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Characterization of leaf blight resistance in barley through isozyme analysis

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ABSTRACT
Variation in the specific activities of polyphenol oxidase, peroxidase and PAL were determined in healthy and inoculated barley genotypes in both resistant and susceptible varieties. Healthy and infected leaf samples were collected at two stages of crop growth i.e. at 30 DAS and 60 DAS. Results indicated increase in the perxodase, polyphenol oxidase activity in the inoculated plants with high Relative mobility (Rm) values. The bands appeared more intense in the resistant varieties than susceptible varieties. The resistant genotypes had higher PAL activity compared to susceptible genotypes.

Key words : Barley, Leaf Blight.

INTRODUCTION

Disease resistance mechanism is a complex phenomenon and in response to invasion by a disease causing organism, plant produces various kinds of reactions. In recent years, it is becoming increasingly evident that several natural and induced defense mechanisms operate in host plants against different diseases. One such defense mechanism is the presence of certain biochemical compounds inhibitory to the pathogen and at the same time activities of various isozymes are also modified. Therefore the isozyme studies were carried out at two different stages i.e. at 30 days after sowing (DAS) and 60 days after sowing (DAS) to understand their role in resistance or susceptibility to blight pathogen.

MATERIALS AND METHODS

Four barley genotypes were selected for the study. Among the 4 genotypes, DWR 28 and PL 760 were moderately resistant to leaf blight pathogen. Another two RD 2508 and RD 2653 were found to be susceptible to leaf blight pathogen by considering the data resulted by field experiments conducted at the Main Agricultural Research Station, University of Agricultural Sciences, Dharwad during Rabi 2003-04 and 2004-05 (Anon, 1995). PL 760 and RD 2508 are six rowed barley genotypes whereas DWR 28 and RD 2653 are two rowed barley genotypes. The genotypes were allotted in Randomized Block Design (RBD) with three replications of 1m x 1 m plots and four rows in each plot. In the field one set was maintained healthy and another set was artificially inoculated with leaf blight pathogen *Helminthosporium sativum* Pam., King and Bakke.

For isozyme studies, top two leaves were collected at 30 and 60 DAS from random plants and composite leaf sample was made for analysis of leaf peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activity in both inoculated and healthy plants. The disease observations were made for leaf blight disease at 30 and 60 DAS using double-digit scale (Kumar *et al*., 1998).

Preparation of acetone powder :

Acetone powder of fresh composite leaf samples were prepared as per the procedure described by Brynt and Forrest (1979).

The isozyme analysis of peroxidase and polyphenol oxidase was done by using vertical slab gel electrophoresis technique with non discontinuous buffer system (Hames, 1990). A vertical slab gel electrophoresis apparatus accommodating upto 13 gel of 5 mm breadth and 30 mm length was used.

Enzyme extraction and sample preparation

Peroxidase (PO) isozymes were extracted by homogenizing 300 mg of acetone powder in 5ml of chilled extraction buffer, pH 8.0 (Farkas and Stahmann, 1966). The isozymes of polyphenol oxidase (PPO) was localized on polyacrylamide gels as per the procedure suggested by Park *et al*. (1980).

Assay and determination of PAL was carried out by adopting the procedure given by Sadasivum and Manikam (1996).

RESULTS AND DISCUSSION

Peroxidase activity of the host was higher in the resistant genotypes than in the susceptible genotype. It

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was noticed that in susceptible varieties like RD 2653 and RD 2508, the number of isozyme bands were comparatively less with low Rm value at both the stages as compared to resistant varieties DWR 28 and PL 760, which recorded more number of bands with high Rm value (Table 1). Also the bands were prominent than the susceptible genotypes. Generally, it was noticed that, when the plants were inoculated with pathogen, bands were increased in numbers in all the varieties. Also the bands of inoculated genotypes were more prominent when compared to healthy plants (Plate 1a, 1b). This statement holds good with the findings of Luthra *et al*. (1998), Averyanov and Lapikova (1995).

Table 1: Relative mobility (Rm) values of peroxidase isozyme as influenced by *H. sativum*

Genotypes	Rm values of peroxidase						
	30 DAS			60 DAS			
	Rm value		No. of bands	Rm value			No. of bands
DWR 28							
a) Healthy	0.35	0.18	2	0.42	-	-	1
b) Inoculated	0.30	0.18	2	0.40	0.18	-	2
PL 670							
a) Healthy	0.38	0.18	2	0.42	0.18	-	2
b) Inoculated	0.31	0.18	2	0.38	0.18	0.16	3
RD 2653							
a) Healthy	0.30	0.16	2	0.34	-	-	1
b) Inoculated	0.30	0.16	2	0.30	0.16	-	2
RD 2508							
a) Healthy	0.30	-	1	0.34	-	-	1
b) Inoculated	0.30	0.16	2	0.30	-	-	1

DAS - Days After Sowing

Polyphenol oxidase is important in the expression of disease resistance mechanism. It was noticed that the activity of polyphenol oxidase was higher in resistant varieties viz., PL 760 and DWR 28. These two varieties expressed more prominent isozyme bands under both healthy and inoculated conditions at both the stages. It means both the varieties had higher inbuilt activity of polyphenol oxidase (Table 2 and Plate 2a, 2b). In contrast the susceptible varieties, RD 2508 and RD 2653 expressed less activity of polyphenol oxidase with less prominent bands when inoculated. The results are in accordance with the results of Luthra *et al* (1988). A sharp increase in PPO and PO activities following infection was observed in all the genotypes, which were largely associated with susceptibility than tolerance. Peroxidase contributes to resistance by oxidation of phenolic compounds and was also reported to be involved in yielding lignin like

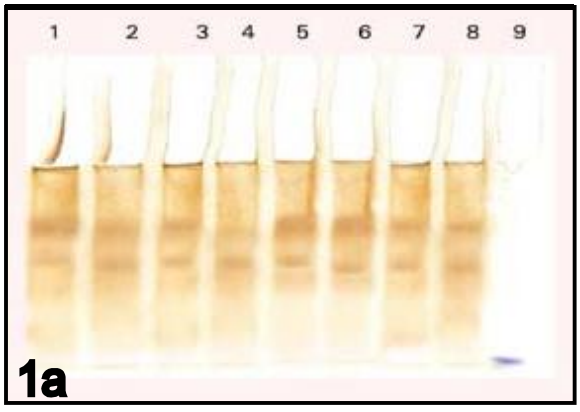

Fig 1a: Isozyme pattern of peroxidase as influenced by *H. sativum* (30 DAS)

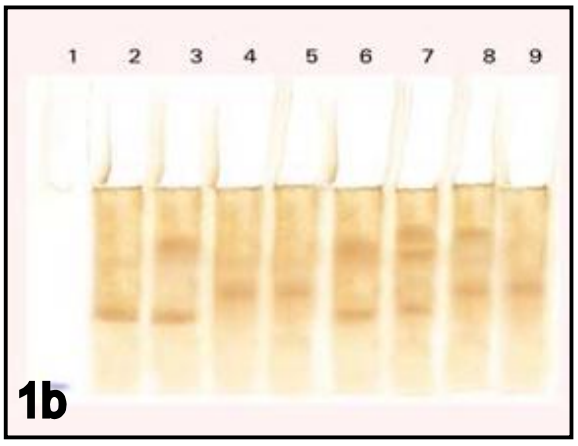

Fig 1b: Isozyme pattern of peroxidase as influenced by *H. sativum* (60 DAS)

Table 2: Relative mobility (Rm) values of polyphenol oxidase isozyme as influenced by *H. sativum*

Genotypes	Rm values of polyphenol oxidase					
	30 DAS			60 DAS		
	Rm value		No. of bands	Rm value		No. of bands
DWR 28						
a) Healthy	0.40	-	1	0.46	0.36	2
b) Inoculated	0.38	0.36	2	0.40	0.30	2
PL 670						
a) Healthy	0.42	-	1	0.46	-	1
b) Inoculated	0.38	0.35	2	0.40	0.30	2
RD 2653						
a) Healthy	0.31	-	1	0.36	-	1
b) Inoculated	0.28	-	1	0.32	-	1
RD 2508						
a) Healthy	0.35	-	1	0.35	-	1
b) Inoculated	0.30	-	1	0.32	-	1

DAS - Days After Sowing

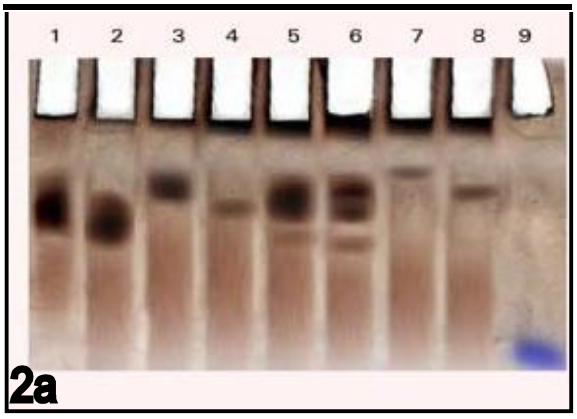

Fig 2a: Isozyme pattern of polyphenol oxidase as influenced by *H. sativum* (30 DAS)

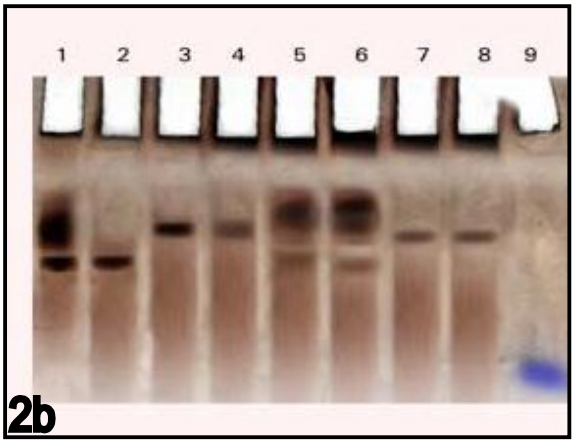

Fig 2b: Isozyme pattern of polyphenol oxidase as influenced by *H. sativum* (60 DAS)

substances. Increased activity of peroxidase upon infection was required for an additional deposition of lignin around the lesions induced by pathogens. The increased activity of PPO has been reported due to either solubalisation of polyphenolases from cellular compounds or activation of latent polyphenol oxidase and also the possibility of its being released by the pathogen cannot be ruled out (Farkas and Kiraly, 1962).

There was reduction in the PAL activity in susceptible genotypes ranging between 13.78 per cent at 60 DAS to 35.09 per cent in the early stage that is at 30 DAS (Table 3). The resistant genotypes had higher PAL activity at the stress condition was due to induced systemic resistance in the genotypes. Which is characteristic of resistant genotype. At initial stage the PAL activity of diseased plant was higher than healthy counter part in resistant genotypes. These results are in agreement with Harms and Terba (1984). However, susceptible plants showed less PAL activity as compared to their healthy counterpart. This difference can be attributed to impaired metabolism of host plant due to destruction of foliage by the pathogen causing decrease in substrate level which in turn reduced enzyme activity. Increase in PAL activity in inoculated resistant genotypes at 30 DAS coincided with corresponding increase in total free phenols. On the other hand, reduced PAL activity in infected leaves coincided with decrease in phenolics. This can be compared with the results of Zuber and Manibhushan Rao (1984). At later stage, the activity of PAL was sharply declined both in resistant and susceptible plants. Inoculated resistant genotypes showed less PAL activity as compared to their healthy counter parts. This

Table 3: Specific activity of Phenylalanine ammonia lyase in leaves of resistant and susceptible barley genotypes as influenced by *H. sativum*

Genotypes	Specific activity of PAL (μ moles cinnamic acid mg^{-1} protein hr^{-1}) 30 DAS			60 DAS		
	Healthy	Inoculated	% increase or decrease over healthy	Healthy	Inoculated	% increase or decrease over healthy
DWR 28	2.432	2.700		0.558	0.527	
PL 670	2.852	2.940		0.560	0.518	
Mean	2.642	2.820	6.31	0.559	0.522	-6.61
RD 2653	1.823	1.760		0.465	0.482	
RD 2508	1.818	1.780		0.449	0.468	
Mean	1.825	1.720	-5.75	0.457	0.475	3.78
% increase or decrease over resistant	-30.92	-39.00		-18.24	-9.00	

was due to decrease in substrate level required by the enzyme for their metabolism at later stage of crop growth. But in contrast, the inoculated susceptible genotypes exhibited high PAL activity as compared to their healthy counter parts. This was due to interaction of pathogen, which culminated in the expression of more PAL activity. This is in agreement with the results of Shiraishi *et al*. (1995).

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