis of pooled data of disease intensity observed at maturity stage.

**Table 1:** Screening results of 63 Pea genotypes under artificially inoculated conditions.

S. No.	Genotype	Disease intensity (%)	Reaction	S. No.	Genotype	Disease intensity (%)	Reaction
1	KDP-25	21.66	MS	33	KDP-55	24.00	MS
2	KDP-43	30.50	S	34	KDP-48	22.00	MS
3	KDP-7	35.13	S	35	HUDP-15	25.33	S
4	KDP-12	44.66	S	36	Shalimar matter	27.16	S
5	KDP-13	22.00	MS	37	SR-71	40.21	S
6	KDP-24	35.33	S	38	PB-18	50.00	S
7	KDP-2	28.66	S	39	Early giant	53.15	HS
8	Shalimar pea-1	43.23	S	40	VRP-361	47.33	S
9	HFP-715	47.33	S	41	KDP-58	39.00	S
10	VRPM-11	44.67	S	42	KDP-59	44.67	S
11	VRPM10	35.92	S	43	KDP-65	41.83	S
12	VRP-29	50.00	S	44	KDP-35	41.33	S
13	VRP-1166	50.65	HS	45	KDP-1	35.92	S
14	KDP-5	27.21	S	46	KDP-32	32.66	S
15	KDP-8	27.33	S	47	KDP-9	45.33	S
16	KDP-11	14.08	MS	48	VL-45	41.31	S
17	KDP-3	15.33	MS	49	Bonville	46.67	S
18	KDP-33	26.00	S	50	KSS-288	35.33	S

19	KDP-60	24.50	MS	51	VRP-287	48.67	S
20	KDP-64	22.00	MS	52	VRP-252	35.33	S
21	Prakash	42.10	S	53	VRP-233	48.67	S
22	Arkal	45.00	S	54	VRP-256	51.45	HS
23	PB-81	34.00	S	55	VRP-284	52.33	HS
24	DHVP-k	40.21	S	56	SHM-54	53.67	HS
25	NO-11	27.16	S	57	VRP-216	51.33	HS
26	Rachna	47.18	S	58	VRP-270	52.31	HS
27	KDP-47	13.23	MS	59	VRPP-2	45.33	HS
28	KDP-46	18.29	MS	60	PB-12	47.96	S
29	KDP-61	18.32	MS	61	SHM-59	53.99	HS
30				62	VRP-293	23.69	MS
31	KDP-22	27.167	S	63	VRPM-12	50.66	HS
32	KDP-62	21.87	MS				

## Discussion

In present investigation, 40 genotypes were found susceptible, 12 moderately susceptible and 8 were found as highly susceptible. The highest disease intensity was found in germplasm SHM – 59 (53.99%) and lowest was in KDP – 47 (13.23%) but none of the germplasm line was found resistant or partially resistant. Several workers also have reported lack of resistance against *Ascochyta* blight pathogens in different parts of the world (Kraft *et al.* 1998) [7]. Warkentin *et al.* (2000) [15] analyzed 335 field pea lines for the relationships between components of partial resistance and yield reduction, these lines originated from more than 30 countries against *Mycosphaerella pinodes* and found that none of them possessed high level of resistance against it. However out of 335 pea lines only seven were able to show less area under AUDPC scores and only cultivar Radley was offered as partially resistant. Similarly Zhang *et al.*, (2006) [17] failed in a comprehensive assessment of field pea germplasm to identify any accession that are highly resistant to *Mycosphaerella pinodes* causing *Mycosphaerella* blight. Fondevilla *et al.* (2005) [3] also reported little resistance in pea against *Mycosphaerella pinodes*. Furthermore, Boros and wawer (2009) [2] reported that genotypic variation in susceptibility to *Mycosphaerella pinodes* was significant but none of the genotype was highly resistant. Work on resistance to this disease is not available in India (Gupta and Paul, 2002) [4]. Present investigation results are in conformity with findings of above workers. Zhang *et al.* (2006) [17] demonstrated differences in susceptibility among different cultivars, but no source of strong resistance has been found for pea. As a result, being found in wild species of pea (Worth, 1998) [16] and also sought in closely related species like *Lathyrus sativus* (Skiba *et al.*, 2004) [12].

## Conclusion

The prominent genotype showed indifferent response to *Ascochyta pisi*. Under the controlled condition, none of the screened material was found resistant or moderately resistant to *Ascochyta* blight thus, indicating lack of diversity for resistance. There was substantial increase in disease intensity from vegetative to maturity stage. The highest disease intensity was recorded in germplasm SHM – 59 (53.99%) and lowest was in KDP – 47 (13.23%).

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