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Variation for infection response to *Bipolaris sorokiniana* and identification of trait specific sources in barley (*Hordeum vulgare* L.) germplasm

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Abstract

The aim of present study was to evaluate un-adapted, exotic and indigenous germplasm of barley to identify useful genetic diversity for spot blotch resistance and other related traits to barley breeders. A total 124 genotypes were evaluated for spot blotch resistance and yield components, for three consecutive years at Varanasi, India. Based on Area Under Disease Progress Curve (AUDPC) score of spot blotch, three germplasm accessions (BCU422, BCU1204 and BCU5092) were found resistant to virulent isolate of spot blotch disease. Trait specific accessions were also identified for yield components traits. To meet breeders' requirements, genetically diverse pairs for specific trait were identified, that can be utilized as parents in breeding programs. Principal Component Analysis (PCA) for AUDPC score and yield components showed that , the first three principal components explained 60.30% (27.22, 18.22 and 14.86% by PC1, PC2 and PC3 respectively) of genetic variation. Identified accessions for spot blotch resistance and yield contributing traits appear to be useful for resistance against *Bipolaris sorokiniana* and improvement in genetic potential of grain yield by providing desirable gene(s)/QTL(s) in barley genetic improvement programme.

Keywords: Spot blotch, AUDPC, Barley, *Bipolaris sorokiniana*, Germplasm.
Abbreviations: AUDPC_Area Under Disease Progress Curve, BLUP_Best Linear Unbiased Predictor, PDI_Phenotypic Diversity Index, PCA_Principal Component Analysis, TKW_Thousand Kernal Weight.

Introduction

Barley (*Hordeum vulgare* L.), one of the world's oldest cultivated crops, is currently the fourth most important cereal of India and the World, both in areas and tonnage harvested, after rice, wheat and maize. This crop is the most widely adapted in the world, grown from the Arctic Circle and high mountainous regions to the desert fringes (Harlan, 1976), having potential to be grown under drought and saline conditions (Paulitz and Steffenson, 2011). Besides its multiple uses in feed, food and malt (Al-Tabbal and Al-Fraihat, 2012), it has also been reported that barley flour fractions are rich in β-glucans, a non-starch polysaccharide with many beneficial health effects (Newton et al., 2011). Spot blotch, caused by *Cochliobolus sativus* (Ito and Kurib.) Drechsl. ex Dastur [anamorph: *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem.], has emerged as a serious concern for cultivation of barley, wheat and other cereals in the warmer parts of the world such as north America (Mathre, 1997), south America and several countries of Asia (Kutcher et al., 1994; Knudsen et al., 1995; van Ginkel and Rajaram, 1998; Sharma et al., 2004). It reduces yield as well as quality of barley grain (Clark, 1979; Kiesling, 1985; Nutter et al., 1985; Mathre, 1997; Kumar et al., 2002). It has been shown that high temperature and high relative humidity are most favourable for the outbreak of this pathogen (Aggarwal et al., 2000). Hence, spot blotch is considered as an increasing threat to barley cultivation under climate change (Verma et al., 2012). Therefore, field resistance in new cultivars need pragmatic approach suitable for farmers (Kutcher et al., 1994), especially in developing countries where most farmers are small to marginal and unable to afford costly fungicides and other technologies. The available barley varieties in India lack desired resistance to spot blotch (Verma et al., 2012). In addition, inter-relationship of different agronomical traits with spot blotch resistance in barley is not well elucidated. However, researches in wheat have shown a negative association between resistance and yield contributing traits (Gilchrist and Pfeiffer, 1991; Rosyara et al., 2009). For last one decade breeders have been searching new resistant sources with desirable agronomic traits after the breakdown of durable resistance in barley cultivar NDB112. Although resistance was identified in wild germplasm (Roy et al., 2010), the sources lack desirable agronomic features and hence are not attractive to breeders. In view of the continued threat of the spot blotch, there is an urgent need to identify and develop genetically improved germplasm for spot blotch resistance and agronomic features. In this study, barley germplasm were evaluated with the aim to identify promising sources for spot blotch resistance and important yield contributing traits. Association between resistance and agronomic traits was also investigated to facilitate the future barley breeding programme.

Table 1. Variance components due to genotypes, year and GxY interaction for 8 quantitative traits in 124 accessions of barley germplasm tested for three years at Varanasi, India.

Traits	Year 1 σ^2 g	Year 2 σ^2 g	Year 3 σ^2 g	σ^2 g(pooled)	σ^2 year	σ^2 gxy
AUDPC	405848.39**	349757.43**	419245.78**	30007.83**	6394.38**	11156.45**
Days to 50% heading	124.27**	145.36**	119.28**	38.82**	24.12**	12.87
Plant height (cm)	130.5**	131.5**	146.7**	87.65**	12.56	17.90**
Awn length	2.05**	1.7**	2.2**	0.08**	<0.001	<0.001
No. of kernels/spike	134.32**	142.43**	143**	148.24**	5.38	12.43**
1000-kernel weight	0.45**	0.43**	0.38**	0.31**	0.04	<0.001
Spike length	6.1**	21.65**	5.1**	7.42**	1.15	1.14
Peduncle length	31.4**	20.8**	17.5**	16.5**	1.52	11.15**

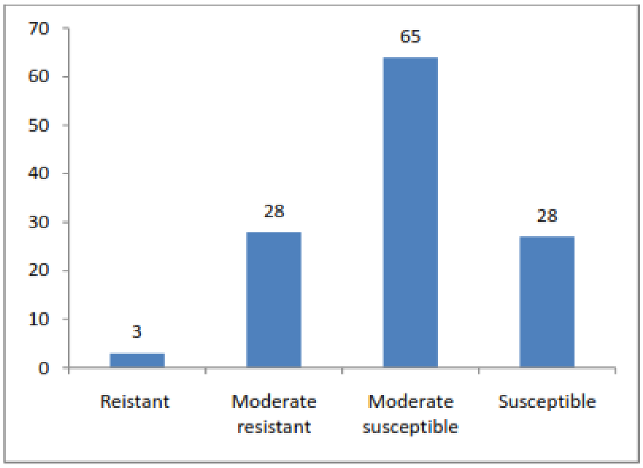

Fig1. Frequency distribution of 124 barely accessions in different groups based on AUDPC score.

Results

GLM analysis of each year and mixed analysis of pooled data of the germplasm indicated significant genotypic variation for resistance and agronomic traits across years and on pooled basis (Table 1). The year variance was significant for Area Under Disease Progress Curve (AUDPC) and days to 50% heading (DTH) while genotype-environment interaction was significant for AUDPC, plant height, number of kernels/spike and peduncle length (Table 1).

Classification of genotypes based on AUDPC

A continuum of genotypic reactions to the virulent isolate of *Bipolaris sorokiniana* from resistant to susceptible was observed (Fig. 1). Based on BLUP value of AUDPC, genotypes having value <1000, 1001-1500, 1501-2000 and >2000 were classified as resistant, moderately resistant, moderately susceptible and susceptible, respectively. Out of 124 genotypes, 3 were in resistant group, 28 moderately resistant, 65 moderately susceptible and 28 were in susceptible group.

Identification of trait specific accessions

Trait specific accessions were identified for spot blotch resistance and important yield contributing traits (Table 2). Three new sources of resistance, BCU422, BCU1204 and BCU5092, were identified. The mean AUDPC score of these three genotypes were 892, 974 and 1020, respectively. The three other germplasm accessions, BCU4412 (60.3), BCU3990 (57.9) and BCU4346 (56.1), had significantly ($P \leq 0.01$) higher number of kernels/spike compared to the best check K603 (52.0). Likewise, five germplasm accessions, viz. BCU4323, BCU4756, BCU3996, BCU4346 and BCU1199 had significantly higher 1000 kernel weight (TKW) than the check variety. Six accessions (BCU5519, BCU6038, BCU3990, BCU5522, BCU4382, BCU5637) with early DTH (<72 days) were also identified. The most diverse pairs of accessions using phenotypic diversity index (PDI) were identified (Table 3). For spot blotch resistance, maximum diversity was shown by BCU5092-BCU387 (0.52), BCU422-BCU387 (0.46) and BCU1204-BCU4384 (0.43) while for number of kernels/spike, it was shown by BCU4412-BCU5638 (0.57), BCU4346-BCU387 (0.56) and BCU3990-BCU387 (0.46). Likewise, diverse pairs for early DTH were BCU3990-BCU387, BCU6038-BCU4436 and BCU5519-BCU5638 having PDI of 0.46, 0.43 and 0.42, respectively.

Correlation analysis

The majority of agro-morphological traits showed very low (positive or negative), non-significant (p>0.05) association with AUDPC (Table 4). DTH, plant height, awn length and spike length showed non-significant (p>0.05) negative correlation with AUDPC while peduncle length and number of kernels/spike showed non-significant (p>0.05) positive correlation. However, AUDPC showed significant (p<0.05) negative correlation with TKW.

Principal component analysis (PCA)

The PCA of germplasm showed that first three components had eigen value more than one and contributed 60.3% of the total variation (Table 5). The most effective traits as the first component were spike length, peduncle length, awn length, plant height and number of kernels/spike, and were loaded

Table 2. Trait specific genotypes of barley identified from 124 accessions tested for three years at Varanasi, India

Specific traits	Identified genotypes
Spot blotch resistant	BCU422, BCU1204, BCU5092
No. of kernels/spike	BCU4412 (60.28), BCU3990 (59.73), BCU4346 (57.06), BCU5069 (56.71), BCU4529 (54.58)
1000-kernel weight	BCU4323 (42.43) , BCU4756 (42.00), BCU3996 (40.50), BCU 4346 (40.10), BCU1199 (39.00)
Days to 50% heading	BCU5519 (69.67), BCU6038 (70.33), BCU3990 (71.00), BCU5522 (71), BCU4382 (71.08), BCU5637 (71.33)

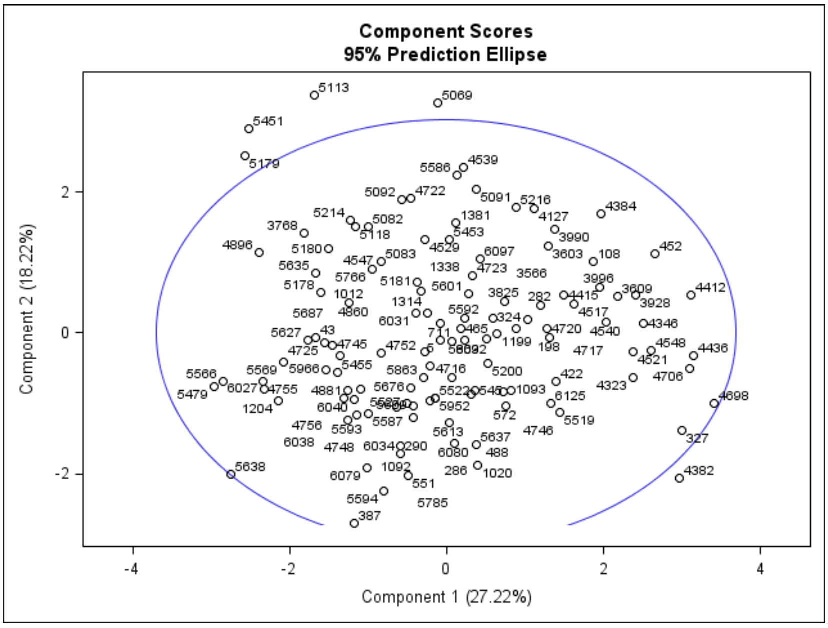

Fig 2. Score plot of Principal component 1 and principal component 2 of the 124 accessions of barley tested for three years at Varanasi, India.

positive. For the second component, peduncle length and number of kernels/spike also loaded positive while awn length, TKW loaded negative, had the effective influence. AUDPC, DTH and plant height had a significant influence on the third component. The result of biplot analysis (Fig. 2) revealed that diverse accessions (BCU387, BCU4384, BCU5638 and BCU4436) were present in the periphery of the circle. This indicated diverseness of the selected accessions and support results obtained by PDI.

Discussion

The value of plant genetic resources is realised only when each and every germplasm line is characterised for relevant traits to reveal new gene combinations for their use in crop improvement (Upadhyaya et al., 2010). Further, an association study on agro-morphological traits with spot blotch resistance will provide a roadmap in making selection strategy. In India, spot blotch is a major threat to barley crop and prevalent varieties do not have adequate resistance (Verma et al., 2012). Potential Resistance sources have been identified in wild germplasm lines (Roy et al., 2010). But, these wild sources possessed many undesirable traits and can reduce agronomic performance of breeding populations if used in crossing program. In the present study, 124 germplasm lines were evaluated in the eastern Gangatic plains (EGP) of India. This region is characterized by high temperature and high humidity, and spot blotch and terminal heat stress are major problems. Germplasm lines showed variation for spot blotch score and morpho-agronomic traits. Superior lines were identified for specific traits with resistance to spot blotch that are likely to serve as new sources of variation in barley improvement program. Based on PDI, the most diverse pairs of accessions were identified for different traits. The inclusion of diverse germplasm in the hybridisation programs might increase the dominance effect and epistatic variations controlling quantitative traits (Halward and Wynne, 1991). These would also result to extend segregation for different traits and in obtaining useful recombinants/transgressive segregants in the subsequent generations. Six rows barley cultivars having resistant to spot blotch are thought to carry resistance source of NDB112 (Steffenson et al., 1996). However, the new pathotype of *B. sorokiniana* is reported to have become virulent against this cultivar and its derivative and resulted into widespread epidemics (Ghazvini and Tekauz, 2007; Ghazvini and Tekauz, 2008). Since spot blotch is highly influenced by environment and the resistance is quantitative (Cohen et al., 1969; Kutcher et al., 1994), achieving higher level of resistance in barely cultivars is difficult (Wilcoxson et al., 1990; Bailey and Wolf, 1994). Therefore, screening of larger number of germplasm is needed to identify new resistant sources (Roy et al., 2010). In the present study three (2.42% of the total evaluated germplasm) new sources (BCU422, BCU1204 and BCU5092) of resistance were identified. Germplasm lines BCU422 and BCU1204 are exotics, introduced from ICARDA and BCU5092 is an indigenous line. Although none of the identified genotypes were immune, they showed low AUDPC score when compared with resistant checks. The line BCU422 was very early (72 days to 50% heading), long spike length (19.33cm), higher number of kernels/spike (50.08), good TKW (41.24gm) and

Table 3. Trait specific diverse accessions of barley identified from 124 accessions tested for three years at Varanasi, India

Trait	Pair of accessions showing maximum diversity	Diversity index
Spot blotch resistance	BCU5092- BCU387	0.52
	BCU 422- BCU387	0.46
	BCU1204-BCU4384	0.43
No. of kernels/spike	BCU4412-BCU5638	0.57
	BCU4346-BCU387	0.56
	BCU3990-BCU387	0.46
Days to 50% heading	BCU3990-BCU387	0.46
	BCU6038-BCU4436	0.43
	BCU5519-BCU5638	0.42

was short statured (72.11 cm) with no lodging. This line could be suitable for incorporation in breeding programme aiming the development of high yielding, early maturing resistant cultivars for south Asia. Early maturity will help in escaping terminal heat stress which causes significant yield losses to barley. Resistant line BCU5092 showed taller plant height (93.77 cm). Such lines generally excel in their capacity to support kernel growth by stem reserve mobilization and contribute to grain yield (Blum et al., 1994) and biomass provided by stem is strong enough to avoid lodging. Evaluation of cultivated barley germplasm lines in another study also revealed a low percentage (5.8%) of spot blotch resistance (Fetch et al., 2008). Evaluations of wild germplasm revealed many potential sources of spot blotch resistance that may be useful for cultivated barley (Roy et al., 2010). However, the potential for exploiting wild barley as a source of novel genes for crop improvement essentially remains untapped. This may be attributed largely to the problems with linkage drag and the time and risk associated with using wild barley in traditional breeding programs that might reduce the agronomic fitness (Jun et al., 2011). Resistant sources identified in this study performed consistently over the years better than the two resistant checks. The trait having the most dominant effect on fitting a plant to its environment for maximum productivity is the appropriate phenological development (Muchow et al., 1994; Passioura, 1996; Richards, 1996). A short grain filling period followed by earlier maturity in the adapted mediterranean germplasm group under terminal drought is genetically associated with high yield. Earliness allows the plants to escape extreme moisture stress, which is often accompanied by heat stress (Mohammadi et al., 2005). Days to heading is considered important for adaptation of barley cultivars in the EGP of India and neighbouring countries where crops get much lesser number of days owing to late planting caused by unfavourable cropping systems and insufficient mechanization. Identified germplasm accessions for early heading can help in escaping terminal heat stress that is quite frequent during early (central and Peninsular India) to mid March (north eastern and north western south Asia). Number of kernels/spike and TKW displayed high phenotypic and genotypic association with grain yield; improving them could result in higher grain yield (Pal et al., 2010; Al-Tabbal and Al-Fraihat, 2012). Birol and Necmettin (2011) suggested that TKW can function as the most important criterion in yield improvement. This study identified three superior accessions for number of kernels/spike and five for TKW. Sarkar et al. (2008) reported that higher TKW has significant correlation with hot water extract, the most important malt quality trait. Identified germplasm accessions may be utilized in barley breeding programme for improvement in grain yield and malting quality of barley. Our study showed weak negative correlation between AUDPC and maturity. In wheat, a negative correlation has been observed between these two traits (Dubin et al., 1998; Mahto, 2001; Rosyara et al., 2009). Both early maturing resistant genotypes and late maturing susceptible genotypes were observed. The results further support possibility to develop early maturing resistant genotypes for Indo-Gangatic plains of south Asia. Furthermore, identification of diverse trait-specific germplasm accessions for spot blotch resistance and yield contributing traits would provide an opportunity to the breeders to use the diverse genotypes in their breeding program, which may facilitate the barley improvement endeavours across the globe.

Materials 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 respectively; in 2008-09 it was 781 mm and 42.3˚C-8.9˚C respectively and in 2009-10 486.3 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 (120:40:40) fertilizers. Research centre is a hot spot of spot blotch and a barley network centre of All India Coordinated Wheat and Barley Improvement Project with specific objective to evaluate barley lines for spot blotch resistance (Verma et al., 2012).

Plant material and experimental design

A total of 124 accessions (Supplementary Table 1) were drill sown in paired rows of four meter length. Line to line and plot to plot distance was 25 and 50 cm respectively. A split plot design with two replications was used. The main plot was genotype while subplots were control and artificial inoculation by virulent isolate of *Bipolaris sorokiniana*. Control plots were sprayed with fungicide (Propiconazole) to ensure full prevention from the spot blotch pathogen.

Isolation and Multiplication of pathogen

The most aggressive pathogen of spot blotch was obtained from the Department of Mycology and Plant Pathology, Banaras Hindu University, Varanasi. This isolate was purified, multiplied on potato dextrose agar (PDA) medium and the mass culture was produced on sorghum grains according to Kumar et al. (2007).

Artificial inoculation of germplasm

Spot blotch was induced by planting most susceptible variety K603 as a spreader row after every tenth plot of germplasm. A spore suspension (approximately 10^4 spores ml^{-1}) containing the surfactant (Tween 20), was uniformly sprayed by a hand held automizer at flag leaf emergence (GS 37) (Zadoks et al., 1974) during the evening hours (Joshi et al.,

Table 4. Phenotypic correlation among 124 germplasm lines tested for three years at, Varanasi, India.

Traits	AUDPC	DTF	PHT	AL	SL	PL	KS
DTF	-0.01						
PHT	-0.05	-0.07					
AL	-0.10	-0.20*	0.23*				
SL	-0.08	-0.22*	0.22*	0.59**			
PL	0.13	-0.23*	0.52**	0.11	0.22*		
KS	0.11	0.02	0.14	0.00	0.18*	0.33**	
TKW	-0.18*	-0.07	-0.03	0.16	0.17	0.00	-0.03

(AUDPC-Area Under Disease Progress Curve, DTH-Days to 50% Heading, PHT-Plant Height, AL-Awn Length, SL-Spike Length, PL-Peduncle Length, KS-Number of Kernels/Spike, TKW-1000 Kernel Weight)

Table 5. Principal components for AUDPC and yield contributing traits of 124 accessions of barley tested for three years at Varanasi, India.

Traits	PC1	PC2	PC3
AUDPC	0.046	0.283	-0.628
Days to 50% heading	-0.276	0.184	0.613
Plant height	0.404	0.254	0.447
Awn length	0.421	-0.448	0.073
Spike length	0.465	-0.372	0.041
Peduncle length	0.464	0.398	0.037
No. of kernels/spike	0.376	0.386	-0.117
1000-kernel weight	0.079	-0.415	-0.071
Variation explained (%)	27.22	18.22	14.86

2007a,b). Field was irrigated after inoculation to maintain high humidity to promote pathogenesis. Cultivars K603 and RD2503 were used as susceptible controls while BCU5592 and BCU5613 as resistant control.

Data recording

Disease analysis

Disease severity- The spot blotch severity was scored on each germplasm line of artificially inoculated plots by using Saari and Prescott (1975) double digit (00 to 99) method. First digit (D_1) indicates vertical disease progress on plant and second digit (D_2) indicates portion of leaf infected by pathogen.
Severity (%) $=D_1/9\times D_2/9\times 100$
Area Under Disease Progress Curve (AUDPC) was based on the estimates of disease severity at different growth stages.

$$\text{AUDPC} = \sum_{i=1}^{n} \left(\frac{Y_i + Y(i+1)}{2} \right) \left(t\,(i+1) - ti \right)$$

Where, Yi = disease level at time *ti*; *t* (*i* + 1) - *ti* = time (days) between two disease scores; *n* = number of dates on which spot blotch was recorded.

Agronomical traits

The yield contributing traits recorded on control plots, were number of kernels/spike, days to 50% heading, plant height (cm), peduncle length (cm), spike length (cm), awn length (cm), number of kernels/spike and 1000-kernel weight (g).
Number of kernels/spike was measured on 50 tiller basis while days to 50% heading was taken on plot basis. The remaining traits were estimated on the basis of mean of five random plants from each replication.

Statistical analysis

Analysis of variance (ANOVA) for the traits was performed separately for each year using SAS 9.2 statistical software (SAS Institute, 2002). The general linear model (GLM) approach was used. 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 the traits of each accession. BLUP value of AUDPC score were used to group the germplasm accessions into resistant, moderate resistant, moderate susceptible and susceptible. Traits specific accessions were identified for resistance to spot blotch, days to 50% heading, number of kernels/spike and 1000-kernel weight. A phenotypic distance matrix was obtained by calculating differences between trait specific accessions and total germplasm lines for all the traits. The PDI was calculated by averaging all the differences in the phenotypic values for each trait divided by their respective range (Upadhyaya, et al., 2010). Based on PDI, the most diverse pairs of accessions were identified. Correlations were calculated on BLUP value of each trait of germplasm lines. Principal component analysis (PCA) was done to investigate the importance of different traits in explaining multivariate polymorphism in barley accessions. BLUP data of all characters were standardized to a mean of zero and variance of one. First and second principal component axes scores were plotted to aid visualization of selected diverse genotypes in trait specific pair of accessions.

Conclusion

The result of present study revealed that barley germplasm has sufficient variability for agronomic traits and infection response to spot blotch pathogen. Trait specific accessions for spot blotch resistance, number of kernels/spike, TKW and days to heading identified and most diverse pairing accessions for specific traits were categorized. Identified accessions may be utilized in barley breeding program.

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