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Research Article

RELATIONSHIP OF AGRO-PHENOLOGICAL TRAITS WITH RESISTANCE TO SPOT BLOTCH IN BARLEY (*Hordeum vulgare* L)

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Abstract- Spot blotch (SB), caused by *Bipolaris sorokiniana*, is one of the most destructive diseases of barley (*Hordeum vulgare*) especially in tropical humid and semi-humid barley growing areas of the world. Agro-phenological traits; plant height (PHT) and days to 50% heading (DTH) are important in barley life cycle and development of superior cultivars. In the present study, association of SB with agro-phenological traits were investigated in 124 germplasm lines and two F3 and F4 populations derived from the two different crosses under SB prone environment. Results showed significant variation for PHT, DTH and SB resistance in the germplasm, parental lines and its F_3 and F_4 populations. Transgressive segregates were observed among the lines for PHT, DTH and SB severity. The t-test's statistical significance of homozygous resistant and homozygous susceptible families selected from F_3 and F_4 populations of both crosses indicates that the differences for mean values of PHT and DTH is not significant however these families showed a wide range of PHT and DTH. The correlation coefficients for AUDPC versus PHT and DTH were weak, *i.e.*, 0.00 to 0.22 indicating that spot blotch resistance was independent of PHT and DTH in germplasm and segregating generations. The basic information generated in present study could help the breeders for development of SB resistant varieties for different geographic regions and different uses.

Key words- Dual purpose, Disease severity, Geographic regions, Spot blotch

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Introduction

Ground Barley is a multipurpose crop used as food, feed and malt. High β-Glucanse activity in barley is very much helpful in lowering the risk of cardio-vascular diseases [1]. It also has capacity to tolerate severe drought and salinity stress [2]. Spot blotch caused by *Bipolaris sorokiniana* (Sacc.) Shoem. (syn. *Helminthosporium sativum*, teleomorph *Cochliobolous sativus*) is an important barley disease in warmer and humid regions [3,4], causing high yield loss with reduced milling and malting quality of grain [5-9]. Warm temperature and high relative humidity being favourable for the disease outbreak, changing climatic conditions has also posed serious threat to barley cultivation [10]. Barley plant type is specific for specific purposes. Development of suitable plant type is essential for exploiting environmental condition prevailing in particular niche as well as its specific uses. Plant height and days to flowering are the important traits to develop an ideal plant type suitable for a specific environment [11]. Variation in flowering time enables plants to optimize the use of resources available in an environment in which they grow [12]. Plant height and heading date also associated with grain yield [13]. Plant height is also positively associated with susceptibility to lodging, reduced grain yield and quality [14]. Plant height is considered most important and use of semi-dwarf genes has greatly improved barley yields by developing lodging resistant genotypes also increased harvest index [15]. The timing of heading is a major trait for the adaptation of cultivars to specific areas, for its best performance. Early heading facilitates long grain-filling period with active photosynthetic components. The post anthesis prolonged photosynthesis in barley improves grain filling and higher yield [16]. Plant height and days to heading are controlled by independent genetic loci [17, 18].

Development of spot blotch resistant cultivars with different allelic combinations of plant height and days to heading requires an understanding of association among these traits. Association of different agro-physio-phenological traits with resistance to spot blotch in wheat were studied earlier [19, 20]. However, no reports on the association of agro-phenological traits to spot blotch resistance in barley is available. Therefore, present study was undertaken to determine the relationship between spot blotch resistance and agro-phenological traits in germplasm and segregating generations. This information will be helpful to barley breeders in developing countries where conventional breeding is practiced.

Materials and methods

Plant materials and location

A total of 124 germplasm lines were evaluated for SB severity and agro-phenological traits. Two different crosses 'BCU5092 × K603' and 'BCU327 × RD2503' were made between resistant and susceptible gentoypes differing for plant height and days 50% heading [Table-1]. Individual plant selection of each line/cultivar was used as parents for crossing. Crosses were made in 2007 at Agricultural Research Farm, Banaras Hindu University (BHU), Varanasi, India (25˚15.29'N latitude, 82˚ 59.01' E longitude and 75.5 m amsl.). The progenies of crosses were advanced to F_2, F_3 and F_4 generations. To maintain the heterozygosity within progeny line of F_4 generations, single seed from 50-60 plants of each F_3 families was harvested and bulked. F_1 and F_3 plants were grown in an off-season nursery at Wellington, Tamil Nadu, India (11.33° N latitude; 76.80° E longitudes, 1854 m above mean sea level) in 2008 and 2009 respectively, to produce F_2 and F_4 seeds.

Around 300 families of F_3 and F_4 were evaluated under induced epiphytotic conditions along with parents during winter crop season 2009-10.

Multiplication of pathogen and creation of artificial epiphytotic condition

The most aggressive pathogen of spot blotch (WPM-29 NCBI deposition number KF358698; culture accession number MTCC11883) was multiplied on sorghum grains [21, 22]. Two susceptible cultivars K603 and RD2503 were planted after every ten plots of F_3 and F_4 generations. Sowing was done during second fourth night of November in order to coincide the post anthesis stage to warm temperature and high humidity in March.

Statistical analysis

Scoring of SB: Severity of spot blotch was recorded in each line on ten randomly tagged plants in the field at three different growth stages (GS) *viz*., GS 63-beginning of anthesis to half complete, GS 69-anthesis complete and GS 77-late milking [23] following the double-digit scale (D1D2, 00-99) [24]. The first digit (D1) indicates vertical disease progress on the plant and second digit (D2) measured spread of disease on the leaf. Disease Severity (%) =D1/9×D2/9×100

Area Under Disease Progress Curve (AUDPC) was calculated using the following formula [25]

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

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

F_3 and F_4 lines were grouped into one of the three classes (1) homozygous, similar to parent P_1, (2) segregating or homozygous different from parental lines, and (3) homozygous, similar to parent P_2 [26]. For grouping lines into category (1) and (3), two criteria were followed. First, the line mean did not differ significantly from the means of the respective parents according to the t-test (p = 0.05). Second, none of the plants within such a line deviated more than twice the standard deviation of the respective parent [27].

Assessment of agro-phenological traits

Plant height and days to 50% heading were assessed for all the tagged plants in germplasm and both F_3 and F_4 generations. Plant height was measured on dough stage (GS 87), while days to heading were counted as the number of days from sowing to 50% flowering (GS 65).

Correlations analysis

Plant height, DTH and AUDPC values of homozygous resistant lines and homozygous susceptible lines obtained in F_3, F_4 generations and germplasm were used to estimate correlations using SAS software [28].

Results

Mean, Range and Coefficient of variation of 124 germplasm lines evaluated for PHT, DTH and AUDPC showed sufficient variability for traits studied [Table-2]. Resistant and susceptible parental lines selected for development of segregating generation showed significant variation (P<0.01) for PHT and DTH [Table-3]. Segregating generations (F_3 and F_4) derived from resistant × susceptible crosses were evaluated for PHT and DTH apart from AUDPC and homozygous resistant and homozygous susceptible families were selected. In the cross between 'BCU327 × RD2503' the selected homozygous resistant families exhibited wide variation in PHT (59.24-98.06 cm) and DTH (62.88-74.16 days) similarly homozygous susceptible families also exhibited wide variation in PHT (57.71-95.86 cm) and DTH (61.84-72.28 days). In the cross between 'BCU5092 × K603' the selected resistant families exhibited wide variation in height (62.80-93.20 cm) and DTH (68.50-90.00 days) similarly homozygous susceptible families also exhibited wide variation in PHT (65.50-94.75 cm) and DTH (75.00-88.89 days). In the paired t test analysis, resistant homozygous families and susceptible homozygous families showed no-significant differences for PHT and DTH [Table-4] Segregating generations derived in both crosses showed transgressive segregants for all traits *viz*., PHT, DTH and spot blotch severity. Out of 124 germplasm lines, 9 selected resistant lines showed wide variation for PHT (60.78-93.24 cm) and DTH (71.08-86.17 days) similarly 15 susceptible lines also showed variation for PHT (61.00-91.89 cm) and DTH (72.00-83.83 days). However, t test analysis showed no-significant differences for PHT and DTH between selected resistant and susceptible lines [Table-4]. The PHT and DTH showed very low (positive or negative), non-significant (p>0.05) correlation with spot blotch severity in segregating generation as well as germplasm lines [Table-5].

Table-1 *Timeline activities of generation development and screening against spot blotch (SB), plant height (PHT), days to 50% heading (DTH)*

Year	Plant material	Season/location	Action/ Data recorded
2007-08	Germplasm	Winter Season / BHU†	Parent selection and hybridization/ SB, PHT, DTH
	F_1generation	Off-season / Wellington	Harvesting and bulking of seed
2008-09	F_2 generation	Winter Season / BHU†	>250 plants of each cross planted and harvested separately
	F_3 generation	Off-season / Wellington	Progeny lines of F2 plants (half seed) planted and single plant from each line harvested separately
2009-2010	Germplasm, Parents, F_3 and F_4 generation	Winter Season / BHU†	Progeny lines of F_2 (remaining half seed) and F_3 plants along with parents and germplasm planted/ SB, PHT, DTH

Table-2 *Descriptive statistics for the spot blotch severity and agronomic characters of the 124 Barley genotypes*

	AUDPC	*Days to 50% Heading*	*Plant height*
Mean±SE	*1770.14±34.99*	*78.59±0.40*	*76.28±0.73*
CV (%)	*22.01*	*5.61*	*10.72*
Range	*829.98-3108.08*	*69.67-88.83*	*55.89-93.78*
Confidence Level(95.0%)	*69.27*	*0.78*	*1.45*

Table-3 *Mean values of morphological traits and response to spot blotch progress of parents included in association study*

Parents	Plant height (cm)	Days to 50% heading	Response to spot blotch
BCU5092	96.00	87	Resistant
K-603	83.40	72	Susceptible
t cal and probability	7.40, <.01	11.55, <.01	
BCU327	85.00	71	Resistant
RD2503	66.90	64	Susceptible
t cal and probability	6.84, <.01	13.47, <.01	

Table-5 *Correlation analysis of Spot blotch severity and agronomic traits*

Cross/germplasm	Generation	AUDPC*DTH	AUDPC*PHT	DTH*PHT
BCU5092×K603	F3	-0.21	0.13	0.23
	F4	-0.03	0.22	0.46
BCU327×RD2503	F3	0.16	-0.06	0.18
	F4	0.09	-0.17	0.09
Germplasm		-0.12	0.00	0.02

Discussion

A successful breeding program is expected to generate genetic gain in yield component and resistance to biotic and abiotic stresses. Enhancement in yield in most situations is more effectively fulfilled on the basis of performance of yield components, which are closely associated with grain yield [29]. Genetic improvement in yield and resistance to biotic and abiotic stresses being the prime goals of a breeder for development of cultivar for a particular region and cropping system.

Table-4 *Plant height, days to 50% heading and Area Under Disease Progress Curve (AUDPC) in homozygous resistant and homozygous susceptible progeny rows of F_3 and F_4 generations and selected resistant and susceptible germplasm lines*

Cross/generations	No of families	Homozygous	Plant height (cm)		Days to 50% heading		AUDPC	
			Mean	Range	Mean	Range	Mean	Range
BCU327×RD2503/F_4	73	Resistant	75.39±0.63	62.62-86.33	68.82±1.01	63.28-74.16	378.78±3.84	302.18-476.26
	25	Susceptible	73.05±1.34	57.71-87.40	67.68±0.91	61.84-71.82	988.80±10.18	893.33-1148.68
t cal and probability			1.76, 0.08		0.63, 0.53		69.01, <0.01	
BCU327×RD2503/F_3	56	Resistant	85.89±1.17	59.24-98.06	69.16±0.92	62.88-73.18	396.29±4.38	311.11-488.89
	17	Susceptible	86.31±1.20	62.00-95.86	66.98±1.61	62.18-72.28	1038.89±11.16	933.33-1244.44
t cal and probability			0.19, 0.85		1.15, 0.25		64.13, <0.01	
BCU5092×K603/F_4	22	Resistant	78.77±0.98	62.80-87.50	82.50±1.32	75.50-90.00	334.75±5.82	217.78-444.44
	10	Susceptible	81.99±1.88	65.50-94.75	80.94±1.97	75.00-88.89	1324.31±14.63	1088.89-1462.22
t cal and probability			6.87, <0.01		0.66, 0.51		76.06, <0.01	
BCU5092×K603/F_3	21	Resistant	83.06±1.77	74.90-93.20	79.50±1.25	68.50-90.00	241.08±7.9	183.20-358.20
	9	Susceptible	81.34±1.87	75.87-91.50	78.88±1.09	75.00-85.50	1062.99±35.03	898.33-1213.33
t cal and probability			0.57, 0.56		0.30, 0.76		32.25, <0.01	
Germplasm	9	Resistant	75.91±2.26	60.78-93.78	81.03±1.15	71.08-86.17	448.75±5.57	329.98-485.57
	15	Susceptible	77.08±2.49	61.00-91.89	78.47±0.97	72.00-83.83	1008.84±11.99	893.71-1106.08
t cal and probability			0.34, 0.73		1.70, 0.09		34.60, <0.01	

Spot blotch resistance, plant height and days to 50% heading are the important agronomic traits to develop a plant type suitable for barley growing region [30]. *Bipolaris sorokiniana* is a weak parasite [31], first colonizes older leaves close to the ground or stressed tissues and progress from lower to upper leaves [32]. Taller genotypes may escape disease due to greater internodes length and slower movement of spores to the upper leaves [31]. Joshi, *et al*., (2002) suggested that scoring for disease severity should be combined with growth stage that will help in effective selection of resistance genotypes in the segregating generations. In the present study disease escape by tall and late maturing genotypes was avoid by artificial inoculation with aggressive strain of SB at different growth stages and data was recorded on specific growth stages. Plant height and DTH showed non-significant association in all segregating generations of both crosses and germplasm. Independent association of PHT, DTH and other traits in barley were also reported earlier [17, 18, 33]. Joshi, *et al*., (2002) reported independent segregation between plant height, days to maturity and spot blotch severity in wheat. The present study suggests that a genetic association of spot blotch resistance with plant height and days to 50% heading is independent, and it is possible to find resistant plants that are tall/dwarf and early/late. This was revealed by, (i) the presence of resistance in both tall and dwarf as well as early and late segregates from crosses, (ii) homozygous resistant and susceptible families obtained in the F_3 and F_4 of crosses showing significant differences for AUDPC but not for plant height and days 50% maturity, and (iii) correlations of plant height and days to 50% heading with disease severity is non-significant. In segregating generations of both crosses we observed the appearance of tall and dwarf or late and early progeny among the homozygous resistant lines showed that resistance was independent of plant height and days to heading. Therefore, it could be possible to develop resistant genotype in both dwarf and tall plants. Similarly, for days to heading a wide variation was observed in both resistant and susceptible group.

Conclusion

Barley is a multipurpose crop with resistance to abiotic stresses. However, it is much affected by spot blotch disease especially in tropical and subtropical regions of world. Genetics of spot blotch resistance is known [27]. However, to develop resistant cultivars for a particular region and cropping system barley breeders require an understanding of association between spot blotch resistance and agronomic traits. In present study association analysis of spot blotch resistance with PHT and DTH showed that these traits are independent. This information could help the barley breeders for development of SB resistant varieties for different geographic regions and different uses. Barley has the potential to be utilised as a green fodder and dual purpose as a food-feed crop under water scarcity conditions in drier parts of Northern plains and hilly regions of India, as it is very fast-growing crop with high biomass in the early stages and requires less water and can grow on poor to marginal soils [34]. Cultivars K603 and RD2503 utilized in crossing programme are popular high yielding grain type varieties but susceptible to spot blotch disease [35]. In present study these cultivars were crossed with resistant, late maturing and tall height germplasm lines. In segregating generations, we observed transgressive segregants for AUDPC, days to 50% heading and plant height in all possible combinations. Tall height and late flowering barley varieties tend to give high dry matter content could be utilized for dual green fodder and grain purposes [36]. These potential transgressive segregants could be utilize for development of spot blotch resistant pure lines having different combinations of plant height and days to 50% heading for development of grain and dual purpose varieties for different barley growing regions of country. Resistant pure lines with early heading and medium height must be evaluated for grain purpose in northern arid regions and late heading and tall for dual food and feed purpose for hilly regions.

Application of research: The result of this study showed that spot blotch resistance in barley are independent of plant height and days to 50% heading. This information could help the barley breeders for development of SB resistant varieties for different geographic regions and different uses.

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Research Category: Plant Breeding

Abbreviations: AUDPC-Area Under Disease Progress Curve, DTH-Days to 50% heading, GS-growth stages, PHT- Plant height, SB-Spot blotch

Research Guide: Prof. V. K. Mishra
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Research project name or number: Inheritance of components of resistance (spore load, latent period and number of spots) of spot blotch caused by *Bipolaris sorokiniana* in Barley

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Author statement: All authors read, reviewed, agree and approved the final manuscript

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Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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