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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.

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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