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Research Article

Genetic evaluation of barley (*Hordeum vulgare* L.) germplasm for resistance components of spot blotch disease

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Abstract
Spot blotch caused by *Bipolaris sorokiniana* is an important fungal disease of Barley in warm humid areas of the world. In present study, 124 genotypes that includes 122 un-adapted germplasm accessions and 2 cultivars of barley were evaluated for three years, to select resistant and susceptible accessions based on five components of spot blotch resistance viz., disease severity, latent period, spore load, number of spots and incubation period. Significant differences were observed among the evaluated accessions for all of the components of resistance. A significant positive correlation was recorded between disease severity, number of spots, and spore load while a significant negative correlation of disease severity was recorded with latent period and incubation period. Multiple regression analysis revealed that number of spots contributed maximum followed by latent period, spore load and incubation period towards the variation in disease severity. Clustering of accessions based on different components identified three groups. Based on the studied components, accessions BCU422, BCU1204 and BCU5092 demonstrated good performance, while BCU711, K603 and RD2506 were the most susceptible to spot blotch pathogen. Identified accessions BCU422, BCU1204 and BCU5092 can be recommended for use in breeding programs that aim to generate barley genotypes resistant to *Bipolaris sorokiniana*.

Key words: Barley, spot blotch, *Bipolaris sorokiniana*, germplasm, disease severity.

Introduction

Barley (*Hordeum vulgare* L.), one of the world's oldest cultivated crops, is currently the fourth most important cereal crop of India. This crop has occupied wide geographic area than any other crop species (Paulitz and Steffenson, 2011). Barley is accepted as a crop having potential to be grown under drought and saline conditions. Besides its multiple uses as feed, food and malt (Jalal and Ahmad, 2011), barley flour are rich in β-glucans, a non-starch polysaccharide with many beneficial health effects (Newton et al., 2011). Spot blotch, a major foliar disease of barley is caused by the fungus *Bipolaris sorokiniana* (Sacc. in Sorok) Shoem. (teleomorph: Cochliobolus sativus (Ito and Kurib.) Drechsl. Ex Dastur). It occurs in the warmer and more humid regions of the world, including North and South America, Europe, Asia, Syria and Australia (Steffenson *et al.,* 1996; Kumar *et al.,* 2002; Arabi and Jawhar, 2007; Tyagi *et al.,* 2008). It reduces yield as well as quality of barley grain (Clark, 1979; Kiesling, 1985; Nutter *et al.,* 1985; Mathre, 1997; Kumar *et al.,* 2002). Temperature more than 25 ^{0}C and relative humidity more than 90% are favourable for the outbreak of spot blotch. Thus, spot blotch is considered to be one of the major threats to barley cultivation under climate change.

Chemical and other control measures are available to manage spot blotch, but these measures are cost ineffective and non eco-friendly. Joshi and Chand (2002) suggested in wheat that cultivar having resistance to spot blotch is most effective and can be easily included in integrated management of spot blotch. In barley a very few resistant lines have been identified and used in breeding programme, resulted a narrow genetic base of spot blotch resistance cultivars (Matus and Hayes, 2002; Condon *et al.,* 2008). Therefore, there is a need to identify new sources of resistance to widen the genetic bases of barley cultivars. Bilgic et al. (2006) reported that spot blotch resistance is partial and controlled by two to three genes. Partial resistance is typically a function of multiple components of resistance that contribute additively to a reduction in the rate of epidemic progress (Parlevliet, 1979). Reports on components of spot blotch in barley are few. Therefore, the aim of this study was to evaluate barley germplasm for spot blotch resistance by using different components of resistance for barley breeding programme.

Material and Methods

Experiment site: The experiment was conducted at agricultural research farm of Banaras Hindu University, Varanasi, India (25˚18'N lat., 83˚03' E long. and 75 m amsl.) for three consecutive years

i.e., 2007-08, 2008-09 and 2009-10. The annual (July-June) rainfall and temperature range (weekly) during 2007-08 was 863.8 mm and 43.9˚C-6.7˚C, in 2008-09 was 781 mm and 42.3˚C-8.9˚C and in 2009-10 was 486.3 mm and 43˚C - 7.1˚C, respectively. The soil type of experimental field was deep alluvial. The experiment was followed by rice crop and conducted under irrigated conditions with recommended basal doses of NPK fertilizers.

Inoculum preparation and inoculation: Aggressive isolates of spot blotch pathogen was obtained from the Department of Mycology and Plant Pathology, Banaras Hindu University, Varanasi. This isolate was purified as suggested by Kumar et al. (2007). The isolate was multiplied on potato dextrose agar medium and its mass culture was produced on sorghum grains. Spot blotch was induced by planting most susceptible variety K603 as a spreader row after every ten germplasm lines. A spore suspension (approximately 10^4 spores mL^{-1}) containing the surfactant Tween 20, was uniformly sprayed by using a hand held automizer at growth stage G 37 (flag leaf emergence) (Zadok et al., 1974), during the evening hours (Joshi *et al.*, 2007a, b). Field was irrigated after inoculation to maintain high humidity to establish the pathogens.

Plant material and Experimental design: A complete randomized block design with two replications was used. Total 122 germplasm accessions received from Directorate of Wheat Research, Karnal along with two checks (K603 and RD2503) were planted in paired row of two meter length, line to line and plant to plant distance were 25 cm and 5 cm respectively, and plot to plot distance was 50 cm. All recommended agronomic practices were followed for expression of genetic potential of each accession.

Data was recorded on following five resistance components of spot blotch disease:

(1) Disease severity: Disease severity for in each genotype was recorded on five randomly tagged plants at three different growth stages (GS) *viz.,* GS 63 (beginning of anthesis to half complete), GS 69 (anthesis complete) and GS 77 (late milking) (Zadoks *et al.,* 1974) using double digit (00 to 99) methods (Saari and Prescott, 1975). First digit (D1) indicates vertical disease progress on plant and second digit (D2) indicates portion of leaf infected by pathogen.

Severity (%) =D1/9×D2/9×100

(2) Latent period: After inoculation, every third day, numbers of spot were counted on flag leaf of five randomly tagged plants till the final spot appeared. Latent period was calculated according to the formula of Parlevliet (1976).

$$A = \sum (Pi - Pi - 1)Tn$$

Where, A=latent period, Pi=per cent of spore appeared on i^{th} day, P_{i-1}=percent of spots appeared on $i\text{-}1^{th}$ day, P_n=per cent of spots appeared on the last day of recording, T_i=days after inoculation.

(3) Number of spots: Number of spots on flag leaf of five randomly tagged plants was counted and total number was divided by the area of flag leaf.

Number of spot on flag leaf/cm^2

$$= \frac{number\ of\ spots\ on\ flag\ leaf}{area\ of\ flag\ leaf}$$

(4) Spore load: Sporulation per spot was measured using the method of Kato and Sasaki (1974). Inoculated leaves bearing sporulating spot were detached to obtained spores numbers. Leaf pieces bearing single spot were taken from flag leaf. The old conidia from the spot was obtained by placing the individual spot in a glass vial with 0.5 ml of water and sealed. Glass vials were incubated for 24 hours at 25^0C then the vials were shaken vigorously to dislodge the conidia. Five spots per leaf were selected randomly examined and total five leaves were sampled. The count of five microscopic slides were considered as one replication. A total three replications were used.

(5) Incubation period: Recorded in days from inoculation to appearance of first spot of spot blotch disease.

Statistical analysis: Analysis of variance (ANOVA) for the each component was performed separately for each year using general linear model (GLM) approach. ANOVA for pooled data was also performed by using mixed model and residual maximum likelihood (REML) method with replication as fixed effect while treatment and year as the random effect. Variance components owing to genotype (Vg) were estimated for each of the years and also as pooled. Best linear unbiased predictors (BLUPs) for combined analysis were worked out for all components of each genotype. Data were analysed using SAS 9.2 statistical software (SAS, 2002).

A matrix of simple correlation coefficients between components of spot blotch resistance were computed (Snedecor and Cochran, 1989). Multiple linear regression and partial coefficient of determination (R^2) was estimated for each disease resistant component (Snedecor and Cochran, 1989) in order to evaluate the relative contribution and to develop the prediction model for disease severity (Y) according to the formula:

Y = a + b1X1 + b2X2 + b3X3 + ...+bnXn

Stepwise multiple linear regression was used according to Draper and Smith (1966) to determine the resistant components accounting for the majority of total variability in disease severity.

Cluster analysis: BLUP data of all components were subjected to hierarchical cluster algorithm

(Ward, 1963) at an R^2 of 0.70 for clustering of accessions.

Principal component analysis (PCA): BLUP data of all components were used for PCA. First and second principal component axes scores were plotted to aid visualization of component differences.

Results and discussion

In all the 3 years and in pooled analysis, both accession and accession × year interaction variances were significant for all traits (Table 1). The combined analysis of variance results revealed a highly significant variation among evaluated accessions.

Results of correlation analysis revealed that all components were significantly correlated ($p < .001$) with disease severity T 2). Spore load and number of spots have positive correlation while latent period and incubation period have negative correlation with disease severity. Neervoort and Parlevliet (1978) suggested that susceptible expression of an accession for one component goes together with susceptible expression for others components. Present study supports the result of previous association study reported by Bashyal *et al.* (2011) and Rehman *et al.* (2011). Present and other association studies suggested that important resistant components to be considered during selection of spot blotch resistant genotypes.

Result of multiple regression indicated that, 89% of the total variation in disease severity could be attributed to these aforementioned 4 components i. e., number of spots, latent period, spore load and incubation period, contributed 65%, 21%, 3% and 0.5% respectively (Table 3). Out of 4 components only 2 *viz*., latent period and spore load contributed 86% of total variation. The overall results reflect the importance of the mentioned components for selection of spot blotch resistance lines in barley breeding programs.

The clustering of 124 genotypes based on BLUP values of all four components grouped the accessions into three clusters (Fig. 2), indicating diversity among the accessions for different components. Majority of accessions in cluster three has lower value for disease severity, number of spots and spore load and higher value for latent period and incubation period; whereas the accessions with higher value of disease severity, number of spots and spore load and lower value for latent period and incubation period were grouped in cluster two. Three accessions (BCU422, BCU1204 and BCU5092) expressed lower value of disease severity, number of spots and spore load and higher value for latent period and incubation period were grouped in cluster three. Three accessions (BCU711, BCU5214 and BCU5216) expressed higher value of disease severity, number of spots and spore load and lower value for latent period and incubation period, along with susceptible check K603 and RD2503 were grouped in cluster one. Cluster two included the accessions have different expression for different components. To determine the patterns of variations and to detect the structure in the relationships between different components of resistance, PCA was carried out (Fig. 1). The first two principal components accounted for 89.9% (PC-1 56.1% and PC-2 33.6%) of the total variation estimated in five components. Factor loading of both principal components determined the relationship with resistant components. PC-1 was related to latent period, incubation period and spore load while; PC-2 was related to disease severity and number of spots.

Result of present study revealed that all the components have significant association with disease severity and could be utilized as selection indices for spot blotch resistant. Among the resistant components latent period and number of spots were most important. Three accessions viz., BCU422, BCU1204 and BCU5092 performed good for all components may be utilise in barley breeding programs aiming development of spot blotch resistant cultivars.

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Table 1. Analysis of variance of components of spot blotch resistance evaluated for three years at BHU, Varanasi

Components	Year 1 σ^2 g	Year 2 σ^2 g	Year 3 σ^2 g	Pooled σ^2 g	σ^2 year	σ^2 g*y
Severity	131.6**	246.08**	221.98**	103.13**	22.45**	96.76**
Latent period	11.61**	11.88**	11.27**	7.78**	<0.001	3.8**
Spore load	50.61**	51.20**	50.67**	47.49**	<0.001	3.34**
No. of spots	94.12**	90.48**	90.27**	88.73**	0.039*	2.86**
Incubation period	8.23**	10.02**	9.23**	6.54**	0.051*	2.88**

*,** significant at $P \leq 0.05$ and $P \leq 0.01$.

Table 2. Simple correlation among components of resistance of barley evaluated for three years at BHU, Varanasi

Components	Disease severity	Latent period	Spore load	Number of spots
Latent period	-0.76**			
Spore load	0.55**	-0.60**		
Number of spots	0.83**	-0.67**	0.54**	
Incubation period	-0.42**	0.49**	-0.26**	-0.29**

** significant at $P \leq 0.01$.

Table 3. Estimated components of spot blotch disease by the multiple linear regression analysis and stepwise multiple linear regression analysis.

components	df	Regression Coefficient (b)	Standard error (SE)	Partial R^2	Model R^2	p-value
Latent period	1	-1.17	0.234	0.21	0.86	<0.0001**
Number of spots	1	0.597	0.05	0.65	0.65	<0.0001**
Spore load	1	0.125	0.090	0.03	.89	0.0422*
Incubation period	1	-0.673	0.371	0.0057	0.895	0.0525*

* and ** significant at 5%, 1% level of probability. Y-intercept (α) = 4.7, SE = 2.08, R^2 = 0.891, Root MSE = 4.30, Adj R^2 = 0.884.

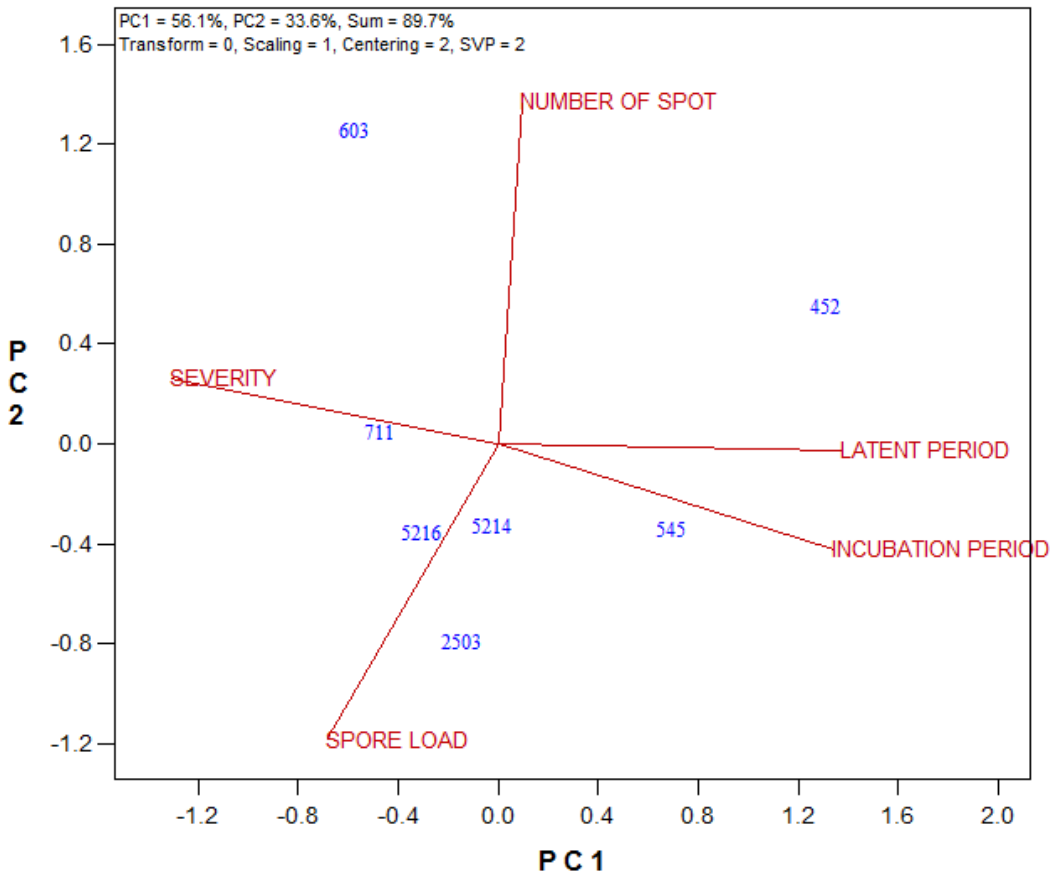

Fig 1. Biplot of first two principal components of barley germplasm for five spot blotch disease resistant components.

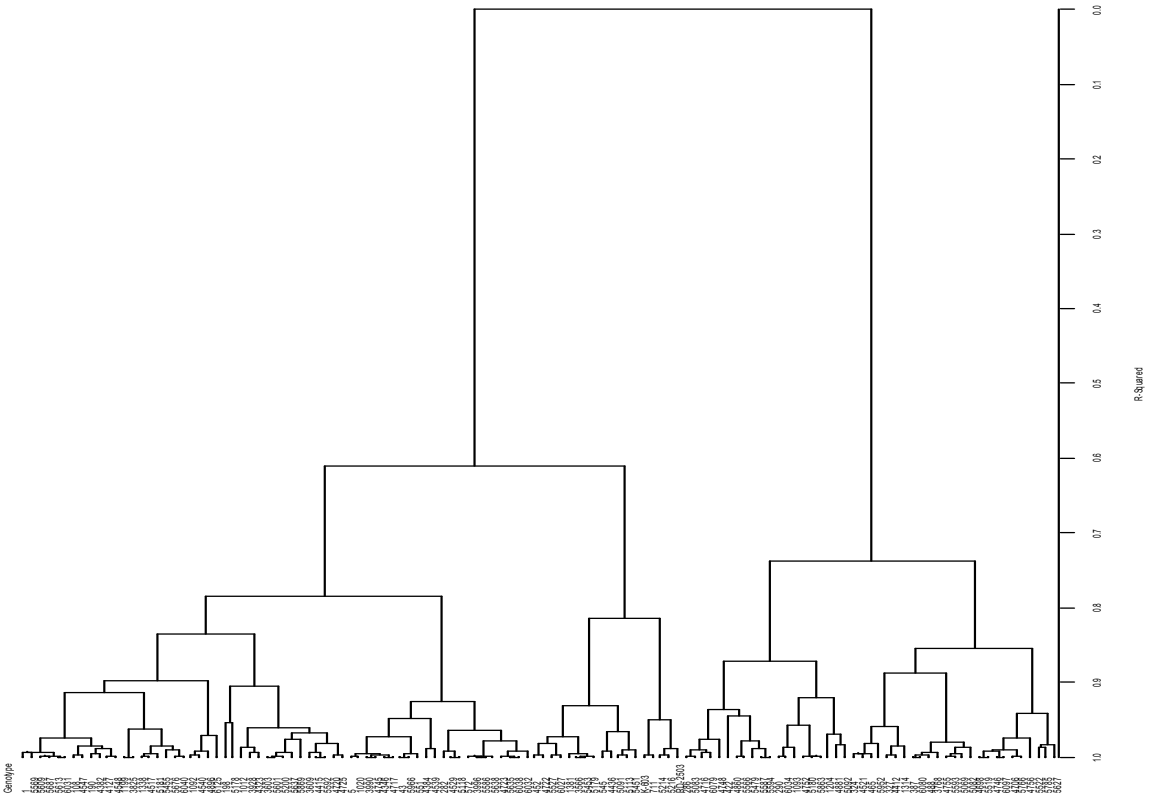

Fig 2: Dendogram generated by Ward's method of cluster analysis for 122 germplasm accessions and 2 checks