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Short Communication

Screening of chilli (*Capsicum annuum* L.) genotypes against *Colletotrichum capsici* and analysis of biochemical and enzymatic activities in inducing resistance

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Chilli (*Capsicum annuum* L.) has its unique place in the world diet in its ripe dried form (as a spice) as well as green fruits (as vegetable). *Colletotrichum capsici* (Sydow) Butler & Bisby, which causes varied disease symptoms viz., anthracnose, die back, ripe fruit rot in chilli, is one of the major production constraints in tropical and subtropical areas. The pathogen caused yield reduction up to 66-84 % in Punjab [1]. Chemical control of anthracnose is hazardous and uneconomical, hence development and use of resistant varieties is the most pragmatic way to keep the disease under check. Before initiating any resistance breeding programme, one must have thorough knowledge on the nature and basis of resistance to anthracnose as they help in formulating effective breeding programme. Importance of some of the biochemical factors such as phenols and their related enzymes in imparting either a resistance or susceptible reaction in the host has been reported in many crops [2, 3]. The present study reports difference in biochemical factors and enzyme activities among different genotypes against anthracnose disease.

The experiment was conducted at Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2005-2006. Seventeen genotypes (seven germplasm lines and 10 hybrids), which includes known resistant (PBC 81) and susceptible (Arka Lohit) genotypes were screened for resistance to anthracnose disease by artificial inoculation method [4]. The experiment was conducted in a completely randomized design with three replications and five fruits per replication. An Coimbatore isolate of *C. capsici* was multiplied on potato dextrose agar medium. 1 ml inoculum of 5 x 10^5 spores per ml was artificially introduced into the epidermis of detached fruits by microinjection method. Inoculated fruits were kept at 25 ± 2^0C in a moisture saturated room. The disease symptoms were recorded fourteen days after inoculation. The degree of disease incidence on fruits was judged by percentage of infected sites (based on lesion development) over total inoculated sites and was scored on 0- 5 scale (0, no disease symptom developed; 1, less than 10% of symptom developed; 2 upto 20% symptom developed; 3, upto 40% symptom developed; 4 upto 70% symptom developed; 5, more than 70% symptom developed). The genotypes with mean disease incidence of 0-10 per cent (corresponding to score 0 - 1) were evaluated as resistant and those with a mean disease incidence of 10-20 per cent (corresponding to score 2) were considered as moderate and others with a mean disease incidence of more than 21 per cent (corresponding to score 3-5) were considered as susceptible and data were statistically analyzed [5]. Standard methods were used for estimation of total phenols [6], Ortho dihydroxy phenols (7) and for assay of peroxidase, poly phenol oxidase and phenyl ammonia lyase [8] and data were statistically analyzed [5].

Screening of parents and selected hybrids for resistance against anthracnose disease under artificial condition

The disease index scale values of accessions ranged

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between 2.67 and 5.00 and were found to be susceptible, except for S1 which was found to be moderately resistant genotype (Table 1). PBC 81, (*Capsicum baccatum*) was evaluated to be resistant with 0.33 scale disease incidence. None of the genotypes involved in the present study was immune to anthracnose disease, when inoculated artificially. The hybrids *viz*., HY 2 (2.33), HY 1 (2.67), HY 4 (2.67) and HY 5 (2.67) were rated as moderately resistant genotypes against anthracnose disease under artificial screening. Typical disease symptoms were developed in fruits of all susceptible genotypes.

Table 1. Disease reaction of parents and selected chilli hybrids to *Colletotrichum capsici* (artificial screening)

Entry	Disease incidence	Response
P1 (Arka Lohit)	4.33±0.36*	S
P2 (MDU Y)	5.00±0.36	S
P3 (S1)	2.67±0.36	MR
P4 (Arka Abir)	4.33±0.36	S
P5 (Bydagi Dabbi)	5.00±0.36	S
P6 (Co 4)	5.00±0.36	S
Acc. 16 (PBC 81)**	0.33±0.36	R
HY 1 (P1 X P3)	2.67±0.36	MR
HY 2 (P2 X P3)	2.33±0.36	MR
HY 3 (P3 X P2)	2.67±0.36	MR
HY 4 (P4 X P3)	2.67±0.36	MR
HY 5 (P6 X P3)	2.67±0.36	MR
HY 6 (P1 X P2)	5.00±0.36	S
HY 7 (P2 X P6)	5.00±0.36	S
HY 8 (P4 X P6)	4.33±0.36	S
HY 9 (P5 X P1)	5.00±0.36	S
HY 10 (P6 X P2)	3.00±0.36	S

*Standard Error
***Capsicum baccatum*
(S = Susceptible; MR = Medium resistant; R = resistant)

Variability for biochemical and enzyme activity

Data on total phenols and activity of three enzymes reveal that variations among genotypes were significant (Table 2). The total phenol content varied significantly among seventeen genotypes, which ranged from 16.4 to 56.1 mg per 100 g. The total phenol content was the highest in the Acc 16, followed by resistant hybrids, moderately resistant and the least in susceptible genoytpes. Higher level of preformed phenolic compounds in anthracnose resistant varieties than those in susceptible varieties was reported previously [9]. These phenol compounds may act as substrates for enzymes which convert them into other compounds that are more directly related to disease reaction.

The orthodihydroxy phenol content among six parents varied from 2.6 mg per 100 g in Bydagi Dabbi to 8.5 mg per 100 g in S1. Among the crosses, the range of orthodihydroxy phenol content was from 3.8 (HY 6) to 14.6 mg per 100 g (HY 5). The orthodihydroxy phenols were significantly higher in the resistant and moderately resistant genotypes as compared to susceptible ones, which had been reported earlier [10].

The peroxidase activity varied from 0.29 (HY 8) to 0.40 activity per min per g (HY 2). The activity of Polyphenol oxidase enzyme was highest in the resistant genotype Acc. 16, S1 and followed by moderate resistant hybrids. Least enzyme activity was recorded in the susceptible genotype HY 6 (Table 2). On infection with pathogen, the activity of the enzyme increased significantly in resistant varieties, which in turn led to formation of more quinones and other oxidation products, resulting in reduced multiplication and inactivation of the pathogen [11]. Peroxidase activity was found to be high in the powdery mildew resistant chilli varieties IIHR 517A and Pusa Jwala [2]. The activity of Phenylalanine ammonia-lyase was highest in the hybrid HY 1, a moderately resistant hybrid followed by Acc.16, a resistant genotype. Susceptible genotypes HY 10 and MDU Y exhibited least activity. Studies on the changes in peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase among different genotypes indicated that there was wide variation in the enzyme activity among different categories of resistance. Thus, the activity of the peroxidase and polyphenol oxidase enzymes are directly related to resistance in the host, which could be due to the conversion of the enzymes into quinones, which are toxic to the pathogen [3]. High phenylalanine ammonia-lyase activity in the resistant genotypes might produce precursors for phenolics and lignin synthesis. Increased activity of this enzyme was also detected in *Colletotrichum capsici* resistant chilli genotypes [12].

It is concluded from the present study that the hybrids *viz*., HY 2, HY 1, HY 4, HY 3 and HY 5 were rated as moderately resistant against anthracnose disease under artificial screening. The total and orthodihydroxy phenols were significantly higher in the resistant and moderately resistant genotypes as compared to susceptible ones. The activity of peroxidase, polyphenol oxidase and phenyl ammonia lyase was also highest in the resistant genotype followed by moderate resistant hybrids. Least enzyme activity was recorded in the susceptible genotype. PBC 81, (*Capsicum baccatum*) was evaluated to be resistant with high level of phenols and enzyme activities.

Table 2. Biochemical factors and enzyme activity in chilli genotypes

Parents/hybrids	Total phenols (mg per 100g)	Enzyme activity			
		Ortho dihydroxy phenols (mg per 100g)	Peroxidase (activity/minute /g sample)	Polyphenol oxidase (activity per minute /g sample)	Phenylalanine ammonia-lyase (activity in µmol /minute/ml)
P1 (Arka Lohit)	39.7	6.2	0.34	0.19	4.98
P2 (MDU Y)	39.6	6.9	0.29	0.21	4.62
P3 (S1)	46.8	8.5	0.37	0.25	5.10
P4 (Arka Abir)	32.7	6.0	0.31	0.22	4.81
P5 (Bydagi Dabbi)	30.2	2.4	0.30	0.24	5.00
P6 (Co 4)	35.4	2.6	0.32	0.20	4.58
Acc. 16 (PBC 81)	56.1	15.1	0.36	0.26	5.14
HY 1 (P1 X P3)	54.2	11.4	0.33	0.28	5.16
HY 2 (P2 X P3)	46.1	12.9	0.40	0.27	5.08
HY 3 (P3 X P2)	48.3	12.1	0.34	0.25	4.98
HY 4 (P4 X P3)	38.8	10.2	0.33	0.23	5.01
HY 5 (P6 X P3)	51.4	14.6	0.35	0.28	5.10
HY 6 (P1 X P2)	16.4	3.8	0.30	0.16	4.59
HY 7 (P2 X P6)	24.3	5.5	0.30	0.20	4.91
HY 8 (P4 X P6)	31.8	4.6	0.29	0.21	4.70
HY 9 (P5 X P1)	20.9	6.2	0.31	0.20	4.61
HY 10 (P6 X P2)	30.6	10.1	0.31	0.18	4.64
SEd	2.868	0.797	0.037	0.024	0.570
CD (0.05)	6.080	1.689	0.079	0.049	1.208
CD (0.01)	8.377	2.327	0.109	0.069	1.665

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