

Phenotypic and Genotypic Evaluation of RILs for Spot Blotch Resistance in Wheat

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ABSTRACT: Spot blotch (*Bipolaris sorokiniana*) is a disease of great concern especially in Latin American and South Asian countries (India, Nepal, Pakistan and Bangladesh), where warm-humid conditions persist during wheat cropping system. Moreover, this disease is now expanding towards non-traditional cooler regions of European countries and India i.e., North Western Plains Zone. It is well recognized that host plant resistance is the best strategy for managing spot blotch disease of wheat. In the present study, recombinant inbred lines (249 RILs) were developed from the cross HUIW234 (susceptible)/YM#6 (resistant) for mapping spot blotch resistance in wheat. Multi-location phenotyping of this material was done at Karnal and two hot spot locations (Coochbehar and Kalyani) of India during 2011-12 and 2012-13 cropping seasons and disease severity was recorded on double digit scale (0-9) taking into account flag leaf and f-1. The frequency distribution of the values for disease severity recorded in all the environments gave a good fit to normal distribution. A number of phenological (days to heading, days to maturity, plant height, canopy color, leaf angle) and yield related traits were evaluated besides disease severity at three locations over two years. The analysis of variance (ANOVA) revealed a significant variation for genotypes and genotype-by-year interaction. A total of 900 SSR makers spanning 21 chromosomes of wheat were utilized to survey the parental polymorphism for spot blotch between parents. A higher percent of polymorphic markers were located on B genome. The mapping population developed for spot blotch resistance are important resources for genetic dissection of this disease in wheat.

Keywords: Spot blotch, disease severity, SSRs, *Triticum aestivum*

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is the most important cereal crop after rice in India and is well recognized as a major staple food of South Asian region countries. Also, India is the world's second largest wheat producer, behind China and ahead of USA. It is a remarkable achievement but India continues to face formidable problem of ever increasing population and future wheat demand will be 110 mt to feed 1.5 billion people of India by 2020. Therefore, there is urgent need for constant improvement of crop yield on one hand and stabilizing yields on other hand. The spot blotch caused by *Bipolaris sorokiniana* also known as *Helminthosporium* leaf blight or foliar blight has emerged as one the most important diseases limiting wheat production in humid and warmer wheat

growing areas causing upto 18-50 % loss in favorable conditions [1,2]. The disease is world widely distributed and is important in Africa, South America, Australia, Canada, Asia and particularly to Indian sub-continent having warm and humid environments [3]. This disease is now expanding towards non-traditional cooler reasons likes NWPZ (India) [4] and European countries.

Information on genetic variability, heritability and other genetic parameters of spot blotch resistance with other agro-morphological attributes is pre-requisite for genetic improvement. It is generally believed that the level of resistance in high-yielding wheat genotypes is still unsatisfactory and needs to be improved significantly in warmer and humid regions of South Asia [4, 5]. Resistance to spot blotch in wheat behaves like a quantitative trait [6], but until recently

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little has been known about its genetics. Some traits shown to have positive association with resistance had been leaf angle [7], stay green trait [and leaf tip necrosis (*Ltn*) which is strongly linked with *Lr34* [9, 10]. The correlations of some morphological and physiological traits with spot blotch resistance have been analyzed [11, 12]. Hence, the present study was undertaken to determine if resistance to spot blotch, independent from the effect of some morpho-agronomic traits, could be found.

MATERIALS AND METHODS

Plant materials

A total of 249 single seed descent (SSD) derived recombinant inbred lines (RILs, F9, F10 generations) of the cross 'HUW 234/Yangmai 6 (YM#6)' were evaluated in field trials for resistance to spot blotch. 'HUW 234' (HUW 12*2/CPAN 1666) is Indian cultivar, susceptible to spot blotch and 'YM#6' (K 8101/K 68) is a Chinese cultivar carrying resistance to spot blotch.

Evaluation of disease severity

The RILs were evaluated along with the two parents in natural field conditions during the crop seasons 2011-2012 and 2012-2013 at DWR, Karnal (alt. 748ft, 29°42'N, 77°02'E) and at two hot spot locations in India, one at the agricultural research farm of UBKV, Coochbehar (alt. 138ft, 25°57'N, 89°54'E), another at BCKV, agricultural research farms, Kalayani (alt. 36 ft, 22°59'N, 88°48'E). The field trials were laid out following a lattice design. Each line was sown in single rows of 1 m under irrigated conditions and sowing was done in two replications. Row-to-row and plant-to-plant distance was 25 and 5 cm, respectively with 0.5 m distance between the blocks. To promote disease build up and spread, one row of the infector was planted around each block. Sowing was done in the first week of December to allow the post-anthesis stage to coincide with warm and humid environment conducive to the disease that occurs in March.

Disease assessment

Spot blotch severity in each plot was visually scored; three times at 7-10 day interval on double digit scale (0-9) taking into account flag leaf (digit D1) and f-1 (digit D2). In the present work the disease recorded at early dough stage has been considered. Per cent disease severity was estimated based on the following formula:

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

Assessment of plant height, days to maturity, days to heading, TKW, canopy color and leaf angle

Plant height and days to maturity were assessed for all lines. Plant height was measured at the dough stage (Zadoks growth stage 87) [13], while days to maturity were counted as the number of days from sowing until the grains were completely hard and possessed moisture levels less than 12% (G.S. 92). Days to heading were counted as the number of days from sowing until 50 per cent of the ear emerges in each line. Thousand kernel weight was evaluated after the harvest. Canopy color was visually scored and divided the lines in three groups as dark green, green and pale green. Leaf angle was measured just after ear emergence in all the lines, dividing the lines in three groups; erect and making an angle of 60 to 90° with respect to the horizontal plane); semi-erect (making an angle of 0 to 60°); and drooping (more than half the length of flag leaf was drooping). This stage corresponded to the 51-55 stage [13].

Molecular marker analysis

Genomic DNA of parents was isolated from 15 days old seedlings using CTAB method. Forty markers from each chromosome spanning the whole genomic region of wheat were used to screen the parents. These markers comprised of *barc*, *gwm*, *wmc*, *cfa*, *cfb* etc. series.

Statistical analysis

Analysis of variance was performed with the PROC GLM procedure in SAS (SAS Institute Inc., v. 9.1.) by treating genotypes, years, locations and replications as random factors. Pair-wise Pearson's correlation coefficients were calculated using the PROC CORR procedure of the SAS software.

RESULTS AND DISCUSSION

Two hundred and forty nine RILs from the cross HUW234/YM#6 were evaluated for seven agro-morphological attributes including percent disease severity over two years and across three locations. The genotype YM#6 was evaluated as highly spot blotch resistant variety while HUW234 is considered as spot blotch susceptible variety. The means and ranges of percent disease severity of parents and RILs are listed in Table 1. The population showed large variation in percent disease severity ranged from 0 to 100 across different environments.

The analysis of variance (ANOVA) for percent disease severity in the population revealed significant variation for genotype and genotype-by-year interaction and highly significant difference between years (Table 2).

Table 1
Range and mean values of spot blotch percent disease severity for the parental lines and the RILs of 'HUW 234/YM#6' population

Genotype	KLY2012	KLY2013	CB2012	CB2013	KNL2012	KNL2013
HUW 234	31±6.71	33±6.10	34±4.41	46±7.37	23±3.39	17±2.66
YM# 6	6±3.17	3±1.76	10±0.33	5±0.66	5±2.0	3±1.33
RILs Mean	40±1.28	28±0.88	38±0.76	40±1.45	12±0.56	13±0.32
RILs range	0-78	2-78	10-100	4-100	4-67	4-35

KLY-Kalyani, CB- Coochbehar, KNL- Karnal

Table 2
Analysis of variance for percent disease severity of spot blotch across environments in the 'HUW 234/YM#6' RIL population

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GENO	248	142593	574.974	4.8	<.0001
YEAR	1	7741.34	7741.34	64.62	<.0001
LOC	2	394961	197480	1648.38	<.0001
GENO*YEAR	248	60064.3	242.195	2.02	<.0001
GENO*LOC	496	145640	293.628	2.45	<.0001
YEAR*LOC	2	32107.2	16053.6	134	<.0001
GENO*YEAR*LOC	496	120984	243.92	2.04	<.0001

LOC-location

The frequency distributions of the values of RILs for disease severity recorded in all the three environments gave a good fit to normal distribution. The mean squares due to RILs and environments were significant for disease severity (DS) (Fig. 1) and the other agro-morphological traits studied (Fig. 2).

In this study, for days to maturity (DM) and days to heading (DH), significant negative correlation was observed while for plant height (PH) no correlation was found with percent disease severity across the three locations and over two years (Table 3). Negative correlation of heading date with AUDPC was also

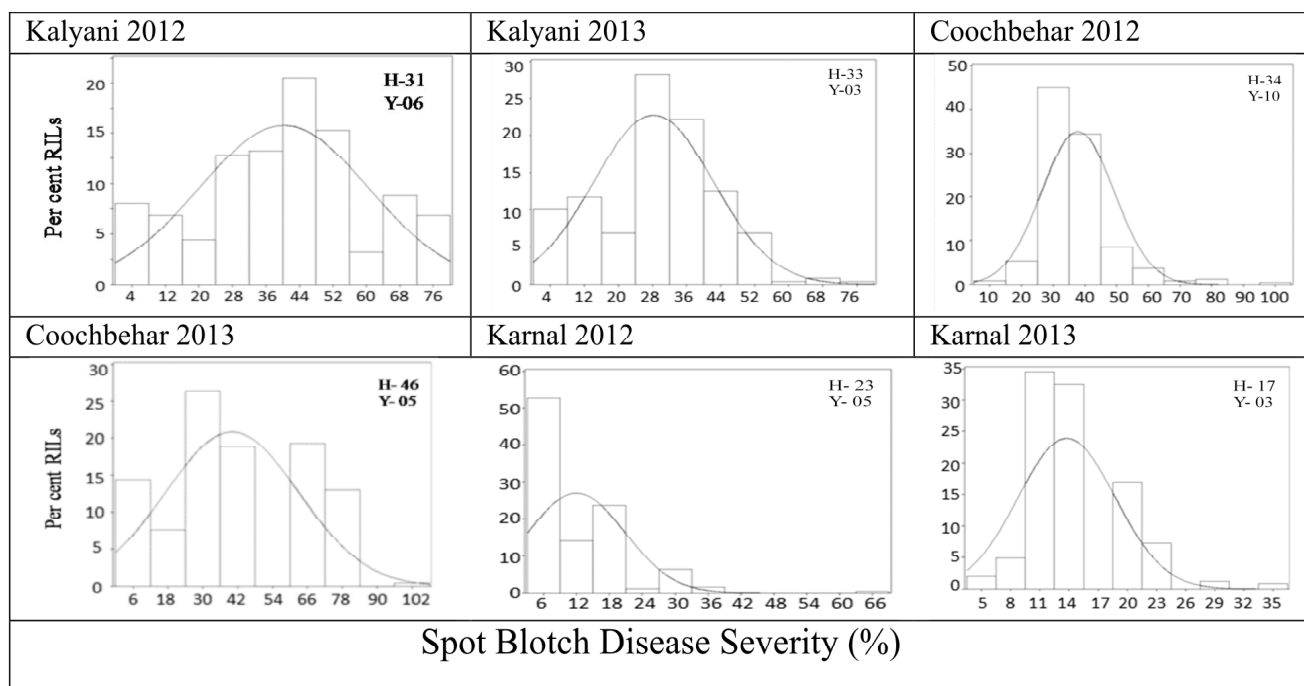


Figure 1: Histograms of % disease severity for spot blotch in the six field trials of the 'HUW 234/ YM#6' RIL population. The mean values for the parents HUW 234 and YM#6 are indicated by the letters H and Y, respectively.

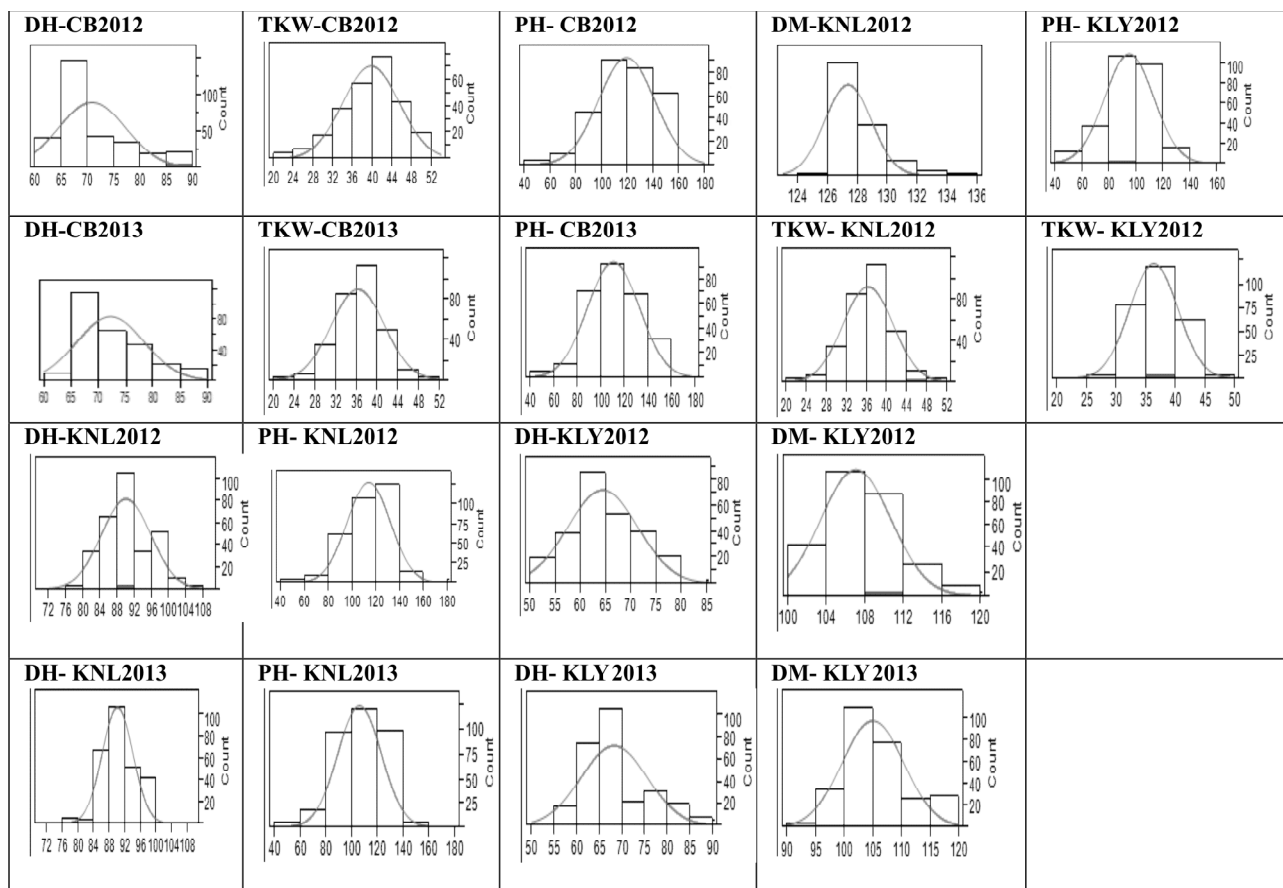


Figure 2: Histograms of morpho-agronomic traits in the six field trials of the ‘HUW 234/YM# 6’ RIL population. KLY-Kalyani CB- Coochbehar KNL- Karnal, DH-days to heading, DM-days to maturity, PH- Plant height, TKW- thousand kernel weight

reported in previous studies [14]. Spot blotch has been reported to be independent of plant height and days to maturity [12] or to have a negative (-0.29 to -0.43, significant or non-significant) correlation with PH in three spring wheat populations [11]. Zhu [15] also found highly significant negative correlations between spot blotch susceptibility and PH or DF (days to flowering) (-0.30 to -0.53).

For TKW and percent disease severity, negative and non-significant correlation was observed. In previous studies also, negative significant correlation was observed for TKW, yield with AUDPC value indicate that spot blotch is the major constraint for wheat production in Eastern Gangetic Plains of India representing major role of environment for disease incidence [16, 17, 18]. No significant correlation was

Table 3
Pearson correlation coefficients between per cent disease severity and different morpho-agronomic traits in the RIL population of the cross ‘HUW 234/YM#6’ during crop season 2011-12, 2012-13 at three different locations

Traits	Disease Severity (%)					
	KLY 2012	KLY 2013	CB 2012	CB 2013	KNL 2012	KNL 2013
DH	-0.69796***	-0.56189***	-0.26667***	-0.65187***	-0.28969***	-0.14564*
DM	-0.66816***	-0.56029***	-	-	-0.25072***	-0.09798 ^{NS}
PH	-0.12867*	-0.0586 ^{NS}	-0.03795 ^{NS}	0.0622 ^{NS}	0.0519 ^{NS}	-0.01909 ^{NS}
TKW	-0.0484 ^{NS}	0.00974 ^{NS}	0.02977 ^{NS}	-	0.14379*	0.12343 ^{NS}
CC	-0.08729 ^{NS}	0.04411 ^{NS}	-0.06117 ^{NS}	0.00637 ^{NS}	-0.07709 ^{NS}	-0.04116 ^{NS}
LA	0.0987 ^{NS}	-0.00341 ^{NS}	0.0732 ^{NS}	-0.00885 ^{NS}	0.03872 ^{NS}	0.02618 ^{NS}

KLY-Kalyani CB- Coochbehar KNL- Karnal DH-days to heading DM-days to maturity PH- Plant height TKW- thousand kernel weight CC- canopy colour LA- leaf angle
* Significant at P<0.05, ** P<0.01, *** Significant at P<0.001, ^{NS} Non-significant

observed for canopy color and leaf angle in the population studied. However, it was previously reported [12] that genotypes with erect or semi-erect leaf angles generally expressed lower disease scores and severities to spot blotch than those with drooping leaves. Dubin [14], found negative correlations between foliar disease and heading dates, but it is not known whether this is due to linkage, pleiotropy or escape [19]. Lack of adequate information on this aspect has prompted wheat breeders to select late and taller genotypes for securing more resistance to spot blotch. Some of the selected morpho-agronomic traits related with spot blotch disease could be used for indirect selection of resistant genotypes which could be used in further wheat breeding programmes.

Molecular Studies

A total of 900 SSR makers spanning all the 21 chromosomes of wheat were utilized to survey the parental polymorphism for spot blotch between parents. Out of these, 17.5% markers were found polymorphic between the parents. A higher percent of polymorphic markers were located on B genome (43%) followed by genome D (29%) and genome A (28%), indicating the chances of presence of QTL for spot blotch resistance on B genome. The putative chromosomes identified after parental screening for spot blotch resistance were 1A, 1B, 1D, 2A, 2B, 2D, 3A, 3B, 4B, 5B, 7B which could further be targeted for identifying the genomic regions associated with spot blotch resistance in wheat.

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