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Genetic variability among wheat (*Triticum aestivum* L.) germplasm for resistance to spot blotch disease

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Abstract

Spot blotch caused by *Bipolaris sorokiniana* (Sacc.) Shoem. is the most devastating disease limiting wheat productivity in warm and humid environments. One hundred and fifty wheat genotypes were evaluated under field conditions in 2013 and 2014 in six different locations in Zambia. The genotypes showed different levels of resistance to spot blotch. Genotypes 19HRWSN6 (Kenya Heroe), 19HRWSN7 (Prontia Federal) and 19HRWSN15 (BRBT2/METSO) were resistant lines across environments. The genotype plus genotype by environment (GGE) biplot grouped the six environments (E) into three mega-environments (ME) with respect to spot blotch severity. ME I contained Golden Valley Agricultural Research Trust (GART) (E6) only. Mpongwe (E4), Mt. Makulu (E5 and E2) and GART (E3) formed ME II, while ME III contained only Mutanda (E1). Genotypes 16HRWYT5, SB50 and 20HRWSN33 were the most susceptible genotypes in ME I, II and III, respectively. Genotype 19HRWSN7 was the most resistant across test locations. The locations in ME III were highly correlated indicating that they provided similar information on genotypes. This suggests that one location could be chosen among the locations in ME III for screening spot blotch resistance each year if the pattern repeats across years. This could aid in reducing the cost of genotype evaluation and improve efficiency as genotypes would be handled in fewer environments.

Keywords: *Bipolaris sorokiniana*, disease, management, screening, host resistance

1 Introduction

Spot blotch caused by *Bipolaris sorokiniana* (Sacc.) Shoem. is the most important disease limiting wheat yields in warm and humid environments (Srivastava & Tewari, 2002; Mikhailova *et al.*, 2004; Khan & Chowdhury, 2011). It occurs worldwide especially in areas with high relative humidity (Mikhailova *et al.*, 2004; Acharya *et al.*, 2011). In Africa, the disease has been reported to occur in Kenya, Malawi, Sudan, South Africa, Zimbabwe (Acharya *et al.*, 2011), Madagascar (Rakotondramanana, 1981), and Zambia (Mukwavi *et al.*, 1990; Tembo *et al.*, 2016). The disease is most severe and damaging under temperatures of between 18 and 32 °C, high relative humidity (Duveiller & Gilchrist, 1994; Mehta, 1997). Spot blotch attacks all plant parts and can cause large yield losses. Yield losses due to spot blotch disease range from 25–43 % in South Asia, 18–22 % in India, 70–100 % in Nepal, 15 % in Bangladesh (Alam *et al.*, 1994) and 15–85 % in Zambia (Mukwavi *et al.*, 1990). Under severe infections the disease spreads to the spikes resulting in shrivelled grains with low grain weight and black points (Raemaekers, 1988; Gubiš *et al.*, 2010). Apart from these effects, spot blotch disease also reduces the grade and quality of wheat (Kumar *et al.*, 2002).

The management of spot blotch disease involving the use of fungicides is not only costly for small-scale farmers, but also difficult in its application and is not environmentally friendly (Iftikhar *et al.*, 2009; Eisa *et al.*, 2013). Use of proper crop rotation is also not feasible amongst small-scale farmers due to small farm sizes. Use of resistant cultivars

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is considered the most economical, cheap, sustainable and environmentally safe method of controlling the disease (Duveiller & Sharma, 2009; Iftikhar *et al.*, 2009; Iftikhar *et al.*, 2012), highlighting the need for the screening of wheat germplasm to identify sources of resistance for use in breeding programmes. Host immunity to *Bipolaris* have not yet been reported in wheat (Duveiller & Sharma, 2013). However, there are different reports on inheritance of resistance to spot blotch. Some research indicated oligogenic dominant resistance while others indicated polygenic resistance (Duveiller & Sharma, 2009). The objective of this study, was thus to screen wheat germplasm in different environments in Zambia to identify sources of resistance that could be used in breeding for resistance against spot blotch disease.

2 Materials and methods

2.1 Plant materials, experimental sites and experimental design

One hundred and fifty wheat genotypes from the Zambia Agriculture Research Institute and the International Maize and Wheat Improvement Centre (CIMMYT, Mexico) were evaluated in Zambia under natural field conditions in 'hot spot' sites (Sites where the disease occurs every year, naturally). The genotypes were screened over two successive years, 2013 (2012/13) and 2014 (2013/14) summer seasons, at three sites in each year. In 2013, the genotypes were evaluated at Mutanda Research Station (Environment 1 – E1), Mt. Makulu Research Station (E2) and Golden Valley Agricultural Research Trust (GART) (E3) (Table 1). In 2014, the genotypes were assessed at Mpongwe Seed-Co Research Farm (E4), Mt. Makulu Research Station (E5) and GART (E6). The genotypes were planted in the second week of November in each year, so that the anthesis coincided with warm temperatures and high humidity that favour disease development and spread. The experimental field was laid out in a 10×15 alpha lattice design with two replications. Each genotype was planted in a 2.5 m long plot of two rows, 20 cm inter row spacing with a plant to plant distance of 10 cm. One row of Loerrie I, a susceptible spreader was planted in the alleyways and borders to create enough disease pressure (Joshi *et al.*, 2004). Standard agronomic practices were followed for good crop management. Fertiliser application involved basal fertiliser (8 % N, 24 % P_2O_5, 16 % K_2O, 0.5 % Zn, 5 % S and 0.1 % B) applied at planting at a rate of 300 kg ha^{-1} and four weeks after planting urea (46 % N) was applied as topdressing to all plots at 150 kg ha^{-1}. Neither pesticides nor fungicides were applied. Weeding was done by hand to eliminate any possible weed competition with the crop.

2.2 Disease assessment

Disease presence was evaluated based on foliar symptoms. Five random plants were tagged at the onset of infection and were checked for disease throughout the experiment. Nonetheless, plants were scored for disease severity at Zadoks' stage ZGS77 (late milking) (Eyal *et al.*, 1987). The disease severity score was based on Saari & Prescott's (1975) scale for assessing foliar disease as cited by Eyal *et al.* (1987). Disease severity on leaves (Nagarajan & Kumar, 1998) of each plot was estimated by averaging the severity ratings of the tagged plants (Joshi & Chand, 2002). The severity was recorded on a 0–9 scale where 0 was scored on leaves with no symptoms while 9 on leaves having many extensive necrotic spots with pronounced chlorosis (Fetch Jr. & Steffenson, 1999). Genotypes falling in the 1–3 category were considered as resistant, 4 as moderately resistant, 5–6 as moderately susceptible and 7–9 as susceptible (Chaurasia *et al.*, 1999).

2.3 Data analysis

A combined analysis of variance for spot blotch severity score was performed using the general linear model procedure (PROC GLM) in SAS version 9.3 (SAS Institute, 2011). The following linear statistical model for combined analysis was used (Annicchiarico, 2002):

$$Y_{ijkm} = \mu + g_i + l_j + (gl)_{ij} + y_k + r_m(l_j y_k) + (gy)_{ik} + (ly)_{jk} + (gly)_{ijk} + e_{ijkr},$$

where Y_{ijkm} = observation of genotype i in location j in year k and replication m, μ = overall mean, g_i = effect of genotype i, l_j = effect of location j, y_k = effect of year k, $r_m(l_j y_k)$ effect of replication m within location j and year k, $(gy)_{ik}$ = genotype $i \times$ year k interaction, $(ly)_{jk}$ = location $j \times$ year k interaction, $(gly)_{ijk}$ = genotype $i \times$ location $l \times$ year k interaction and e_{ijkr} = residual effect.

A genotype main effect (G) plus Genotype × Environment interaction (GE) (GGE) biplot was used to visualize patterns amongst genotypes as either resistant and/or susceptible in each environment (location x year) and group of environments, to distinguish mega-environments and to explore relationships among test environments in their ranking of genotypes in relation to spot blotch (Yan & Tinker, 2006). A mega-environment refers to a group of environments that consistently share the best genotypes (Yan *et al.*, 2007). The vertex genotype for this sector is the winning genotype for these environments. The discriminating ability of the test environment was also determined by the length of the vector. The length of the environment vector measures the discriminating ability of the test environment. Test environments with long vectors have more discriminating ability compared to those with shorter ones (Badu-Apraku

Table 1: *Mean climatic conditions for the six environments during 2012/13 and 2013/2014 season.*

Location	*Environment*	*Temperature* (°C)		*Mean RH* † (%)	*Rainfall* (mm) *Seasonal total*	*Number of days with rain*	*Latitude (South)*	*Longitude (East)*
		Maximum	*Minimum*					
2012/13								
Mutanda	1	26.0	17.0	80.6	941.9	80	12°25.959′	26°12.620′
Mt.Makulu	2	27.0	17.0	76.5	868.8	57	15°32.946′	28°15.078′
GART ‡	3	26.0	17.0	77.0	695.8	50	14°58.185′	28°06.134′
2013/14								
Mpongwe	4	28.8	20.4	87.5	1292.7	105	12°06.622′	28°09.181′
Mt. Makulu	5	28.7	17.5	79.5	931.2	67	15°32.946′	28°15.078′
GART	6	27.1	17.9	79.5	737.8	57	14°58.185′	28°06.134′

† RH: Relative humidity, ‡ GART: Golden Valley Agricultural Research Trust.

et al., 2013). The cosine of the angle between the vectors of two locations estimates the relationship between these (Yan & Tinker, 2006) with respect to spot blotch severity. According to Yan *et al.* (2007), an angle of $< 90°$(acute angle) indicates positive correlation, an angle of 90°or −90°, correlation of zero, an angle of $> 90°$, negative correlation, while wide obtuse angles indicates strong negative correlation. The GGE biplots were computed in Genstat version 14 computer software VSN International Ltd (Payne *et al.*, 2011). The GGE biplot analysis model equation was:

$$Y_{ij} - \mu_j = \lambda_1 \xi i_1 \eta j_1 + \lambda_2 \xi i_2 \eta j_2 + \varepsilon_{ij} \quad \text{(Yan, 2001)},$$

where Y_{ij} is the average yield of ith genotype in *j*th environment; μ_j is the average disease severity score across all genotypes in *j*th environment; λ_1 and λ_2 are the singular values for principal component 1 (PC1) and PC2, respectively; ξi_1 and ξi_2 are the PC1 and PC2 scores, respectively, for *i*th genotype; ηj_1 and ηj_2 are the PC1 and PC2 scores, respectively, for *j*th environment; ε_{ij} is the residual of the model associated with the *i*th genotype in *j*th environment.

3 Results

3.1 Combined analysis of variance

Highly significant differences ($P < 0.001$) were observed among genotypes (G) for their reaction to spot blotch disease (Table 2). Locations (L), years (Y), genotype (G) × location (L), genotype (G) × year (Y), L × Y, and G × L × Y were also significant ($P < 0.001$).

3.2 Reaction of the wheat genotypes to spot blotch disease across years

During 2013, the 150 genotypes screened for spot blotch disease had a mean severity score of 4.3 with the range of between 2.0 and 8.0. Mutanda (E1) showed a mean severity score of 3.0, Mt. Makulu (E2) of 4.5 and GART (E3) of 5.0. In 2014, the disease severity score ranged between 3.0 and 8.0 with a mean of 7.0. Mpongwe (E4) had a mean severity score of 7.3, Mt. Makulu (E5) of 7.0 and GART (E6) of 6.7. The mean disease severity score of genotypes was higher in 2014 season than in 2013 season. For example genotype number 5 (16HRWYT20) showed a mean severity score of 5 in 2013 season and 7 in 2014 season, number 12 (19HRWSN2) had a mean score of 5 in 2013 and 7 in 2014 season, and number 25 (20HRWYT11) had scores of 4 in 2013 and 7 in 2014 (Table S1 in the Supplement). During both years, disease symptoms were first observed on the lower leaves and progressed upwards as the season advanced. The symptoms were visibly uniform on most plant parts at flowering stage.

Table 2: *Analysis of variance for 150 wheat genotypes for spot blotch disease severity score tested in 2013 and 2014.*

Source of variation	*Degree of freedom*	*Mean square*
Year (Y)	1	500.56 ***
Location (L)	2	303.19 ***
Y × L	2	17.13 ***
Replication (Y × L)	6	645.64
Genotype (G)	149	2.65 ***
G × Y	149	1.38 ***
G × L	298	1.69 ***
G × Y × L	298	1.54 **
Error	894	0.67
Corrected total	1799	
CV (%)	15.40	
Mean	5.32	
R^2	93.43	

***, ** indicate significance at $P < 0.001$ and $P < 0.01$, respectively

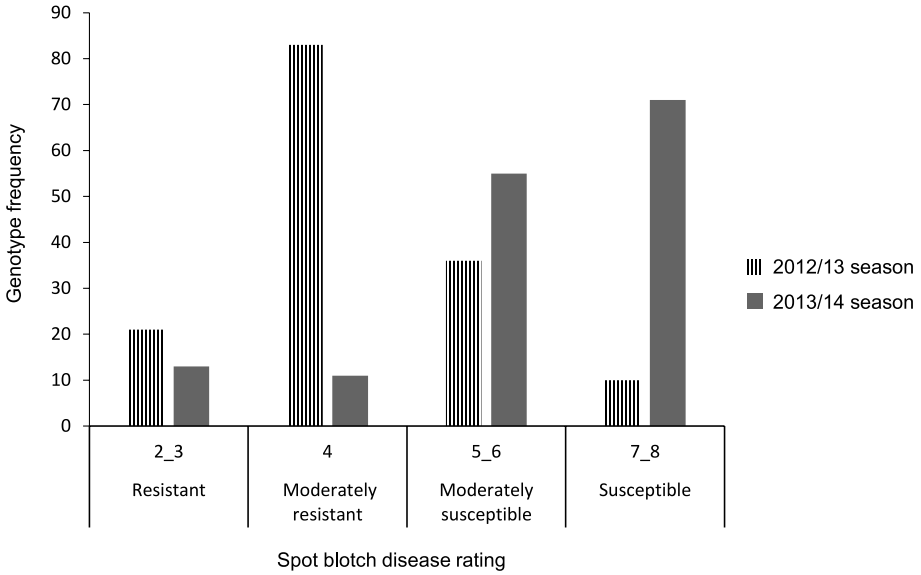

Fig. 1: *Frequency distribution for spot blotch disease severity during 2012/13 and 2013/14 seasons.*

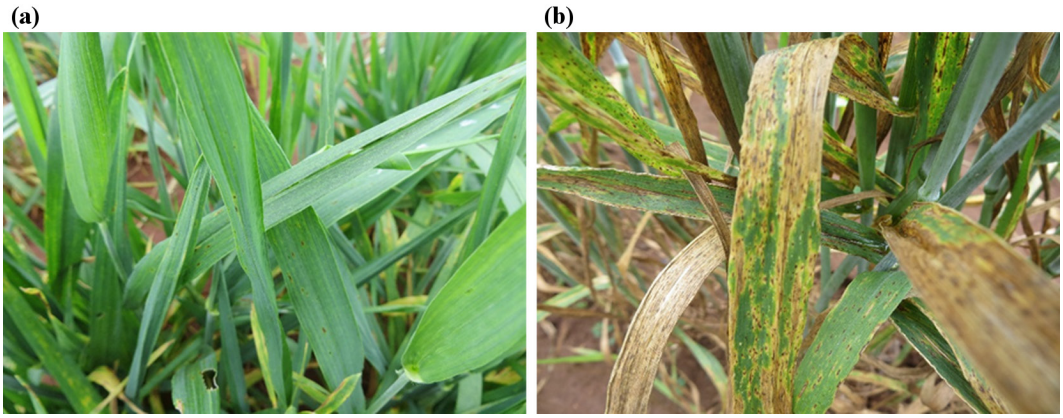

Fig. 2: ***(a)*** *Genotype resistant to spot blotch disease.* ***(b)*** *Susceptible genotype.*

Based on the 0–9 scale, none of the genotypes was symptomless during both seasons. In 2012/13 season, 21 genotypes out of 150 screened were found to be resistant (R) and 83 moderately resistant (MR), 36 were moderately susceptible (MS) and 10 were susceptible (S) (Fig. 1). During 2013/14 season, 13 genotypes were found to be resistant, 11 MR, 55 MS and 71 susceptible.

The most resistant genotypes (Fig. 2a) across environments were from CIMMYT-Mexico and included 19HRWYT6 (Kenya Heroe), 19HRWSN7 (Prontia federal) and 19HRWSN15. Some of the most susceptible genotypes (Fig. 2b) across environments were Sonalika (SB50) from CIMMYT-Mexico, and UNZAWV2, Pwele and Loerrie II from Zambia. Most of the Zambian genotypes evaluated had disease scores ranging between 5.0 and 8.0 (moderately susceptible and susceptible, respectively) across environments. No genotype from Zambia was resistant across environments.

3.3 GGE biplot analysis of environments and genotypes on spot blotch severity

The biplot (Fig. 3) explained 51.0 % (PC1 = 31.8 % and PC2 = 19.2 %) of the total genotype (G) and genotype × Environment (GE) variation. The polygon (Fig. 3) was divided by the rays into five sectors. The genotypes fell into all the sectors but the locations fell in three of them. This shows that the environments comprised of three different mega environments (I, II, and III). ME I consisted of environment 6. ME II had four environments (E) 2, 3, 4, and 5 while environment 1 appeared in mega-environment III. The vertex genotype in mega-environment I was genotype number 6 (16HRWYT5). The vertex genotypes in mega-environment

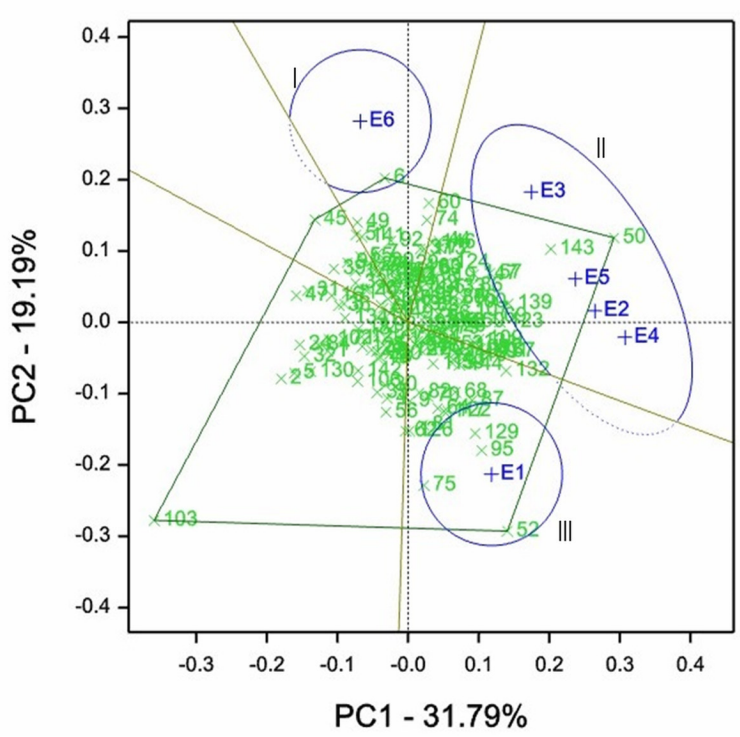

Fig. 3: *Polygon view of the total genotype (G) and genotype × environment (GE) variation (GGE) biplot based on the performance of wheat genotype with respect to spot blotch disease and also showing the mega-environments in relation to the disease. Genotypes are labelled 1 to 150. Mega-environments are labelled I, II, and III. Details for genotypes are given in Table S1. Details for environments are given in Table 1.*

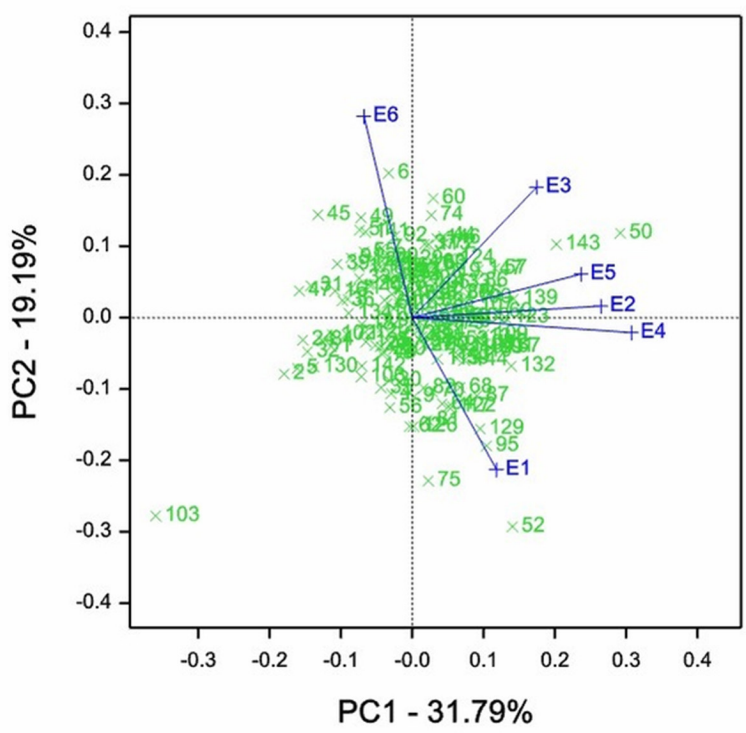

Fig. 4: *Total genotype (G) and genotype × environment (GE) variation (GGE) biplot showing relationships among test environments in discriminating genotypes in relation to spot blotch disease. Environments are labelled E1 to E6. Details for environments are given in Table 1.*

II and III were genotypes number 50 (Sonalika) and 52 (20HRWYT3), respectively. Genotype number 103 (19HRWSN7) and 45 (20HRWYT30) were the vertex genotypes in a sector where there was no environment. However, genotype number 103 was located very far away from the test locations.

In this study, all environments except E6 had positive PC1 scores. Environment 6 had a negative PC1 but close to the origin. Environments 2, 4, and 5 had positive PC2 values close to zero. Environments 6 and 3 had large positive PC2 values while environment 1 had negative PC2 scores (Fig. 4). The angle between E2, E3, E4 and E5 was less than 90°. The largest angle (> 90°) was between E6 and E1 followed by the angle between E4 and E6. With respect to vector length from the origin of the biplot, E4 had the longest vector. This was followed by E6, E1, E3, E2, and E5.

4 Discussion

Highly significant differences observed among genotypes in their reaction to spot blotch disease indicated that genetic variability existed in the material under study which provides an opportunity for further genetic improvement. The significance of years, locations, genotype × location interaction (G × L) suggests that genotypes responded differently to locations and years. Significant genotype (G) × year (Y), G × L × Y interactions indicate that the performance of genotypes was inconstant over years (Gomez & Gomez, 1984). Therefore, screening of genotypes over locations and years is worthwhile to identify genotypes with stable resistance to spot blotch disease.

Genotypes 19HRWSN6 (Kenya Heroe), 19HRWSN7 (Prontia federal) and 19HRWSN15 were found to be resistant across seasons and sites, and therefore could be utilised in wheat breeding programme to improve resistance to spot blotch. High disease severity in 2013/14 season compared to 2012/13 could be attributed to highly conducive climatic conditions such as favourable temperatures, leaves remaining wet for quite a long period of time due to frequent rainfall and dew which favoured sporulation, multiplication and spread of the disease. The results are in line with the work done by several scientists who reported a close association between weather conditions and spot blotch disease severity (Kumar *et al.*, 2002; Sharma & Duveiller, 2007; Duveiller *et al.*, 2007; Acharya *et al.*, 2011).

Genotype 6 (16HRWYT5) was the most susceptible in ME I (E6) as it is located at the vertex of the polygon. Genotype 50 (Sonalika) was the most susceptible genotype in mega-environment II (E2, E3, E4 and E5) followed by genotype 143, whereas genotype 52 was the most susceptible in mega-environment III (E1). The grouping of these genotypes in separate mega-environments was very consistent with their mean performance to spot blotch disease in the aforementioned environments. Genotype 103 (19HRWSN7) exhibited high levels of resistance to spot blotch disease across all test environments as it fell in a sector without any environment (Yan *et al.*, 2001). Genotypes on the vertex of the polygon in each sector are either the best or worst performing as they are further from the biplot origin (Yan & Tinker, 2006). Additionally, they are the most responsive compared to those located within the polygon (Adu *et al.*, 2013). However, those within the polygon but close to the origin, show average reaction across all environments (Yan & Falk, 2002). In this case, genotype 12, 58, 120 and 134 were some examples of genotypes that showed average reaction to spot blotch severity across all locations. Hence, therefore, GGE biplot analysis is an important tool for visualizing patterns amongst genotypes as either resistant and/or susceptible in each environment and group of environments.

In terms of environmental correlations, environments within ME II were highly correlated in their ranking of genotypes as indicated by the angle between them which was less than 90° (Yan *et al.*, 2007). This indicates that similar information about genotypes was obtained from this mega-environment, suggesting that one location within this mega-environment could be chosen for genotype evaluation in each year if the pattern repeats across years (Yan *et al.*, 2007). This would help to reduce on the cost of evaluating genotypes and improving efficiency of screening for resistance. The angle between environments E6 and E1, and between E6 and E4 was quite large showing that the environments were not correlated.

In terms of location versus season relationships, Mt. Makulu locations (2 and 5) in both seasons fell in one sector suggesting repeatable performance of genotypes in this location. Repeatability is very essential for assessing a test location that is representative of all test locations over years (Badu-Apraku *et al.*, 2013). Thus, a location is considered highly representative if its genotypic rankings are repeated across years, so that genotypes selected in one year will have greater performance in forthcoming years (Yan *et al.*, 2011). GART environments (E3 and E6) fell in different sectors both years, suggesting that there was no repeatability of genotypes in this location.

All locations except environment 6 had positive PC1 scores, an indication that they were discriminating genotypes. However, environments Mutanda (E1), Mpongwe (E4) and GART (E6) were considered highly discriminating among genotypes as shown by the length of their vectors from the biplot origin. The length of a vector of a test environment estimates the discriminating ability of gen-

otypes (Badu-Apraku *et al.*, 2013). The longer the vector the higher the ability to discriminate genotypes and the shorter the vector the lesser the discriminating ability (Yan & Tinker, 2006). Mt. Makulu environments (E2 and E5) had short vectors indicating that they had the least discriminating ability of genotypes. Yan *et al.* (2010) indicated that environments with shorter vectors could be considered as independent test environments, treated as unique and essential test environment.

The GGE biplot showed that environments 1 (Mutanda), 3 (GART2012/13) and 6 (GART2013/14) contributed most of the genotype by environment interaction (GEI) variability in terms of genotype reaction to spot blotch disease as these were located further apart in the biplot (Joshi *et al.*, 2007). This implies that a genotype could have huge positive interaction with some environments while having large negative interactions with some other environments (Yan & Hunt, 2001). The GEI could affect the efficiency of breeding for resistance. Pinnschmidt & Hovmøller (2002) reported that GEI affects breeding for high levels of resistance due to inconsistency in the phenotypic expression of the disease. Moreover, it complicates selection of desirable genotypes (Farshadfar *et al.*, 2012).

In conclusion, genetic variation existed among genotypes to the reaction of spot blotch disease. Most of the resistant and moderately resistant genotypes were identified among CIMMYT-Mexico lines. Some of the resistant genotypes identified across locations included 19HRWSN6, 19HRWSN7 and 19HRWSN15. These resistant genotypes could be used as valuable source in breeding for resistance to the disease. The GGE biplot analysis identified genotype 19HRWSN7 as the most resistant across all test environments. Therefore, GGE biplot analysis could efficiently be used to identify genotypes resistant to spot blotch disease over locations.

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