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Biochemical studies on *Phytophthora capsici* inoculated and uninoculated plants of *Piper* spp.

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Abstract: Biochemical studies on susceptible, tolerant and immune genotypes of blackpepper to *Phytophthora capsici* were analysed for total and OD phenols, reducing and non-reducing sugars and free amino acid contents. As compared to uninoculated plants, the contents of total phenol and free amino acids in the inoculated plants increased in Panniyur 1. In contrast Kalluvally reported to be tolerant to *P. capsici* registered increase in the contents of total phenols only. While the immune genotype had exhibited decrease in contents of OD phenol, reducing and non-reducing sugars as compared with uninoculated plants. It is suggested that biochemical differences observed could be used for screening disease resistance genotypes to *P. capsici*.

Key words : Blackpepper, Piper spp., Phytophthora capsici, Disease resistance.

Introduction

Blackpepper popularly known as "the king of spices" is an important export oriented crop among spices grown in India. Its cultivation is under serious threat due to culmination of Phytophthora foot rot disease during rainy season. There is no effective control measure and also there are no resistant varieties to tackle this malady. However an introduced genotype *Piper colubrinum* L. shows immune reaction to *Phythphthora capsici* (Sarma *et al.* 1991). The resistant / tolerant varieties posses various physical and chemical barriers to restrict the entry and growth of the pathogen in the host cells. In many plantpathogen interactions, phenomena involved in the defense action of plants to pathogen have been found and reported. Principal factors involved in the expression of resistance is based on genetic composition of the host and expression of the resistance genes may be influenced by many factors like aggressiveness of the pathogen, availability of specific nutrients, accumulation of inhibitory substances at the infection site, metabolism of the host constituents including the activity of numerous enzyme systems, host nutrition and environment (Barnett, 1959). Present study was conducted during 1997-98 at the College of Horticulture, Vellanikkara, Thrissur. In the present study three different genotypes *viz.* Panniyur 1, kalluvally and *P. colubrinum* reported to be susceptible, tolerant and immune respectively to *Phytophthora capsici* were studied with respect to phenols, sugars and amino acids.

Materials and Methods

Rooted cuttings of three blackpepper genotypes *viz.* Panniyur 1, Kalluvally and *P.colubrinum* were raised in polybags and were maintained in the glasshouse at ambient conditions.

Pure culture of *P. capsici* was isolated from the infected leaves of blackpepper and was inoculated to Potato Dextrose Agar (PDA) medium after surface sterilisation. Culture was purified by 'hyphal tip method' and was maintained in PDA medium by repeated subculturing. Pathoginicity test was conducted with 5 mm culture disc by 'detached leaf bioassay' as reported by Kueh and Khew (1980). Then pathogen was reisolated from the artificially inoculated leaves by the method already described. Artificial inoculation of *P. capsici* on leaves was conducted as per the procedure reported by Kueh and Khew (1980). Mature third leaf from top was washed thoroughly and surface sterilised with 70 per cent ethyl alcohol. With sterile needle minute pinpricks were made on the lower side of the leaf and 5 mm culture discs of the pathogen were inoculated.

Similarly, stems were inoculated as per the method suggested by Sarma *et al.* (1991) by making minute pinpricks at the center of

Table 1. Comparison of total and OD phenol content (mg^{-1} g) in *Phytophthora capsici* inoculated and uninoculated plant parts of *Piper* spp.

	Piper nigrum L.						*P. colubrinum* L.			
	Panniyur 1		Kalluvally		* Mean				Mean	
	UI	I	UI	I	UI	I	UI	I	UI	I
Total phenol content (mg^{-1} g)										
Leaf	2.123	6.413	3.245	7.328	2.684	6.871	5.539	7.504	3.636	7.082
Stem	2.239	5.628	3.645	6.926	2.942	6.277	3.437	4.973	3.140	5.842
Mean	2.231	6.021	3.445	7.127	2.838	6.574	4.488	6.239	3.388	6.462
CD (0.05) = 0.948										
OD phenol content (mg^{-1} g)										
Leaf	5.093	4.973	7.627	3.306	6.360	4.140	7.667	1.303	6.796	3.194
Stem	4.537	2.223	7.060	1.157	5.799	1.690	5.997	2.433	5.864	1.938
Mean	4.815	3.598	7.343	2.231	6.079	2.915	6.832	1.868	6.330	2.566
CD (0.05) = 0.859										

* Worked out from means of Panniyur 1 and Kalluvally
UI - Uninoculated I - Inoculated

Table 2. Comparison of reducing non-reducing sugar content (mg^{-1} g) in *Phytophthora capsici* inoculated and uninoculated plants parts of *Piper* spp.

	Piper nigrum L.						*P. colubrinum* L.			
	Panniyur 1		Kalluvally		* Mean				Mean	
	UI	I	UI	I	UI	I	UI	I	UI	I
Reducing sugar content (mg^{-1} g)										
Leaf	2.375	1.753	3.325	1.690	2.850	1.722	3.711	1.516	3.137	1.653
Stem	2.474	3.654	2.374	1.963	2.424	2.810	3.304	2.224	2.717	2.614
Mean	2.425	2.704	2.849	1.827	2.637	2.266	3.508	1.870	2.927	2.134
CD (0.05) = 0.682										
Non-reducing sugar content (mg^{-1} g)										
Leaf	1.652	1.787	1.488	1.440	1.570	1.614	1.440	0.232	1.528	1.154
Stem	2.464	1.595	1.976	0.330	2.220	0.962	1.792	0.074	2.077	0.066
Mean	2.058	1.691	1.732	0.887	1.895	1.289	1.618	0.153	1.803	0.910
CD (0.05) = 0.401										

* Worked out from means of Panniyur 1 and Kalluvally
UI - Uninoculated I - Inoculated

the second node from the top, by placing 5mm culture disc and tied with polythene after placing wet cotton wading. High humidity was maintained in the bell jar throughout 72 h period of incubation and thereafter plants were taken for analyses.

Both healthy and inoculated leaves and stems of the three genotypes were extracted

Table 3. Comparison of total free amino acids (%) in *Phytophthora capsici* inoculated and uninoculated plants parts of *Piper* spp.

	Piper nigrum L.						*P. colubrinum* L.			
	Panniyur 1		Kalluvally		* Mean				Mean	
	UI	I	UI	I	UI	I	UI	I	UI	I
Leaf	0.030	0.029	0.035	0.049	0.033	0.039	0.047	0.099	0.037	0.059
Stem	0.017	0.031	0.032	0.016	0.025	0.024	0.039	0.037	0.030	0.043
Mean	0.024	0.030	0.034	0.032	0.029	0.031	0.043	0.068	0.034	0.038
CD (0.05) = 0.012										

* Worked out from means of Panniyur 1 and Kalluvally
UI - Uninoculated I - Inoculated

in 80 per cent methanol and the extracts analysed for the total phenols and free amino acids as per the method suggested by Sadasivam and Manickam (1996). Reducing and non-reducing sugars and OD phenol in the sample were analysed as per the procedure reported by Mahadevan and Sridhar (1986). The data generated were calculated for the level of significance as per Panse and Sukhatme (1985).

Results and Discussion

Total phenols

Following *Phytophthora capsici* inoculation total phenol content increased in all the genotypes irrespective of plant parts analysed (Table 1). Higher increase was found in Panniyur 1 followed by Kalluvally and *P. colubrinum*. Of the different plant parts, leaves had shown higher increase compared to stems. Tepper and Anderson (1984) reported that phenolics in high concentrations are toxic to plant cell themselves and hence will normally be present in small quantities. The higher content of total phenol observed in *P. colubrinum* might be responsible for checking lesion development after inoculation with pathogen. The increased production of phenols which are cytotoxic might have contributed to increased cell death in Panniyur 1 and Kalluvally and thereby its hypersensitive reaction to the disease. Markose (1996) reported higher content of total phenols in susceptible chilli varieties to bacterial wilt.

OD phenol

The enzymes *viz.* polyphenol oxidase and peroxidase oxidise the colourless dihydroxyphenols to give the coloured ortho quinones. While certain dihydroxy phenols get conjugated with each other or with glucose hydroxyl groups to form tannins, both form constituents of plant melanins. (Mayer and Harrel, 1979 and Bell, 1981). These tannins and *ortho* quinones have toxicity to microorganisms (Hunter, 1978).

Inoculation of pathogen on leaves and stems resulted in significant decrease of OD phenol content within 72 h in the three genotypes studied (Table 1). Maximum decrease was observed in *P. colubrinum* compared to *P. nigrum*. Similarly, decrease was found to be more in Kalluvally as compared to Panniyur 1.

As in the present investigation, Addy (1976) reported that on inoculation of *Malus pumila* with *Erwinia amylovora* resistant varieties leached dihydroxy phenols more rapidly than the susceptible one. Similarly flag smut resistant wheat varieties had higher levels of OD phenols, but on inoculation with fungi its level decreased (Sindhan *et al.* 1996).

Reducing sugars

There was significant reduction in quantity of reducing sugars in both species of *Piper* on inoculation (Table 2). More reduction in reducing sugar content was observed in *P.colubrinum* compared to *P. nigrum*. Among P. nigrum genotypes, decrease in reducing sugar was observed only in Kalluvally while in Panniyur 1, sugar content increased.

Sugars provide carbon skeleton for phenol synthesis. The higher content of sugars present in *P. colubrinum* may be responsible for higher content of total phenols and thereby contributes to its immunity. On inoculation, the decrease in sugar content observed in *P. colubrinum* might be due to its conversion to polyphenols. Since the sugars are the preferred nutrients for the pathogen development, decrease in sugar content observed in *P. colubrinum* on inoculation might be responsible for its immunity. On the other hand, increase in sugar content observed in Panniyur 1 on inoculation might be responsible for its susceptibility by giving ample substrate for the pathogen to grow. The decrease in sugar content observed in Kalluvally as in *P.colubrinum* might be responsible for its tolerance. Easwaran (1967) observed reduction in quantity of reducing sugars in susceptible as well as in moderately susceptible varieties on inoculation with bacterial wilt pathogen. Prasad *et al.* (1972) also observed reduction in reducing and total sugars in bacterial leaf blight resistant rice varieties inoculated with pathogen. Reduction in sugar content had been reported by Sindhan *et al.* (1996) in flag smut resistant wheat varieties.

Non-reducing sugars

Significant depletion of non-reducing sugars was observed in inoculated plant parts of the three genotypes studied (Table 2). The decrease was more in *P.colubrinum* compared to *P. nigrum*. Maximum decrease in non-reducing sugar content was observed in *P. colubrinum* followed by Kalluvally and Panniyur 1.

The co-existence of phenols and sugars results in glycolisation of phenols by sugars forming phenolic glycosides which are more soluble in cell sap and thus are involved more efficiently in resistance expression (Walker, 1975). The carbohydrates may also be utilised for meeting the energy requirement of host plants due to increased respiration. The reduction in sugar contents in stems of all genotypes and leaves of Kalluvally and *P. colubrinum* can be attributed to the fact that a major part of these sugars are probably shifted for polyphenol synthesis as reported by Niesh (1964).

Abraham (1986) attributed the resistance of betelvine cultivars to bacterial leaf spot pathogen as pre-inoculation higher levels of reducing, non-reducing and total sugars and post-inoculation increase of non-reducing sugar content in susceptible cultivars. Decreased photosynthetic efficiency and content of chlorophyll, reducing, non-reducing and total sugars and starch content in capsicum leaves inoculated with *Alternaria solani* was reported by Veermohan *et al.* (1994). Paul (1998) reported higher levels of soluble sugars in bacterial wilt resistant varieties of chilli and tomato.

Total free amino acids

On inoculation there was significant increase in amino acid content in *P. colubrinum* and *P. nigrum* (Table 3). Maximum increase was found in *P. colubrinum* and Panniyur 1, whereas decrease was observed in Kalluvally. In general there was increase in amino acid content due to inoculation in stems as well as in leaves irrespective of the genotypes studied.

An increase in amino acid content may be due to decomposition of host protein or decreased protein synthesis. Similarly synthesis of amino acid by growing fungus would also cause an increase in the amino acid content whereas decrease in content was attributed to the utilisation by pathogen (Andel, 1965). There was decrease in amino acid content in less susceptible cultivar of betelvine due to inoculation of Phytophthora leaf rot pathogen (Chile and Vyas, 1983). In brinjal the resistant genotype recorded higher content of amino acids under healthy condition and its level decreased under diseased environment of bacterial wilt pathogen (Paul, 1998).

Keeping in view of changes in biochemical contents in different genotypes, the present findings will be useful in disease resistance screening and selection of parents for breeding programme.

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