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BIPLOT ANALYSIS FOR SPOT BLOTCH AND YIELD TRAIT USING WAMI PANEL OF SPRING WHEAT

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ABSTRACT

Wheat (*Triticum aestivum* L.) is a staple food worldwide. Spot Blotch (SB) has been a major disease for cultivation of wheat in Eastern Gangetic Plain (EGP). The goal of this study was to evaluate genetically diverse, advanced elite lines of wheat association mapping initiative (WAMI) population to identify useful genetic diversity and other related traits for spot blotch resistance present in WAMI panel for wheat improvement. A panel of 289 elite lines of WAMI population were assessed for spot blotch resistance, days to heading and plot yield for three consecutive years (2012-13, 2013-14 & 2014-15). The significant differences among genotype, year and genotype $\times$ year for area under disease progress curve (AUDPC), days to heading and plot yield were exhibited. The negative correlation between days to heading and spot blotch AUDPC was observed whereas, AUDPC and days to heading was significantly and negatively correlated with plot yield. Based on GGE biplot, the first two principal components explained 100%, 100% and 84.5% of the total variation for AUDPC, days to heading and plot yield respectively. Germplasms 9122, 9049 and 9239 were showed most resistant under study. The highest mean value for plot yield was observed for the panel 9061, 9070, 9203, 9216, 9118 and 9227 which were found relatively more stable for grain yield. These genotypes could be used in wheat improvement programmes for developing superior genotypes for yield and spot blotch resistance contributing to food security in south Asia.

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1 Introduction

Wheat is most important and widely cultivated cereal crop throughout the world and remains a vital source for human food (FAOSTAT, 2016), contributes substantially to human daily calories and food security (Braun et al., 2010). Gradually and consistently wheat demand has been always increasing in the developing world. The world population expected to increase over 9 billion by 2050, the demand of wheat consumption will be increase by 60% compared with 2010. To fulfill this demand, global annual yield of wheat must increase from the current 1% per year (2001–2010) to 1.6% per year (2011–2050) (Giraldo et al., 2019). Biotic and abiotic stresses are major constraints leading to more impacts in productivity of wheat. Among biotic stresses, spot blotch has emerged as major challenge in the eastern parts of India; some reports indicated that this disease also spread into the cooler traditional rice-wheat production areas (Chand et al., 2003).

Spot blotch is a fungal disease caused by pathogen *Bipolaris sorokiniana* and it is considered as most important and destructive disease in the region having warm and humid climate of the world, restraining wheat production in the areas such as eastern India, South East and Latin America, the tarai of Nepal, China and Africa (Raemakers 1988; Saari 1998). Due to wide spread losses, this disease is considered as the most significant disease of wheat in north-eastern plain zone (NEPZ) of India encompassing eastern Uttar Pradesh, Bihar, West Bengal and Orissa (Saari 1998; Joshi et al., 2007b). Due to losses caused by this disease, wheat breeders rank this disease as number one disease in NEPZ of India (Saari, 1998; Joshi & Chand, 2002). Disease severity is affected by crop management, soil fertility, planting density, the development stage of the plant and the weather conditions experienced by the host during later part of life cycle (Joshi et al., 2007b). Particularly in grain filling stage, this disease becomes more severe (Joshi & Chand, 2002) and in Indian subcontinent, the crop losses have been reported to be in range of 15–25% (Dubin & Van Ginkel, 1991), but level of losses can be much higher in individual fields. Reports from several countries indicated that average yield loss due to spot blotch is estimated to be 15–20% but can reach 40–70% in susceptible genotypes (Fernandez et al., 2014).

Many effort has been done to control spot blotch disease but only integrated approach is considered most effective component (Joshi & Chand, 2002; Duveiller, 2003). However, in some instances, only resistant varieties prove to be controlling this disease. Wheat variety that possess high yield, early maturity along with resistance to spot blotch disease could be best in NEPZ. However, a negative correlation between spot blotch susceptibility and early maturity was observed by many researchers (Dubin et al., 1998; Shrestha et al., 1998). The primary focus of disease control relies on genetic resistance that involves race non-specific mechanism. Use of resistant cultivars is the best way to control this disease (Bai &

Shaner, 1994; Chaurasia et al., 1999). Although in recent years, significant effort has been made by wheat breeding programs targeting Indian gangetic plains, most cultivars grown in the subcontinent still harbor relatively low level of resistance to foliar blight (Joshi et al., 2004b).

Keeping in view of increasing threat of spot blotch disease and more heat stress to the wheat crop, there is necessity to identify and develop genetically improved germplasm incorporating earliness, tolerance and resistance to spot blotch for EGP of south Asia. In this study, an attempt has been made to identify the germplasms belong to south Asia which are tolerant to spot blotch disease by analyzing the genotype x environment interaction in WAMI panel of wheat.

2 Materials and methods

The experimental materials comprised of 289 genetically diverse, high-yielding, advanced elite lines of wheat association mapping initiative (WAMI) population of wheat obtained from Matthew Reynolds, CIMMYT Mexico.

2.1 Experimental design and trait evaluation

The WAMI population was evaluated 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., 2012–13, 2013–14 and 2014–15. The mean temperatures (November–April) during 2012–13, 2013–14 and 2014–15 was 26.12°C, 25.41°C and 23.21°C respectively and mean annual rainfall was 737.7, 932.7 and 1009.4mm respectively. The experiment field was followed by rice-wheat cropping system and conducted under irrigated conditions. The sowing of crop was done from 1st to 10th December during three consecutive rabi season. The experiment was laid out in alpha lattice design with two replications and each genotype was sown in two rows of two meters long plot under irrigated conditions maintaining row-to-row distance 20 cm and plant to-plant 5 cm. The genotypes were allocated randomly to each replication using Fisher and Yates Random Table (Panse & Sukhatme, 1985). Recommended fertilizer dose (120 kg N: 60 kg P₂O₅: 40 kg K₂O in per ha) were applied in field. Full amount of K₂O and P₂O₅ were supplied as single dose at sowing, while Nitrogen was given in three split doses: 60 kg per ha at sowing, 30 kg at the time of the first irrigation (21 days after sowing–DAS) and remaining 30 kg at the time of the second irrigation (45 DAS). Data was recorded for spot blotch severity, days to heading, plot yield (grain yield of 50 spikes) and 1000 grain weight.

2.2 Inoculation of pathogen and disease assessment

For creating artificial epiphytotic conditions, the most aggressive isolate of *B. sorokiniana* (NABM MAT1; NCBIJN128877, BHU, Varanasi, India) obtained from the department of mycology and

plant pathology, Banaras Hindu University, Varanasi (Joshi & Chand, 2002) was used. The isolate was purified, multiplied on potato dextrose agar (PDA) medium and the mass culture was produced on sorghum grains. A spore suspension adjusted to 10^4 spores per ml of water using haemocytometer and applied during the evening hours as per the method of Chaurasia et al. (1999). The field was irrigated immediately after inoculation to ensure spore germination and disease development. Frequent irrigation were given to provoke environmental conditions facilitate to spot blotch.

For area under disease progress curve (AUDPC), disease severity (%) was recorded on each germplasm line of artificially inoculated plots at three different growth stages (GS) viz., GS 63 (beginning of anthesis to half complete), GS 69 (anthesis complete) and GS 77 (late milking) by using Saari & Prescott (1975) double digit (00 to 99) method. The first digit (D1) indicates vertical disease progress on the plant and the second digit (D2) indicates severity measured in diseased leaf area. Disease severity was then used to estimate the AUDPC by following formula given by Shaner & Finney (1977).

$$AUDPC = \sum_{i=0}^{n-1} [(Y_i + Y_{i+1})/2] \times (t_{i+1} - t_i)$$

Where, Y_i = disease severity at time t_i , $(t_{i+1} - t_i)$ = Time (days) between two disease scores, n = number of dates at which spot blotch was recorded.

2.3 Statistical analysis

Analysis of variance (ANOVA) for the traits was performed using proc GLM procedure of SAS version 9.2 statistical software to determine the contribution of genotypes, year and their interactions. The association study among traits was estimated by

using the Proc CORR procedure in SAS in terms of Pearson's correlation coefficients.

GGE biplot GUI package of R software was used to estimate the genotype $\times$ year interaction, to test the most discriminating years, which-won-where/what pattern, identify most stable lines over years using first two principal components (PC1 and PC2) derived from principal component (PC) analysis of environment-centered data (Yan, 2001).

3 Results

The analysis of variance of 289 WAMI panels tested over 3 years at Varanasi, India showed significant differences among genotype, year and genotype $\times$ year for AUDPC and, days to heading and plot yield (Table 1). A negative and non-significant phenotypic correlation was observed between AUDPC and days to heading whereas, AUDPC was significantly and negatively correlated with plot yield and while days to heading revealed significant negative associated with plot yield (Table 2).

Out of three years, highest mean of relative humidity was recorded in the year 2014-15 at BHU, Varanasi. The highest mean value for AUDPC was observed for the genotypes during the year 2014-15 as shown in GGE biplot (Figure 2A & B). It is evident that the high humidity during booting to milking stage of wheat provokes spot blotch (Figure 1B). As the minimum temperature increases during night resulted more chances of disease occurrence (Table 3). It was observed that humidity is more important than the temperature for spot blotch development whereas, resistant genotype under study shows independent of years. Hence, the selection of these lines will be beneficial for the farmers.

Table 1 Pooled variance components due to genotypes, year and G $\times$ Y interaction for 3 quantitative traits in 289 WAMI panels of wheat tested in three years at BHU, Varanasi, India

Source	d.f.	Mean sum of squares of 3 years		
		AUDPC	Days to heading	Plot yield
Genotype	288	102858.93*	11.25*	444.45*
Year	2	549899.51*	4072.36*	1709.12*
Replication	1	10776.34	122.56*	231.31*
Genotype $\times$ Year	576	18904.76*	3.50*	118.03*
Error	866	3319.37	1.18	34.39

*Significant at the p-value ($P < 0.01$) probability level.

Table 2 Correlation among AUDPC, days to heading (DH) and plot yield (PY).

	AUDPC	DH
DH	-0.01	
PY	-0.21*	-0.07*

* Significant at $P < 0.05$.

Figure 1 (A) Mean temperature (B) Relative humidity (%) during wheat growing period over three year at BHU, Varanasi.

Table 3 Monthly mean minimum temperature (Tmin. in °C) and Relative humidity (RH %) at the test center in three years at Banaras Hindu University, Varanasi

Month	Year 2012-13			Year 2013-14			Year 2014-15		
	T max.	T min.	RH	T max.	T min.	RH	T max.	T min.	RH
Nov.	26.74	11.53	70.16	26.61	11.71	66.78	27.16	11.46	63.63
Dec.	21.99	10.35	66.16	23.81	11.65	67.66	21.14	9.4	73.02
Jan.	20.8	7.75	66.73	19.54	11.34	79.19	16.58	10.31	83.06
Feb.	24.43	12.3	72.52	22.33	12.5	71.46	17.91	13.51	69.88
Mar.	31.28	16.67	53.11	29.68	16.5	61.82	29.57	16.95	61.32
Apr.	37.79	20.36	38.93	36.77	20	34.47	34.62	20.39	52.27
Overall mean	26.12	12.58	62.23	25.41	13.61	66.09	23.21	13.21	69.35

3.1 GGE biplot results

The first principal component (PC1) accounted for 75.13% of total variation and the second principal component (PC2) accounted for 24.87%. Cumulatively; these two principal components explained approximately 100% variation for AUDPC (Figure 2A, B and C). The polygon is formed by connecting the markers of the WAMI

panel that are further away from the biplot origin, such that all other panels come within the polygon (Figure 2A). The polygon also contains number of lines perpendicular to each side of the polygon. These perpendicular lines divide the biplot into sectors. The vertex panel in each sector represents the highest AUDPC panel in the year that falls within that particular sector (Yan & Tinker, 2005; Yan et al., 2010). The figure 1A has nine sectors

Figure 2 (A) 'Which-won-where/what view of genotype $\times$ year biplot (B) The mean vs. stability view of genotype $\times$ year interaction effect (C) Ranking of genotype for AUDPC over the years.

with vertex panels 9296, 9177, 9235, 9094, 9105, 9148, 9040, 9046, 9147 and 9104. The vertex panels 9296, 9177 and 9235 fell in the sector of year 2013 indicating that these panels gave highest AUDPC for that particular year. Panels 9105, 9094 and 9148 were the highest AUDPC panels in the year 2014 while panels 9040, 9046, 9147 and 9104 were the highest AUDPC panels in the year 2015. Panels that fell within the polygon were less responsive than the vertex panels. The three years fell into three different sectors. This pattern implies that the target years may consist of three different

environments and that different panel should be selected and utilized for each. Using the mean vs. stability GGE biplot of AUDPC for the 289 WAMI line as shown in Figure 2B, the vertical line separates lines with below-average means from those with above-average means. The mean AUDPC is estimated by the projections of their markers on the average-tester axis. The stability of the panels is determined by their projection onto the middle horizontal line. The greater the absolute length of the projection of a panel, that panel indicates less stable. In present study, the highest mean value for AUDPC was observed for

the panels 9104 followed by 9158, 9012, 9132, 9014 and 9176 that were consistently more susceptible by being longest vector on the right side of the origin of the biplot on the performance line. On the other hand, panels 9122, 9049 and 9239 were consistently most resistant by being longest vector on the left side of the origin of the biplot on the performance line. The performance of lines 9040, 9288, 9235 and 9204 were highly variable over the years, but the resistant panels (9122, 9239 and 9049) were more stable over the years.

The first principal component (PC1) accounted for 57.44% of total variation and the second principal component (PC2) also accounted for 42.56% for days to heading (Figure 3A). The vertex genotypes were 9175, 9228, 9131, 9130, 9001, 9147, 9112, 9180 and 9193. These

genotypes had the longest distance from the origin of the biplot and showed the late or early headed genotypes over the years under study. Genotypes 9180, 9193, 9112, 9137 and 9227 had the late heading by being longest distance from the origin of the biplot on the right side of the performance line, while genotypes 9131, 9195, 9130 and 9045 had the earliest days to heading. The performance of lines 9198, 9133, 9216 and 9235 were unstable for days to heading by being greater the absolute length of the projection of a panel in either side of horizontal line (Figure 3B). The ideal genotype showed both highest mean and stable thus using ideal genotype as the center, concentric circles were drawn to help visualize the distance between each genotype and the ideal genotype (Figure 3C). On the basis of relative position of ideal

Figure 3 (A) "Which won where/what" of genotype × year biplot (B) The mean vs. stability view of genotype × year interaction effect (C) Ranking of genotype based on days to heading the year.

genotype, the ranking of genotype are done. Therefore, genotypes placed closer to the ideal genotype are more desirable than others. Thus genotype 9195, 9131 and 9045 were regarded as ideal genotype and more favorable than all the other genotypes. The other genotypes were distantly placed from ideal genotypes declared unfavorable one.

The first & second principal component explained 64.3% and 20.2% variation respectively. In total, both principal components explained 84.5% of the total variation for the plot yield (Figure 4A, B and C).

In this polygon, perpendicular lines are drawn from the biplot origin gave 10 sectors with vertex genotypes 9036, 9170, 9131, 9104, 9070, 9061, 9203 and 9255 of the polygon (Figure 4A). The property of biplot polygon view is that each vertex genotype has higher yield than the other genotypes that fall in the related sector (Yan, 2002). GGE biplot were used for graphically visualization of performance and stability of genotypes (Figure 4B). The highest mean value for grain yield was observed for genotypes 9061, 9070, 9203, 9216, 9118 and 9227. Genotypes with highest mean value could be

Figure 4 (A) "Which won where/what" view of genotype $\times$ year biplot (B) The mean vs. stability view of genotype $\times$ year interaction effect (C) Ranking of genotype based on plot yield over the year.

selected, whereas the rest were discarded. The ideal genotypes identified were 9070 followed 9216, 9227, 9118, 9183 and 9183 for plot yield (Figure 4C).

4 Discussion and Conclusion

Spot blotch caused by a fungus *B. sorokiniana* is an important disease in wheat production. It has become more devastating disease in the warmer areas of the world particularly the Indian subcontinent characterized by prevalent warm and humid conditions (Aggarwal et al., 2010). Further, the wide spread use of conservation tillage practices may also be favorable for spot blotch incidence in the south east Asia (Duveiller & Sharma, 2009). It has been reported that this pathogen spread into the cooler traditional rice-wheat production areas (Chand et al., 2003). The extensively adopted rice-wheat cropping system facilitates suitable environment for the survival and multiplication of foliar blight pathogens in south Asia. 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 humid regions of south Asia (Sharma & Duveiller, 2006; Joshi et al., 2007a). Consequently, an integrated approach, with host resistance as a major component, is generally considered best for controlling the disease (Hetzler et al., 1991; Mehta et al., 1992). The resistance to spot blotch in prominent cultivars has been slow due to quantitative nature of resistance which has a large influence of environment (Joshi et al., 2004a; Kumar et al., 2009). This is one of the reasons why selection of spot blotch resistant plants in breeding programmes becomes complicated. The agricultural research farm of Banaras Hindu University, Varanasi testing center used in this study fall under EGP having characteristics of warm-humid weather particularly at reproductive stage of the crop which provokes spot blotch disease.

Result of current study indicate significant differences among genotype, year and genotype $\times$ year for AUDPC percent, days to heading and plot yield exhibit considerable phenotypic variations. The negative correlation among earliness and spot blotch susceptibility was found (Dubin et al., 1998; Mahto, 2001; Rosyara et al., 2009) while AUDPC and days to heading was significantly and negatively correlated with plot yield. The similar results of Saxesena et al. (2017) also showed by developing recombinant inbred line population from cross of two parents YS#58 $\times$ YS#24 for spot blotch resistance. The value of plant genetic resources are realized only when each and every germplasm line is characterized 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.

According to Heidari et al., 2016, GGE biplot facilitated visual comparison and informative methods to detect genotypes stability and in the preferential genotypes recommendations. This approach provides an easy and comprehensive analysis of genotype by years' interaction such that not only it allows effective evaluation of genotypes but also a comprehensive understanding of the target environment (year) (Yan & Tinker, 2005). Genotypes showed variation for spot blotch score and morpho-agronomic traits. Current study identified superior lines for specific traits with resistance to spot blotch disease that are likely to serve as new potential sources of variation in wheat improvement programme. For AUDPC, the first two principal components explained 100% (PC1=75.13% and PC2=24.87%) of the total variation of the year-centered data implying complex interaction between genotypes and year. The stability analysis of wheat genotypes is explained by performance line passing through the origin of the biplot showed that the germplasm 9122, 9049 and 9239 was farthest to the left of the biplot origin and near to performance line indicating lower AUDPC mean. The more stable a genotype will be more near to the performance line as reported earlier by Yan et al., 2000 hence, these lines could be considered stable for spot blotch resistance across the years. Identification of genotypes with stable resistance for disease is an important component that ensures selection of appropriate sources of resistance for breeding programmes (Sharma et al., 2012; Singh et al., 2015). Using the ideal environment (year) as the centre, concentric circles were drawn to help visualize the distance between each environment and the ideal environment (Yan et al., 2000; Yan & Rajcan, 2002).

The variation in plot yield was mainly affected by genotype and genotype $\times$ year interactions. The PC1 vs PC2 of genotypes and environments (years) explained 84.5% of the total variance. When a genotype is near to performance line and environment on the right side of origin of biplot, this indicates that the genotype is specifically adapted to that environment (Kumar et al., 2016). The highest mean value for plot yield was observed for the panel 9061, 9070, 9203 and 9216 which were found relatively more stable while 2014 was most discriminating year for plot yield. In breeding, an ideal genotype is regarded as the one that has the highest mean yield and is stable across environments (Yan & Kang, 2003). Results of this study implied that genotypes possessing good spot blotch resistance, high yield potential and adaptation under spot blotch conducive environment could be used in wheat improvement programs for developing superior genotypes for yield and spot blotch resistance contributing to food security in south Asia.

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Conflict of interest

There is no conflict of interest to declare.

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