

Assessment of genetic variability and correlation among agro-morphological traits and spot blotch disease in a RIL population of wheat

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Abstract

A study of genetic variability for various agro-morphological traits including spot blotch disease severity (DS) and grain yield (GY) involving complete set of 214 recombinant inbred lines (RILs) population (cross 'Kanchan / Chirya 1) was conducted during 2014-15 and 2015-16 crop seasons. Combined analysis of variance (ANOVA) over years indicated that genotypes, environments and G × E interactions were significant, indicating that the population exhibited significant differences for the traits studied. Similarly, variability analysis indicated wide genetic variation for all the traits among parents and recombinant inbred lines. Significant but negative correlation of disease severity (DS) with days to heading (DH), days to maturity (DM), plant height (PH) and grain yield (GY) indicated negative impact of high disease pressure on the expression of these traits. Among other agro-morphological traits, days to maturity showed significant positive correlation with plant height, grain yield and 1000-grain weight. High estimates of phenotypic and genotypic coefficient of variation (PCV and GCV) were found for disease severity (32.09 and 26.12 during 2014-15 and 30.79 & 29.99 during 2015-16, respectively) followed by grain yield and 1000-grain weight. High estimates of heritability coupled with moderate to high values of genetic advance were recorded for most of the traits during both the years. The results indicated sufficient genetic variability, high estimates of heritability coupled with genetic advance along with desired correlation among traits, and therefore selection strategy might be effective in identifying promising genotypes possessing desired level of disease resistance, high yield and wider adaptability for target environments..

Keywords: Genetic variability, heritability, RILs, spot blotch, wheat

1. Introduction

Wheat (*Triticum aestivum* L. subsp. *aestivum*) is the most important cereal crop affected by various biotic and abiotic factors. In recent past, reports of new and emerging challenges, especially adverse effects of climatic changes by abiotic stresses, increasing disease severity that significantly reduce grain quality and yield (Gurung *et al.*, 2012) need attention to ensure sustainable production of wheat. In wheat, spot blotch (SB) caused by *Bipolaris sorokiniana* is attaining serious concern due to climate

changes, crop rotation system and other traditional farming practices (Singh *et al.*, 2008). It is considered as a major biotic constraint in South Asia including eastern parts of India that leads to reduction in grain size, yield and also grain quality. The disease is more prominent in the areas having favourable weather conditions and sudden rise in temperature (28°C or more) the infection rate increases abruptly (Singh *et al.*, 2007). The pathogen produces disease symptoms on leaves that are

characterized by small, dark brown lesions varying 1 to 2 mm long with or without chlorotic margins. The average yield losses due to spot blotch are reported ranging 18-50% under favourable conditions (Singh *et al.*, 2015).

Among the viable options to manage this disease, use of resistant cultivars is likely to be a robust, economical and environment friendly approach. The most important criteria to develop and select SB resistant cultivars through resistance breeding approach largely depend on extent of genetic variability for target traits. To estimate extent of genetic variability, various parameters namely; mean, range, heritability and genetic advance of agromorphological traits associated with spot blotch resistance is useful and has been practiced across crops (Singh *et al.*, 2007). The expression of a character is determined by heritability which is a useful quantitative parameter for selecting genotypes possessing resistance and other traits. High heritability coupled with high genetic advance helps in making selection from segregating generations (Sarfraz *et al.*, 2016). Besides, significant and positive correlation between economic traits is essential (Singh *et al.*, 2008). The character association proclaims the magnitude of correlation among resistance, yield components with yield and among themselves, which is found to be effective while selecting desirable genotypes.

Sharma *et al.* (2006) studied area under disease progress curve (AUDPC) and highest disease score (HDS) of spot blotch and also studied the genetic control of this disease. Singh *et al.* (2014) identified several promising lines that possessed high degree of spot blotch resistance coupled with desired genetic background. These lines will be useful for direct testing and also serve as potential donors for improvement of spot blotch resistance and other agronomic attributes in future wheat genotypes. In warmer humid regions of South Asia, utilization of available and potential resistance against spot blotch for developing high yielding and disease resistant wheat genotypes is not fully explored. Singh *et al.* (2008) carried out a study with large number of genotypes to assess the genetic parameters of spot blotch resistance for making effective selection. Duveiller *et al.* (2005) studied the selection for resistance in segregating generation and highlighted the need to broaden the source of variability for identification of new resistant material. In the present study, attempts were made to phenotype a complete set of RILs population evolved from a cross - 'Kanchan /

Chirya1' for assessing genetic parameters like genetic variability, heritability and genetic advance that will enable breeders in making feasible and possible progress for spot blotch disease resistance.

2. Material and methods

2.1 Experimental material: The experimental material for the present study was a population of 214 recombinant inbred lines (RILs) that were developed involving spot blotch resistant parent Chirya1 (CS/Th.cur//GLEN/3/ALD/PVN/4/NINGMAI-4/OLESEN//ALD/YANGMAI-4) and the agronomically superior but susceptible cultivar Kanchan (HUW 202/K7537/MUTANT of HD 2160). The RILs along with parents were planted at ICAR-Indian Institute Wheat and Barley Research, Karnal during two consecutive crop seasons (2014-15 and 2015-16) in field conditions following alpha-lattice design. Sowing was done slightly late (third week of December) so as to coincide post-anthesis stage with warm temperature and high humidity favouring high disease conditions. Each line was planted in a single row of one meter length keeping distance between rows to 25 cm with plant to plant spacing of 5 cm. Border rows of each block (15 genotypes) were planted with blend of susceptible genotypes (infectors Raj 4015, Sonalika and Kanchan) to provide equal chance of infection for each test entry and also to ensure high disease pressure build up and its spread under field conditions for proper disease screening. During both the years, field experiments were conducted under irrigated conditions following recommended package of practices.

2.2 Creation of artificial epiphytotic conditions in the field: A pure culture of aggressive isolate of *Bipolaris sorokiniana* multiplied on sorghum grains was uniformly sprayed at three different growth stages flag leaf emergence (GS37) and anthesis (GS65) on Zadoks scale (Zadoks *et al.*, 1974) during evening hours. A mixture of virulent isolates of *Bipolaris sorokiniana* collected from different hot spot locations namely Faizabad, Coochbehar, Pantnagar and Kalyani was used in the present study. The field was irrigated immediately after inoculation to provide a favourable environment for the development of spot blotch disease.

2.3 Disease assessment and agronomic data recording: Observations on various agronomic traits including spot blotch disease were recorded at appropriate stages. For spot blotch disease, three recordings following double

digit (DD) score method as proposed by Singh and Kumar (2005) were made based on per cent leaf area covered due to spot blotch on the flag leaf (digit D1) and the penultimate leaf (digit D2) on the basis of infection response. The disease severity (DS) of RILs and checks was calculated as given below;

$$\text{Disease severity (\%)} = (D1 / 9) \times (D2 / 9) \times 100$$

Observations on agro-morphological traits namely days to heading (DH), days to maturity (DM), plant height (PH), 1000-grain weight (TKW) and grain yield (GY), were recorded. Days to heading was measured at the hard dough stage at Zadoks growth stage 87 (Zadoks *et al.*, 1974). DH were counted as the number of days from sowing until 50 % of the ear emerges fully from the boot of flag leaf in each plot. Plant height (cm) was measured at maturity stage from ground level up to the tip of spike of main tiller excluding awns. DM were counted as the number of days from sowing till the grains get utterly hard and retains moisture levels less than 18%. For TKW (gm), 1000 seeds were counted randomly by seed counter and weighted by electric balance. Grain yield per plot (gm) was recorded taking weight of all harvested and cleaned seeds from each plot.

2.4 Statistical analysis: Data recorded for various traits was used for the analysis of variance (ANOVA) following PROC GLM of SAS software (SAS Institute Inc., v. 9.1.). Pearson correlation coefficient between disease severity and other traits was calculated using the PROC CORR function. Genotypic and phenotypic coefficient of variation was estimated as per method suggested by Burton (1952) and Singh and Chaudhary (1985). Heritability (broad-sense) and genetic advance were estimated using the formula suggested by Johnson *et al.*, (1955).

3. Results and discussion

3.1 Analysis of variance: The analysis of variance (ANOVA) estimates based on data recorded on various agro-morphological traits including disease score during both the years presented in Table 1 that revealed significant differences for all the studied traits. Combined ANOVA over genotype and environment exhibited significant G × E interaction, indicating that the RILs population differed genetically and traits studied are influenced by environment and phenotyping of such material for these traits and exclamation with genotyping confirms precision over environments.

3.2 Agro-morphological traits: The results indicated wide variability among RILs and parents as evident by mean performance during both the years and this was consistent across years for disease severity and other traits. As expected, mean disease severity score value in the susceptible parent (Kanchan) was much higher (68 and 77) during both the years, while it was low in the resistant parent (19 and 22) and intermediate for the RILs (32 and 35), respectively (Table 2). Like-wise, mean performance of both the parental lines (Kanchan and Chirya1) during both the years (2014-15 and 2015-16) also revealed wide variation for all the other agro-morphological traits (Table 2). It was found that during first year (2014-15) the estimated values of both parents revealed sizable differences for heading duration (68 and 79 days), maturity duration (103 and 121 days), (plant height (101 cm and 89 cm), 1000 grain weight (35.5g and 46.5g) and grain yield per plot (92.1g and 100g). Similarly, during second year (2015-16) mean value of the two parents varied for days to heading (66 and 78 days), days to maturity (101 and 123 days); plant height (100 cm and 88cm), 1000-grain weight (35.4g and 44g) and grain yield per plot (89g and 102g), respectively (Table 2). Results indicates that parental genotype 'Kanchan' had much lower mean value across years for days to heading (67 days) as compared to the resistant parent Chirya1 (78 days), demonstrating thereby that early heading lines might show greater frequency of disease severity in comparison to late heading lines which is consistent with the previous studies (Rosyara *et al.*, 2009; Singh *et al.*, 2015). Among the agro-morphological traits, plant height is one of the key traits for wheat breeders for several other reasons and thus it will be easier to bring improvement in associated traits including spot blotch. Whereas, estimates of mean values of RILs for all the traits were much wider (beyond parental range) thereby indicated that RILs population had segregation that was fixed in early generations.

It was found that mean values of disease severity score for RILs ranged (0-91 and 0-95) during 2014-15 and 2015-16 crop seasons, respectively. These values were variable in comparison to parental values and resulted in large number of lines possessing high level of resistance even better than the donor parent 'Chirya1' which might be useful for direct selection. Some lines showed 0 or no disease, indicating that these lines may have transgressive segregation towards resistance, which is beneficial. Also, a higher values of DS was also reported in some of the lines which is indicative of good disease pressure.

Table 1. Analysis of variance for spot blotch severity and other traits during 2014-15 and 2015-16

Traits	df	DH	PH	TKW	GY	DM	DS
Source		MS	MS	MS	MS	MS	MS
E	1	3312.9**	1498.3**	27.7**	3312.1**	3598.3**	1817.6**
G	215	186.4**	1627.8**	66.2**	47.2**	1851.8**	856**
G x E	215	44.2**	118.8**	6.1**	23.34**	98.8**	18.36**
Error	215	18.3	32.35	2.5	15.3	53.35	12.3

** Significant at 0.01 per cent; and * significant at 0.05 per cent, Where, DH =days to heading, PH = plant height, TKW = thousand kernel weight, GY=grain yield, DM =days to maturity and DS= disease severity, (%) respectively.

Table 2. Mean, range of parents and RILs for spot blotch disease severity and other traits during 2014-15 and 2015-16

Parents/traits		KANCHAN (Mean)	CHIRYA-1 (Mean)	RILs (Mean)	Range
DH	2014-15	68±1.5	79±0.2	72.1±0.1	89-56
	2015-16	66±0.1	78±1.5	73±1.1	84-52
PH	2014-15	101±2.2	89±5.3	83.2±1.1	104-67
	2015-16	100±2.2	88±2.5	85±1.3	103.5-69
TKW	2014-15	35.5±1.1	46.5±1.3	39.6±0.2	49-24
	2015-16	35.4±0.2	44±0.7	38.3±0.1	43-21
GY	2014-15	92.1±1.1	100±1.4	93.2±2.1	105-51
	2015-16	89±0.1	102±1.1	92±0.1	99-53
DM	2014-15	103±1.1	121±0.1	106±0.5	96-116
	2015-16	101±0.2	123±1.1	101±0.2	98-120
DS	2014-15	68±1.3	19±2.1	32±1.7	0-93
	2015-16	77±1.2	22±1.3	35±1.2	0-95

Where, DH =days to heading, PH = plant height, TKW =thousand kernel weight, GY=grain yield, DM = days to maturity and DS= disease severity, respectively.

3.3 Correlation coefficients: The estimates of correlation coefficient between disease severity, grain yield and other traits presented in Table 3, indicated significant but negative correlation of disease severity score with days to heading (-0.51), days to maturity (-0.33), plant height (-0.60) and grain yield (-0.20), thereby reflecting adverse effect of high disease score on these traits. On the other hand a weak (non-significant) positive correlation of disease severity with 1000-grain weight was observed. This may be due to large variability in the population showing low effect of high disease score on grain size. Spot blotch is generally known to reduce the grain size and weight.

Positive significant correlation of days to maturity with plant height (0.31), grain yield (0.30) and grain weight (0.26) suggested that making selection for either trait will have direct influence on the performance of others. Whereas, negative and significant correlation observed between days to heading and disease severity (-0.51)

indicated good scope for improving disease resistance by selecting early flowering types. Negative correlation of spot blotch disease severity with plant height is not very commonly reported feature because of the fact that the disease progression is from lower to upper leaves and the tall types will score lower disease value.

During both the years the mean plant height value for the susceptible parent Kanchan is higher than the resistant parent Chirya1. Several lines with low spot blotch severity possess short PH and vice versa. Singh *et al.* (2016) reported that plant height reduces the rate of movement of disease due to splashing of spores. The result showed negative and significant correlations between DS and PH (-0.605, $p < 0.001$). Similar results were reported by Sharma *et al.* (2006). It is known that susceptible lines are comparatively early maturing and have less mean value for DM compared to resistant parent indicating that late maturing genotypes are less prone to disease infection. The results observed are similar to the findings of Singh *et al.*, (2015). In the present study, negative and significant correlation between grain yield and disease severity (-0.191) was estimated and this was also evident by high mean value of grain yield observed in the resistant parent as compared to the susceptible parent. It shows

that increase of severity in susceptible type plant leads to decrease in the yield (Table 2). Similar findings of negative and significant correlation of spot blotch disease with grain yield had been reported previously by Singh *et al.* (2008), which is reflecting the significance of the disease tolerance. Further more, the susceptible parent had lower mean value for grain weight as compared to the resistant parent (Table 2). However, 1000 grain weight showed weak positive correlation with disease severity (0.174). Findings of lower phenotypic correlations for hundred kernel weight (HKW) with AUDPC during the grain filling duration were also reported by Rosyara *et al.* (2009). The magnitudes of correlation coefficient between disease severity and grain size are usually lower and results showed that resistance of spot blotch and grain weight can be improved independently, which is similar to previous results of Sharma and Duveiller (2003).

Table 3. Correlation coefficients among spot blotch disease severity and other traits during 2014-15 and 2015-16

Traits	DM	PH	GY	TKW	DS
DH	0.122	0.145	0.125	0.197	-0.51***
DM		0.312***	0.308***	0.264***	-0.33***
PH			0.126	0.156	-0.605***
GY				0.173	-0.203 *
TKW					0.174

*significant at $p < 0.05$, **significant at $p < 0.01$, ***significant at $p < .001$

Table 4. Estimates of genetic parameters in RILs population for spot blotch disease and other traits during 2014-15 and 2015-16

Traits	Years	DH	PH	DM	TKW	GY	DS
PCV (%)	2014-15	11.8	22.52	27.38	23.3	29.7	32.09
	2015-16	11.99	21.56	27.01	25.11	31.15	30.79
GCV (%)	2014-15	9.37	11.24	16.99	19.99	25.84	26.12
	2015-16	7.74	12.28	16.13	18.57	24.24	29.99
H ² (%)	2014-15	73	80	85	95	90	93
	2015-16	72	82	93	88	92	96
GA	2014-15	7.56	18.81	21.46	23.01	25.73	28.76
	2015-16	4.44	17.25	22.65	26.53	22.52	26.52

GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation, H²: heritability, GA: Genetic advance.

3.4 Estimates of genetic parameters: Genetic parameters estimated as measures of variability includes; genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2) and genetic advance (GA) under disease stressed condition for two years are presented in Table 4. High estimates of PCV and GCV ($>20\%$) were recorded for disease severity followed by grain yield and 1000-grain weight during 2014-15 and 2015-16. While, days to heading showed lower values of PCV and GCV in both the years (Table 4), PCV and GCV values for disease severity (32.09 and 26.12, and 30.79 and 29.99) during 2014-15 and 2015-16, indicated that there is high variability and thus ample scope for selecting resistant genotypes among the RILs of the present population. These results follow the same pattern as reported by Chethana *et al.* (2017).

Moderate estimates of GCV and high value of PCV for plant height and days to maturity during both the years indicated presence of high variability among the RILs populations for these two traits. In general, the magnitude of PCV was higher than GCV in both the years, which indicates the effect of environmental conditions on the expression of the traits studied. Although, PCV and GCV alone might not give a very clear picture of the extent of genetic gain to be achieved for the selection of phenotypic traits, unless the heritable fraction of the trait(s) in question is known. The knowledge of heritability of a particular character is indicative of role and influence by the environmental effect in its expression, and the existing sufficient genetic variability for that trait leads to the suggestion that selection would be effective for improvement of such traits. Through direct selection for grain yield related traits increases the chance of improving wheat grain yield. The estimates of heritability have been classified as low (below 30%), medium (30-60%) and high (above 60%) as suggested by Johnson *et al.*, (1955). Following above classifications, present study has reports for higher estimates of heritability ($>60\%$) for all the traits (Table 4). However, this may not be a common feature for at least few traits that are highly influenced by the environment. It is also suggested that since

heritability alone does not represent the extent of genetic improvement, knowledge of heritability estimates coupled with genetic advance is more useful, as some important inferences can be drawn for these traits and selection for trait(s) with high heritability coupled with high genetic advance would be more effective and better approach than following high heritability with low genetic advance.

It may be concluded that the results from the present set of RILs exhibited genetic variability, high estimates of heritability coupled with high genetic advance were recorded for disease severity followed by grain yield, days to maturity and 1000-grain weight, whereas plant height showed high heritability with moderate genetic advance during both the years. Heritability values are indicative of preponderance of additive effects but if associated with low values of genetic gain for particular trait(s), indicates non-additive component and in such cases genetic gain would be low (Panse and Sukhatme 1967). The high values of heritability for disease severity (93 and 96 per cent) with moderate values of genetic advance (28.76 and 26.52) during 2014-15 and 2015-16, respectively, indicates that this trait in this particular set of material might be governed by additive gene action and thus could be improved through either pure line selection or simple mass selection. Whereas, days to heading showed high heritability along with low advance in both the years which indicates that the trait is governed by non-additive gene action and selection would not be very effective for this trait under population. The traits (disease severity, grain yield, thousand grain weight etc.) that showed high genetic correlation with spot blotch disease could be taken as complementary traits for development of genotypes for identifying spot blotch tolerant material from this set of material and environments. The lines that exhibited high GCV, heritability and genetic advance for combination of traits could be directly tested in multilocation trials and identified as promising lines showing improvement for these characters for yield, resistance and adaptation in varying conditions.

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