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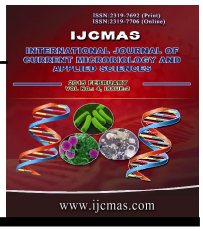

Original Research Article

Induction of Phenol and Defence-Related Enzymes During Wilt (*Fusarium udum* Butler) Infestation in Pigeon Pea

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Keywords

Pigeon pea, Wilt, Phenol, Polyphenol oxidase, Phenylalanine ammonium lyase

A B S T R A C T

The present investigation was carried out on wilt (*Fusarium udum* Butler) resistant (ICPL 87119, BDN 2) and susceptible (T_{15-15} & ICP 2376) genotypes of pigeon pea at 0 day after infection (DAI), 1 DAI, 4 DAI and 7 DAI in infected and non-infected tissues to identify some biochemical markers like phenol and enzymes related to phenol metabolism viz., PPO and PAL for wilt resistant. The phenol content increased 1 DAI to 4 DAI in leaves and root tissue and decreased later in leaves tissue only during infection of wilt pathogen. The phenylalanine ammonium lyase (PAL) and polyphenol oxidase (PPO) activity were highest in resistant genotypes at all the stages. The root tissues recorded 5 to 10 fold higher PAL activity than leaves in all genotypes.

Introduction

Pigeon pea [*Cajanus cajan* (L.) Mill sp.] , is the second most important pulse crop after chickpea, grown in India and plays an important role in subsistence agriculture. It ranks sixth in total world's production of grain legume. Globally it is cultivated on 4.75 million hectares producing 3.68 million tones with productivity of 774.30 kg/ha (FAO, 2010). During its life span, pigeon pea is attacked by more than 100 pathogens (Nene *et al.,* 1989). The wilt is caused by *Fusarium udum* Butler and is one of the serious and oldest known diseases (Butler, 1906).

Plants are equipped with a variety of defense mechanism against such pathogen or biotic stress to protect against the attack, some of this are constitutive while others are induced upon the attack of pathogen like accumulation of phenolics or induction of antioxidant and its related enzymatic system (Kandoliya and Vakharia, 2013a). Thus this experiment was design to study with phenol and its related enzyme like PPO and PAL and its relation with the host pathogen interaction in pigeon pea in laboratory condition.

Materials and Methods

The four pigeon pea (*Cajanus cajan* L.) genotypes (BDN-2 and ICPL-87119; resistant and ICP-2376 & T_{15-15}; susceptible differing in their reaction to wilt pathogen *Fusarium udum* Butler obtained from Model Farm, Vadodara, Anand Agricultural University were used in present experiment. Pigeon pea genotypes were raised in plastic bag in sterilized soli and sand in 1:1 ratio in 15x15 cm plastic bag in green house. Ten days old seedlings were uprooted from plastic bag. The root system washed in running tap water and rinsed in sterilized distilled water. One seedling transferred into each test tube having 20ml of 6.5×10^5 spores/ml. Cover all tube with cotton plug in order to hold the seedling in position and minimized the contamination. The whole stand was wrapped with black paper to eliminate the effect of light. Sterilized distilled water was added to the tube every two days to make up the loss. Seedling grown in sterilized distilled water serves as control. Seedling was collected after 1 DAI, 4 DAI and 7 DAI for analysis. The phenol content in pigeon pea seedling was determined by method of Malik and Singh (1980).

The PPO activity was analyze as per Malik and Singh (1980) using three hundred milligram of leaves and roots ground in 3 ml of 0.1 M sodium phosphate buffer, pH 6.0. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was used for enzyme assay. Protein from the extract was measured by Folin Lowry method which was used for expression of specific activity of the enzyme.

The PAL activity was analyzed using three hundred milligram of leaf and root tissues homogenized with a pre-chilled mortar and pestle in 3 ml of extraction buffer containing 50 mM borate-HCl buffer (pH 8.5) and 0.04% ß-mercaptoethanol. The homogenate was centrifuged at 10,000 rpm for 15 min. The clear supernatant was used as the enzyme source for the assay of PAL (Mahadevan and Sridhar, 1996). Protein from the extract was measured by Folin Lowry method which was used for expression of specific activity of the enzyme.

Result and Discussion

The results presented revealed that the highest phenol content was observed in the roots (1.26 mg g^{-1}) of susceptible genotype (T_{15-15}) than the other genotypes at pre-infection stage (10 DAG or 0 DAI). At 1, 4 & 7 DAI, the phenol content was higher in infected leaves of all the genotypes as compared to non-infected leaves (Fig. 1). Among all genotypes, the resistant genotype ICPL 87119 recorded highest phenol content (2.17 mg g^{-1}) in leaves than the other genotypes at 4 DAI. The per cent reduction was more in resistant genotypes than the susceptible ones. In roots, the phenol content was increased in both infected and non-infected tissues of all the genotypes as compared to 0 DAI. However, phenol content was slightly increased in infected roots of susceptible genotypes and decrease in the resistant genotypes. The phenol content in infected leaves tissue was significantly higher in resistant genotypes as compared to susceptible genotypes at 1 and 4 DAI (Table 1). In present study, a marked increase in phenolic content might be due to accumulation from surrounding healthy tissues (Farkas and Kiraly, 1962 and Jaypal and Mahadevan, 1968). Similar findings were reported by Singh *et al*. (2003) and Kandoliya and Vakharia (2013b) in chickpea resistant lines against *Fusarium* wilt, Vir et al. (1975) in resistant chickpea cultivar infected by *A. rabiei*, Jabeen *et al*.

(2009) in chilli under the environment of fusarium wilt and Bashan *et al*. (1985) in tomato plants in relation to resistance against *P. syringae*. At 7 DAI, the phenol content was decline in both infected and non-infected leaves of all genotypes. Rathod and Vakharia (2011) reported similar results in chickpea cultivars under diseased environment of *F. oxysporium f. sp. ciceri*. The increase in phenol content was more in susceptible genotypes roots compared to resistant ones.

The resistant variety had reported higher PPO activity compared to susceptible genotype both in leaves and root tissue, and enhanced upon infection with fungus (Fig. 2, Table 2). In leaf tissue, the highest PPO activity 8.43 ΔOD mg^{-1} protein was recorded in resistant genotype ICPL 87119 at 7 DAI. The resistant genotypes have higher enzyme activity under infected and non-infected condition against the susceptible. The rise of 84.24% in enzyme activity was observed in resistant genotypes at 7 DAI compared to 1 DAI, as against the rise of 55.87% in susceptible genotypes. At 7 DAI the resistant genotypes have 73.52 % higher PPO activity compared to susceptible genotypes. In root tissue, the higher PPO activity 16.61 (ΔOD mg^{-1} protein was recorded in resistant genotype ICPL 87119 at 7 DAI. The resistant genotypes have higher PPO activity compared to susceptible, and also after infection. The resistant genotypes have 38.23% higher enzyme activity against the susceptible at 7 DAI. The rise of 39.26% in PPO activity was observed in resistant genotype at 7 DAI compared to 1 DAI. The root tissue has 2 to 5 fold higher enzyme activity against the leaves tissue both in resistant and susceptible genotypes. The per cent increase in PPO activity was more in resistant genotypes compared to susceptible ones. The higher activity of PPO in resistant genotypes must have resulted due to more oxidation of phenolic substances to form more toxic quinones and the reversed disproportionate of quinones to semiquinone radicals that may lead to generation of reactive oxygen species (ROS). These oxidative products are toxic substances for the extra-cellular enzymes produced by the pathogen (Mahatma *et al*., 2008). Therefore, it is likely to govern same biochemical mechanism for resistance in the present study. On the other hand, lower enzyme activity in the susceptible genotypes, produce less amount of toxic quinones or other oxidative products to that extent as found in resistant. In the later stage the enzyme activity was very less in the susceptible ones providing no protection against *Fusarium* wilt ultimately leading to death of the plants. In addition to the direct defense response, PPO-generated H_2O_2 could also be a component of signaling processes by acting as a diffusible inducer of cellular protectant genes, phytoalexin biosynthesis, and salicylic acid and ethylene production, as well as a trigger of cell death resulting in restricted lesions delimited from surrounding healthy tissue (Thipyapong *et al*., 2007).

The results of change in PAL activity in leaves and root tissue upon *Fusarium udum* Butler infection are presented in Fig.3 and Tables 3. Higher PAL activity was observed in resistant genotypes compared to susceptible one under infected and non-infected condition in both leaves and root tissue. In leave tissue, the highest PAL activity 119.65 µg trans cinnamic acid h^{-1} mg^{-1} protein was observed in resistant genotype (ICPL 87119) at 7 DAI. The resistant genotypes have 66.43 % higher enzyme activity compared to susceptible one at 7 DAI. In resistant genotypes leaves tissue 163.10 % (1.6 fold) rise in enzyme activity were recorded at 7 DAI compared to 1 DAI.

In root tissue, the resistant genotypes have higher enzyme activity compared to susceptible one and was enhanced upon fungus infection. At 7 DAI the resistant genotypes have 58.12 % higher PAL activity as against the susceptible genotypes. The highest PAL activity 288.11 ug trans cinnamic acid h^{-1} mg^{-1} protein in root tissue was observed in resistant genotypes (ICPL 87119) at 7 DAI. The root tissue has recorded 5 to 10 fold higher PAL activity in resistant and susceptible genotypes as compared to leaves tissue. The higher PAL activities in resistant genotypes were observed compared to susceptible one, and the root tissue had higher enzyme activity as against the leaves tissue.

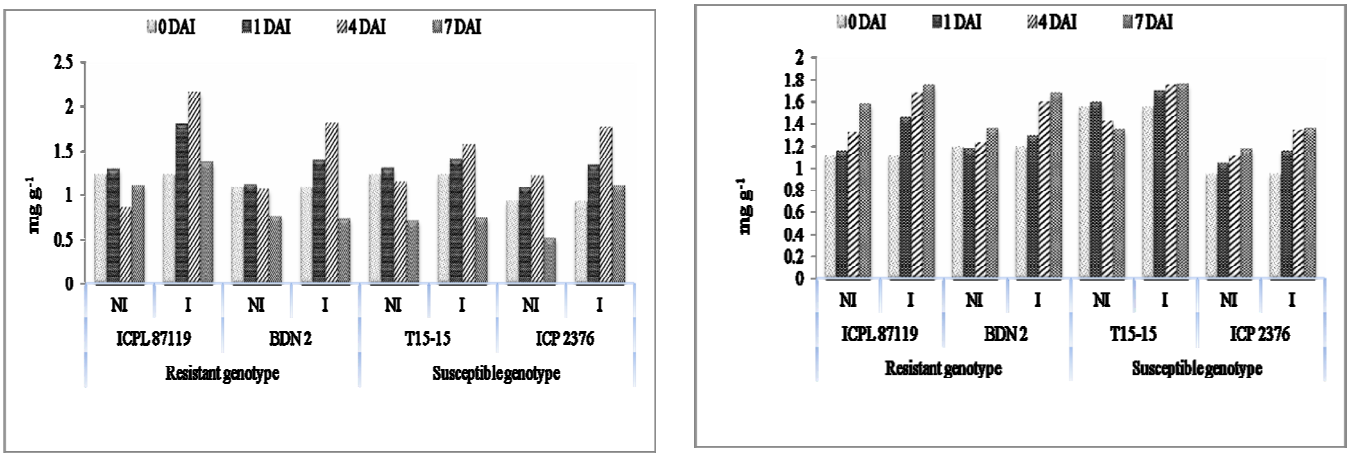
Table.1 Changes in total phenol content in leaves and root tissue

Genotypes	Reaction	Phenol (mg g^{-1} FW)						
		Pre inoculation (0 day)	Disease Development Stages					
			1st DAI		4th DAI		7th DAI	
			NI	I	NI	I	NI	I
A: Leaves								
		Resistant						
ICPL 87119		1.20	1.30	1.81	0.86	2.17	1.11	1.37
BDN 2		1.08	1.12	1.40	1.06	1.82	0.76	0.73
		Susceptible						
T_{15-15}		1.23	1.31	1.41	1.15	1.57	0.71	0.74
ICP 2376		0.93	1.08	1.34	1.22	1.78	0.52	1.11
S. Em.		0.03	0.02	0.02	0.03	0.04	0.04	0.18
C.D. at 5%		0.09	0.06	0.07	0.08	0.12	0.13	0.54
C.V.%		4.43	3.44	4.37	2.78	3.70	4.96	5.69
B: Root								
		Resistant						
ICPL 87119		1.11	1.16	1.46	1.32	1.68	1.58	1.75
BDN 2		1.12	1.18	1.30	1.23	1.60	1.36	1.68
		Susceptible						
T_{15-15}		1.26	1.60	1.70	1.42	1.75	1.35	1.76
ICP 2376		0.95	1.05	1.16	1.11	1.34	1.18	1.36
S. Em.		0.04	0.02	0.02	0.03	0.04	0.02	0.11
C.D. at 5%		0.12	0.06	0.07	0.08	0.13	0.08	0.33
C.V.%		5.91	3.27	4.37	2.96	4.96	2.78	5.30

Table.2 Changes in PPO activity in leaves and root tissue

Genotypes	Reaction	Polyphenol oxidase ((ΔOD mg^{-1} protein)					
		Disease Developing Stages					
		1st DAI		4th DAI		7th DAI	
		NI	I	NI	I	NI	I
A: Leaves							
		Resistant					
ICPL 87119		3.88	4.52	5.12	7.14	4.39	8.43
BDN 2		3.31	3.73	4.22	5.62	4.41	6.77
		Susceptible					
T_{15-15}		2.71	2.73	3.24	3.69	3.41	4.72
ICP2376		2.84	2.89	3.30	3.81	3.62	4.04
S. Em.		0.028	0.04	0.021	0.038	0.023	0.029
C.D. at 5%		0.09	0.12	0.06	0.12	0.07	0.09
C.V.%		4.43	5.91	3.44	3.70	5.12	5.32
B: Root							
		Resistant					
ICPL 87119		11.29	11.40	13.63	15.17	12.32	16.61
BDN 2		10.12	10..55	10.79	13.29	11.20	13.76
		Susceptible					
T_{15-15}		9.25	9.31	9.55	10.85	9.96	11.09
ICP2376		9.06	9.17	9.84	10.13	9.88	10.88
S. Em.		0.39	0.44	0.35	0.23	1.42	1.63
C.D. at 5%		1.24	1.40	1.10	0.73	4.48	5.16
C.V.%		4.21	4.56	3.82	5.05	1.04	1.02

Fig.1 Total phenol in (A) leaves and (B) root at different time after inoculation

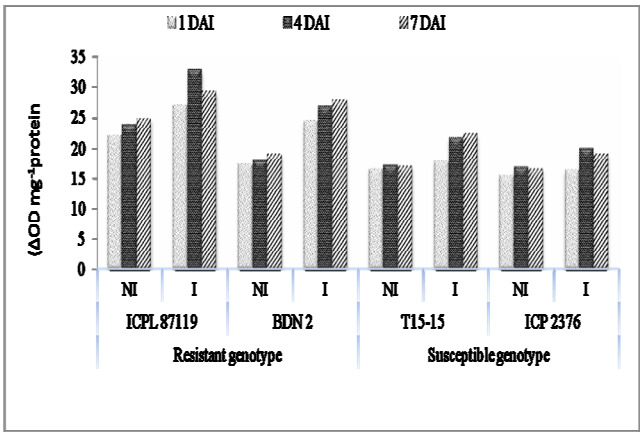


Note: NI- Non-infected, I- Infected

Table.3 Changes in PAL activity in leaves and root tissue

Genotypes	Reaction	Phenylalanine Ammonia Lyase (ug trans cinnamic acid h^{-1} mg^{-1} protein)					
		Disease Developing Stages					
		1st DAI		4th DAI		7th DAI	
		NI	I	NI	I	NI	I
A: Leaves							
		Resistant					
ICPL 87119		31.21	45.65	49.76	70.21	65.82	119.65
BDN 2		25.23	32.43	33.48	62.11	55.10	85.74
		Susceptible					
T_{15-15}		14.21	20.76	25.91	37.34	41.25	64.10
ICP2376		16.23	19.43	27.48	43.24	32.86	59.31
S. Em.		0.49	0.66	0.54	0.63	0.39	0.27
C.D. at 5%		1.57	2.09	1.71	2.01	1.23	0.85
C.V.%		5.48	2.40	5.14	5.54	4.90	5.83
B: Root							
		Resistant					
ICPL 87119		120.12	145.87	146.75	181.23	185.90	288.15
BDN 2		104.34	112.43	110.24	129.56	131.30	187.51
		Susceptible					
T_{15-15}		84.74	98.31	96.12	119.80	113.47	157.61
ICP2376		79.54	100.20	85.73	113.43	110.29	143.21
S. Em.		1.76	1.51	1.64	1.45	1.61	1.82
C.D. at 5%		5.56	4.76	5.17	4.57	5.08	5.75
C.V.%		1.59	0.97	0.72	0.46	0.42	0.60

Fig.2 Change in PPO activity in [A] leaves and [B] root after inoculation



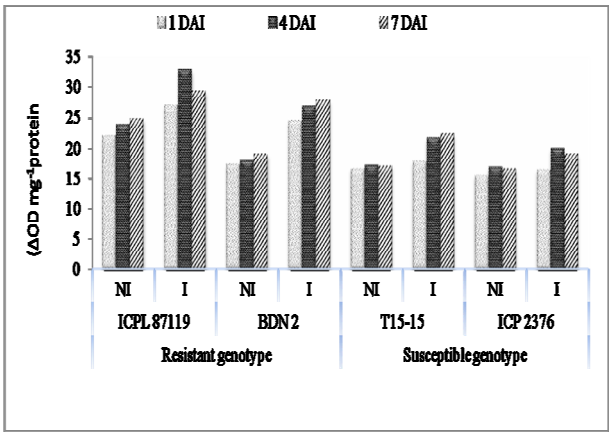
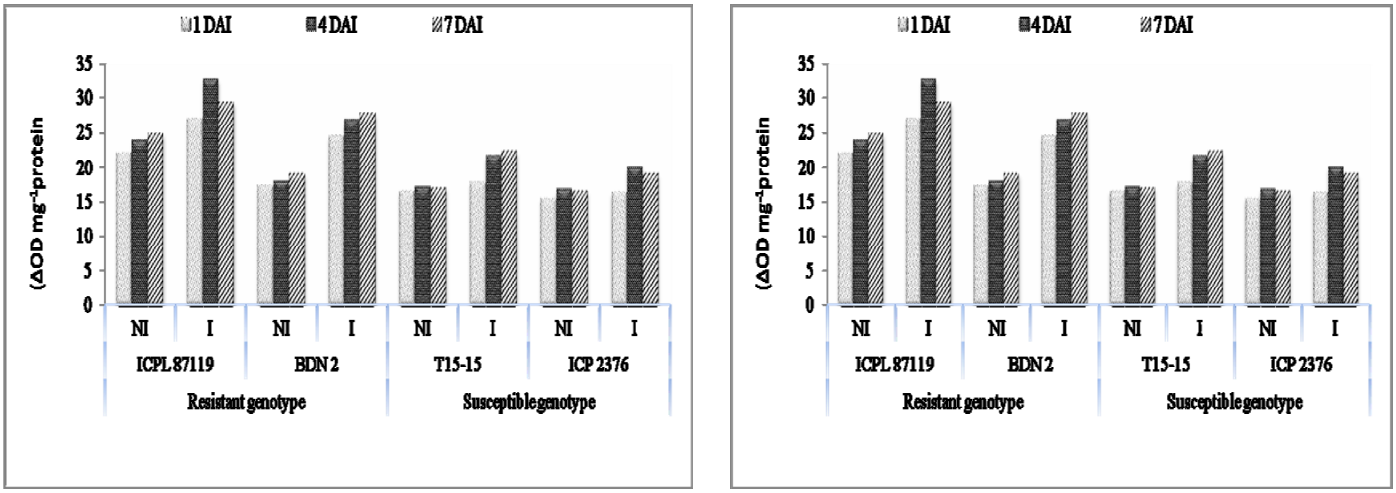


Fig.3 Change in PAL activity in [A] leaves and [B] root after inoculation



More over as this enzyme is involved in lignin and phytoalexin synthesis its continuous presence in higher quantity may be essential till host plant becomes sure of strong structural and metabolic defense. Two enzymes studied in relation to phenol metabolism are concerned, *i.e.* Phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO), among the varieties, JCP-27 and WR-315 showed higher activity, while susceptible varieties JG-62 showed the lower value for both the enzymes when chickpea genotype treated with *Fusarium oxysporum f. sp. Ciceri* (Kandoliya, 2011). Raju *et al.* (2008) observed higher levels of enzymes related to phenol metabolism (polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL)) in roots and shoots of resistant chickpea cultivar than that of susceptible cultivar on treatment with elicitors and wilt pathogen. The chickpea seedling exposed to cell wall protein of *Fusarium oxysporum* f. sp. *Ciceri* showed enhanced synthesis of phenol, pathogenesis related protein and activities of PAL and peroxidase relative to control. The rapid increase in PAL activity in pathogen treated seedling is well reported. The phenolic compound may contribute to enhance the mechanical strength of host cell wall and may also inhibit the fungus growth, as phenolics are fungi toxic in nature. Increased activity of PAL was also recorded in palm with bioformulations of *Pseudomonas* applied in palm (Karthikeyan *et al.*, 2006). Several studies have shown that PAL activity is induced in plants upon treatment with *Pseudomonas fluorescens* (Chen *et al.*, 2000; Sundaravadana, 2002; Saravanakumar *et al.*, 2003).

The phenol content increased 1 DAI to 4 DAI in leaves and root tissue indicates systemic induction and decreased later in leaves tissue due to higher activity of PPO as result of infection of wilt pathogen. The phenylalanine ammonium lyase (PAL) and polyphenol oxidase (PPO) activity were remains higher in resistant genotypes at all the stages and root tissues recorded 5 to 10 fold higher PAL activity than leaves in all genotypes indicates constitutive characteristic of resistant variety to defend against pathogen.

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