Simultaneous Selection Index as a Tool for Identification of Stable High Yielding Maydis Leaf Blight Resistant Maize Prebreeding Lines

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Abstract

Maize is a crop possessing high adaptability however, large differential genotypic responses have been reported when evaluated under multiple environments. Using randomized complete block design with two replications a total of 169 teosinte derived maize backcross inbred lines (BILs) were evaluated in three different environments namely, E2, E4 and E6 for maydis leaf blight (MLB) resistance and grain yield. Out of these, 73 BILs were identified displaying resistance to MLB in at least one of the environments and were subjected to additive main effect and multiplicative interaction (AMMI) analysis and genotype and genotype X environment (GGE) biplot analysis for identification of lines showing stable and high MLB resistance and grain yield. Highly significant effects of genotype, environment and genotype X environment interaction (GEI) were observed for both the traits studied. AMMI ANOVA for percent disease index (PDI) revealed that highest percentage of total sum of squares (SS) was attributed to GEI (40.55%) while 32.86% and 26.59% was contributed by genotype and environment, respectively. For grain yield largest contribution of 68.02% towards SS was done by genotype component followed by GEI (17.50%) and E (14.48%). GGE biplot analysis identified two mega environments for both PDI (E2, E4/E6) and grain yield (E2/E4, E6). Based on AMMI stability value (ASV), genotype MT-90 (32) was observed to be most stable for PDI. While for grain yield highest stability was displayed by genotype MT-83 (28). Simultaneous selection index (SSI) helped in identification of ten stable high yielding MLB resistant genotypes namely, MT-120 (45), MT-14 (2), MT-166 (62), MT-148 (55), MT-190 (72), MT-37 (9), MT-19 (3), MT-114 (42), MT-77 (27) and MT-94 (35) which could be used in future breeding programmes either as donor of MLB resistance and grain yield or after combining ability analysis these genotypes could be used as parents for development of superior yielding MLB resistant hybrids.

Introduction

Maize, exhibiting highest genetic yield potential amongst different cereals thereby earning for itself the title “queen of cereals” is a crop of global repute. Grown in more than seventy countries (Anonymous 2018) along with rice and wheat maize provides 60% of the world’s energy intake (Anonymous 2008) and contributes 39% of grain production globally. In Indian context, maize is the third largest produced and consumed crop after wheat and rice (Kumar et al. 2013) and contributes significantly towards the poultry industry (Hellin and Erenstein 2009). Like other crops, maize cultivars are also confronting with various factors during different developmental phases to realize its genetic potential while grown commercially at farmers’ field or grown at experimental site. Consequently, the crop growth and development is severely affected leading to sub-optimum outputs. Apart from abiotic factors that constitute all the non-living components and interacts right from the seed germination to maturity stages, maize crop is also affected by biotic factors, many of them are beneficial while others are harmful, interfering at different stages, from germination to maturity and during storage, leading to loss of green biomass, green ears including baby corn and sweet corn and, grain yield. Diseases caused by a fraction of biotic factors are 61 in numbers and have been reported to cause about 13.2% loss in economic product per annum (Payak and Sharma 1985; Kumar et al. 2013). Maydis leaf blight (MLB) is one of the diseases significantly affecting maize production in India and abroad.

A disease of historical significance due to its epidemic propositions in 1970 in the US, MLB is caused by a necrotrophic ascomycete fungi *Bipolaris maydis*. The disease can cause as high as 70% yield losses to maize production (Mubeen et al. 2017). Almost all the yield losses in India are attributed to race O of the pathogen as race C is confined only to China (Wei et al. 1988) and race T affects Texas male sterile cytoplasm (cmsT) maize (Carson et al. 2004) which is not widely cultivated in the country. Though chemical fungicides can be used for controlling the disease, yet the additional input requirement and the associated ill effects necessitate the search of novel genes and alleles and their deployment to incorporate gene-based protection mechanism in the plant. In fact, development of tolerant genotypes by integrating novel alleles/genes seems to be the most feasible, attractive, cost effective and

long-term alternative for management of MLB in maize. Resistance to MLB in maize is reported to be both qualitative (Faluyi and Olorode 1984; Zaitlin et al. 1993; Chang and Peterson 1995)and quantitative (Pate and Harvey 1954; Kumar et al. 2016) in nature. The development of MLB resistance using quantitative genetic factors is advantageous in many ways as it not only provides resistance against many pathogenic races but also prevents the evolution of new and more virulent pathogen variants. A number of MLB resistant varieties have been derived using maize germplasm belong to primary gene pool however prospects of wild relatives and biological progenitor of maize in incorporating resistance to MLB has remained largely unexplored. The narrow genetic base of cultivated maize is an important factor limiting the breeding of new maize varieties for high-yield and disease resistance (Wallace et al. 2014). Sourcing resistance from wild relatives is advantageous because they possess a plethora of novel resistance alleles on account of being exposed to difficult environments from times immemorial and still surviving, flourishing and thereby continuously evolving under the existing and emerging climatic conditions. The breeding goals would be easier to address if the enormous genetic variation present in wild progenitors is available to the breeders in a form, they could use in their breeding programs.

Teosinte (*Zea mays* ssp. *parviglumis*), the nearest and most probable wild progenitor of maize is interfertile with maize and produces viable hybrids (Doebley et al. 1984; Singh et al. 2017) and in certain parts of the world is still believed to be exchanging genes with maize naturally. Teosinte can be used as donor of pre-domestication alleles for the improvement of maize with respect to different traits (Liu et al. 2016; Kumar et al. 2019; Adhikari et al. 2019, 2021; Singh et al. 2021; Joshi et al. 2021, Sahoo et al. 2021). This could be done by creation of diverse prebreeding lines which would serve as donor of novel genetic variation and could be employed to breed for high value characteristics such as MLB disease resistance. Only a single study has been performed by Lennon et al. (2017) in which maize wild relative *Zea mays* spp. *parviglumis* was used as a donor of MLB resistance alleles.

Development of resistant genotype is essential, however, besides being resistant, a genotype must be high yielding and stable in performance in order to be commercially viable and substantially accepted by maize growers over a wide range of agroclimatic conditions. Yield alike MLB resistance is a complex quantitative trait and greatly influenced by external environment which may result in shift in scale or rank of the genotype performance when grown in diverse environments (Dia et al. 2016a). Differential genotypic response with respect to yield and disease resistance when grown in diverse environments have also been observed by Aina et al. (2007) and Ssemakula and Dixon (2007). Popularly called as the GxE interaction, the differential sensitivity of genotype performance to environments complicates the identification of superior genotype across the environments (Dia et al. 2016b). The presence of GxE interaction reduces the correlation between genotype and phenotype therefore slows the progress due to selection (Chalwe et al. 2017). Therefore, the nature and magnitude of GxE interaction must be taken into consideration during the identification of superior genotypes. Multi environment trials are an effective tool for identification of GxE interaction. A number of different methods (Wricke 1962; Eberhart and Russell 1966; Perkins and Jinks 1968; Shukla 1972; Francis and Kannenberg 1978; Lin and Binns 1988) are available for stability assessment however, the most commonly used methods for studying GxE interaction is the additive main effect and multiplicative interaction (AMMI) model and the genotype and genotype x environment (GGE) biplot (Yan et al. 2001).

The GGE biplot is a statistical tool utilized for examining the performance of genotypes tested in different environments. The which-won-where biplot helps in identification of mega environments and the winning genotypes for each mega environment (Yan 2001). The AMMI model combines analysis of variance for additive main effects with principal component analysis for the multiplicative interaction. Stability of a genotype can be assessed by calculating the AMMI stability value (ASV) (Hagos and Abay 2013) which is based on interaction principal components axes 1 (IPCA1) and 2 (IPCA2) (Purchase et al. 2000). A widely adapted genotype displays least ASV value (Adjebeng-Danquah et al. 2017). Selection of genotypes with stable MLB resistance and grain yield over a range

of different environmental conditions and in the presence of variable disease pressure is an ideal strategy for making optimal progress in resistance breeding (Gyawali et al. 2019). Keeping all these observations in perspective a study was planned to generate diverse pre breeding lines by crossing maize inbred line DI-103 with MLB resistant teosinte (*Zea mays* spp. *parviglumis)* and using the MLB resistant inbred lines so produced for AMMI analysis for grain yield and MLB reaction score parameters with objective to identify genotypes that displays stable and high grain yield combined with resistance to MLB. Lines identified to be high yielding and MLB resistant can be used as potential germplasm in maize breeding programme.

Materials And Methods

Experimental materials

The genetic materials for the experiment were developed at Norman E. Borlaug Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. Wild relative of maize, teosinte (*Z. mays* ssp.*parviglumis*) was crossed as pollen parent with a superior maize inbred line DI-103 used as a seed parent. The F_1s thus produced were subjected to a single generation of backcrossing with DI-103 followed by selfing for four consecutive generations leading to development of BC_1F_5 backcross inbred lines (BILs) population. A total of 169 BILs were subsequently utilised for the purpose of experimentation.

Experimental Layout

The evaluation of experimental material consisting of 169 BC_1F_5 lines was done in *Kharif* 2018-2019. Each line was planted in a single row 2 m long and 75 cm apart. These lines were evaluated in Randomized Complete Block Design with two replications in three different environments namely E2, E4 and E6. The details of study environments along with magnitude of whether parameters during the crop season is presented in table 1.

Observation procedure

Data on MLB reaction and grain yield was recorded for all the 169 BILs in three different environments. For the purpose of screening of BILs for MLB response by creating artificial epiphytotic conditions in E2 and E4 the causal organism *Bipolaris maydis*, was aseptically isolated from locally collected infected maize leaves. The pathogen was initially cultured on sterilized solidified Potato Dextrose Agar (PDA) medium containing petri plates. For the purpose of mass multiplication of the pathogen the autoclave bags containing sorghum grains were seeded with 1.5x1.5cm rectangular pieces of *Bipolaris maydis* mycelium containing solidified PDA medium under aseptic conditions and incubated at a temperature of 25-27^{0}C for 10 days with intermittent shaking after every 2-3 days to facilitate uniform mycelium growth on grains. After shade drying 15-20 mycellium covered sorghum grains were placed in the leaf whorls of 35 days old plant. The field was frequently sprayed with water for effective spore germination and disease development. The entire process of mass multiplication and field inoculation of pathogen have been presented in Fig. 1. For natural spread of MLB in E6, a susceptible check was sown after every ten BILs and also as a border crop so as to ensure uniform disease inoculum for all the lines. Data on disease severity was recorded 35 days after inoculation for each line in all three environments by following 1 to 9 rating scale of Hooda et al. (2018) (Table 2). Disease rating was converted into percent disease index (PDI) by using the following formula:

$$\text{PDI}=\frac{\text{Sum of all the numerical rating}}{\text{Total number of observation}\times\text{maximum rating}}\times 100$$

In order to assess grain yield per plant, ears from three plants per entry per replication per environment were harvested after physiological maturity and weighed. Average grain yield over three plants was calculated and expressed in gm and further utilized for analysis.

Statistical Analysis

Combined Analysis of Variance

Combined Analysis of variance with respect to both PDI and grain yield was done. For both the traits the data was heterogenous at 5% level of significance as revealed by Bartlett's Chi-square test therefore, the data was transformed before combined ANOVA was carried out.

AMMI and GGE biplot analysis

AMMI analysis was conducted for both PDI and grain yield in order to divide the total variation into variation due to genotype, environments, and interaction as per the model given by Gauch and Zobel (1996) as mentioned below:

$$Yger = \mu + \alpha g + \beta e + \sum \lambda n ygn \delta en + \rho ge + Eger$$

where,

Yger = Performance of genotype g in environment e for replicate r, μ = grand mean, αg = genotype mean deviation (genotype means minus grand mean), βe = environment mean deviation, n = number of principal component analysis (PCA) axes retained in the model, λn = singular value for PCA axis n, ygn = genotype eigenvector values for PCA axis n, δen= environment eigenvector values for PCA axis n, ρge = residuals, Eger = error term.

Both AMMI and GGE biplot analysis was done with the help of GEA-R (Genotype x Environment Analysis with R for Windows) software version 2.0.

AMMI stability value (ASV)

Stability of genotype with respect to MLB resistance and grain yield was assessed across environments by means ASV as suggested by Purchase et al. (2000). ASV was calculated as per the formula given below and was used to rank genotypes according to trait stability. Lower ASV signifies more stable genotypes.

$$ASV = \sqrt{\left(\frac{IPCA1\ sum\ of\ square}{IPCA2\ sum\ of\ square}\ X\ IPCA1\ score\right)^2 + (IPCA2\ score)^2}$$

Genotype Stability Index (GSI)

The rank of genotype (RT) based on overall mean of the genotype for a specific trait across seasons and ASV rank of the genotype for that particular trait (RASV) were added up to calculate the GSI as given below and suggested by Chalwe et al. (2017).

GSI= RASV + RT

Genotype with lowest GSI was considered to be superior and stable for the trait under consideration.

Simultaneous Selection Index (SSI)

GSI for both MLB resistance and grain yield were summed up to calculate SSI for each genotype.

$SSI = GSI_{MLB\ resistance} + GSI_{grain\ yield}$

A genotype with lower SSI was considered to be superior and stable with respect to both MLB resistance and grain yield.

Results And Discussion

Categorisation of BC_1F_5 lines into different disease resistance classes when evaluated in multiple environments

Differential response for MLB resistance was observed amongst the evaluated lines (Fig. 2) and on the basis of rating scale proposed by Hooda et al. (2018) the BILs were categorised into four groups *i.e.,* susceptible, moderately susceptible, moderately resistant and resistant (Table 3). When evaluated under E2 the PDI varied from 22.2 to 95.49% and a mean PDI of 59.46% was observed. Of the 169 BILs investigated, 26, 77, 51 and 15 were scored as susceptible, moderately susceptible, moderately resistant and resistant to MLB, respectively. In E4, the mean PDI of 70.96% was observed with PDI value ranging from 29.63% to 85.19 %. A total of 64, 91, 10 and 4 lines were scored as susceptible, moderately susceptible, moderately resistant and resistant to MLB, respectively. Screening for MLB resistance under E6 revealed a mean PDI of 54.48% with PDI ranging from 23.5 to 94%. A total of 62, 63, 33 and 11 lines were grouped into susceptible, moderately susceptible, moderately resistant and resistant categories with respect to MLB response, respectively. Ott (2008) also observed variability for northern leaf blight resistance in the teosinte derived maize introgression lines. As the parental maize inbred line DI-103 was susceptible to MLB, the MLB resistance in the derived BILs were thought to be the result of teosinte genomic introgression. In accordance with our study, the near isogenic lines containing teosinte genomic introgression in the background of the maize inbred B73 were also developed by Liu et al. (2016) and when screened for grey leaf spot (GLS) resistance by Lennon et al. (2016) the lines were identified with different GLS resistance alleles sourced from teosinte. The unimproved germplasm though a source of novel resistant alleles is scarcely used in breeding programme due to associated inferior characteristics as low productivity, high plant and ear height and high stalk and root lodging (Teixeira and Guimarães 2021). The MLB resistant pre-breeding lines developed in this study can serve as a link between unimproved and improved germplasm and can be utilized effectively in future breeding activities as source of MLB resistance. When all three environments were considered together a total of 73 BILs which showed resistance in at least one of the environments were identified. As the objective of our study was mainly to detect superior and stable genotypes for MLB resistance and grain yield and also to reduce clutter in biplots, based on the preliminary MLB resistance evaluation, a subset of only 73 resistant BILs were subsequently utilised for combined ANOVA, AMMI analysis, GGE biplot analysis and computation of $GSI_{MLB\ resistance}$, $GSI_{grain\ yield}$ and SSI.

Combined Analysis of Variance for PDI and grain yield

Combined Analysis of Variance of the shortlisted 73 resistant BILs was done for PDI and grain yield using the data obtained after evaluation under three environments. Combined ANOVA (Table 4) across the environments revealed presence of highly significant differences ($P < 0.01$) for genotypes, environment and GXE interaction (GEI) for both PDI and grain yield. In accordance with our study significant G, E and GEI effects were also reported by Sibiya et al. (2012) for grey leaf spot disease resistance and grain yield in elite African maize germplasm. (Gyawali et al. 2019) also reported significant G and GEI while evaluating barley genotypes in multiple environments for resistance to spot form of net blotch. For grain yield, significant G, E and GEI in maize genotypes were also detected by Mafouasson et al.

(2018) and Aktas and Ure (2020). Highly significant environmental effect demonstrated that the experiments were carried out under differing climatic conditions affecting both PDI and grain yield of genotypes. The presence of significant GEI indicates that MLB resistance response and the yield of the evaluated BILs varies with the environments which reduces the correlation between genotype and phenotype for a specific trait thereby making the selection of genotype less effective and hence ultimately reducing the speed of crop improvement. Therefore, reducing the magnitude of GEI is important. Reduction in GEI can be achieved by means of stability analysis and selection of only those genotypes which shows wider adaptability (Yaghotipoor and Farshadfar 2007).

AMMI analysis of variance for PDI and grain yield

AMMI ANOVA for PDI revealed that highest percentage of total sum of squares (SS) was attributed to GEI (40.55%) while 32.86% and 26.59% was contributed by genotype and environment, respectively (Table 5). GEI was further divided into interaction principal components axes (IPCA) the first two of which were statistically significant. The first IPCA (IPCA1) had maximum share of 64.09% whereas IPCA2 had share of 35.91% in the total GEI sum of square. In accordance with our study, significant G, E and GEI was also observed by Persaud et al. (2019) for sheath blight resistance in rice with GEI contributing as high as 39.52% to the total SS. They also identified two significant principal components contributing greater than 70% of the total GEI effect. Highly significant G and GEI were also observed for spot form of net blotch disease in the evaluated 340 barley genotypes by Gyawali et al. (2019). As GEI effect was larger than the genotype effect the genotypes were ascribed to show variable performance for MLB resistance in different environments which makes the identification of stable genotypes more crucial.

For grain yield, AMMI ANOVA revealed the largest contribution of 68.02% towards SS by the genotype main effect followed by GEI (17.50%) and E (14.48%) as presented in Table 5. The contribution by GEI to SS was 3.88 times smaller when compared to genotype component however it was greater than contribution due to environment. Two statistically significant principal components namely, IPCA1 and IPCA2 accounted for 96.25% and 3.75% of the GEI effect, respectively. Significant G, E and GEI was also observed earlier by Mafouasson et al. (2018) while evaluating yield stability of single cross maize hybrids. A large SS for genotypes displayed that the diverse nature of genotypes with greater differences among the mean grain yield was responsible for causing most of the variations. GEI was 3.89 times smaller than that for the sum of square for G. Further, sum of square due to G x E was 1.2 times larger than that for E. This clearly showed that grain yield was mainly ascertained by the genotype and GEI and E had a very little role to play thereby suggesting the possible existence of different genotype groups (Mohammadi et al. 2011). It is evident from table 5 that for both PDI and grain yield using biplots in interactions was very advantageous as the first two PCA axes explained 100% of the total interaction effect. The conclusion drawn on genotype stability based on these two axes was therefore very reliable. Different researches have also indicated that GEI was precisely predicted by two PCAs (Gauch and Zobel 1996; Nayak et al. 2008; Mukherjee et al. 2013). Accordingly, the stability values and index were calculated by retaining two PCA axes in the model for both the traits.

Predictions from AMMI1 biplot

AMMI1 biplot consists of main effects for the trait in question displayed on the X axis and IPCA1 scores displayed on the Y axis. On the basis of mean of the trait and the IPCA1 scores the genotypes and environments are plotted on to the biplot. The central vertical line on the biplot denotes grand mean of the genotypes for the trait while the horizontal line indicates an IPCA1 value of zero. IPCA1 scores are an indication of genotype stability. Lesser the IPCA1 score more stable the genotype. An IPCA1 score of zero indicates highly stable genotypes across all the environments. The larger the IPCA scores, either positive or negative, the more specifically adapted a genotype is to certain environments (Mafouasson et al. 2018). AMMI1 biplot (Fig. 3a) between IPCA1 and PDI revealed that four genotypes namely MT-40

(10), MT-107 (39), MT-120 (45) and MT-151 (56) falling almost on vertical line had PDI equivalent to the PDI grand mean of all genotypes while seven genotypes namely MT-19 (3), MT-114 (42), MT-120 (45), MT-148 (55), MT-151 (56), MT-172 (64) and MT-190 (72) falling almost on horizontal line had equal GEI effects with IPCA1 scores of almost zero signifying a small interaction with the environments and were considered to be the most stable with respect to MLB resistance. Out of these MT-148 (55) was located on the extreme left and therefore was identified as line having high degree of resistance and lower fluctuations to MLB. On the other hand, MT-172 (64) was located on the extreme right showing stable and susceptible disease response. The highly resistant genotype MT-148 (55) identified in the present study could be used as potential source for disease resistance while MT-172 (64) could be used as susceptible host for producing pathogen inoculum for MLB screening notwithstanding the diverse environments. Similar identification and utilisation of stable blast resistant and susceptible genotypes was also proposed by Persaud and Saravanakumar (2018).

Thirty four genotypes i.e., 1, 4, 7, 11, 12, 13, 14, 17, 18, 19, 20, 21, 22, 23, 24, 27, 30, 31, 32, 33, 34, 37, 38, 44, 46, 47, 51, 53, 59, 64, 67, 68, 70 and 71 falling on the right side of the vertical line were expected to be more susceptible to MLB while thirty three genotypes namely, 2, 5, 6, 8, 9, 15, 16, 25, 26, 28, 29, 35, 36, 40, 41, 43, 48, 49, 50, 52, 54, 55, 57, 58, 60, 61, 62, 63, 65, 66, 69, 72 and 73 situated on left side of the vertical line were expected to have higher degree of resistance to the disease. The situation of the E4 on right side of the vertical axis indicates that the environment was more suitable for development of MLB while the E2 was least suitable. Environment E6 on the other hand had intermediate suitability for MLB development. The E4 therefore could be preferentially used for screening of genotypes for MLB resistance. The suitability of E4 to support greater disease development can also be validated from the initial MLB screening experiments conducted in all three environments which showed that highest number of 155 susceptible lines were identified in E4 followed by E6 with 125 susceptible lines and E2 with 103 susceptible lines. The E6 with low IPCA1 scores of 0.193 had little interactions, while a greater GEI was recorded for E2 and E4 with IPCA1 score of -1 and 0.807, respectively. A significantly larger number of genotypes recorded low IPCA1 scores of -0.5 to 0.5 and showed small interactions, which led to clustering of the genotypes on the biplot. Genotypes MT-184 (68) and MT-9 (1) showed the highest IPCA1 score of -1 and -0.945, respectively. Genotypes possessing stable response to disease severity across the environments are possessing quantitative disease resistance while genotypes showing specific adaptations to particular environments are expected to harbour monogenic or oligogenic qualitative resistance (Gyawali et al. 2019). Based on this criteria genotypes, MT-9 (1), MT-27 (6), MT-45 (12), MT-55 (14), MT-60 (17), MT-89 (31), MT-107 (39), MT-184 (68), MT-187 (70) and MT-188 (71) were identified harbouring qualitative resistance against MLB. These lines can be investigated further in order to identify novel genes imparting MLB resistance in maize.

For grain yield (Fig. 3b) a total of three genotypes MT-77 (27), MT-114 (42) and MT-174 (65) were situated on the vertical line and displayed similar means. While genotypes namely, MT-14 (2), MT-40 (10), MT-83 (28), MT-85 (30), MT-89 (31), MT-105 (38) and MT-120 (45) with IPCA1 scores of nearly zero lied near the horizontal line and showed stable grain yield across environments. Out of these MT-120 (45) was situated on the extreme right side of the vertical line and near the horizontal line signifying high and stable grain yield. This genotype after assessment of combining ability can therefore be used as parental line for production of high yielding hybrids or as a component line in development of synthetic varieties. A total of thirty-one and thirty-seven genotypes were located on the right and left side of the vertical line. Genotypes located on the right side namely, 1, 2, 3, 5, 6, 7, 8, 9, 11, 15, 16, 17, 18, 20, 23, 24, 30, 34, 35, 40, 45, 47, 51, 53, 55, 62, 64, 66, 67, 68 and 72 displayed higher grain yield while those located on left side i.e., 4, 12, 13, 14, 19, 21, 22, 25, 26, 29, 31, 32, 33, 36, 37, 38, 39, 41, 43, 44, 46, 48, 49, 50, 52, 54, 56, 57, 58, 59, 60, 61, 63, 69, 70, 71 and 73 showed inferior yield. Environment vector for E6 was situated on the right side while those for E2 and E4 was situated on the left side of the vertical axis thereby indicating that these environments were associated

with higher and lower grain yield, respectively. AMMI1 biplot for PDI also revealed that E6 environment was associated with reduced development of MLB compared to E2 and E4 and lower disease development could be one of the reasons for higher yield in E6. The E6 vector had the longest length followed by E4 and E2. The length of the environment vector serves as an indication for the IPCA1 score of the environments. E2 and E4 with low IPCA1 scores of 0.498 and 0.502, respectively, had little interactions, while a greater GEI was recorded for E6 with IPCA1 score of -1. A significantly larger number of genotypes recorded IPCA1 scores of -0.667 to 0.334 leading to clustering of the genotypes on the biplot. Genotypes MT-176 (66) and MT-72 (24) showed the highest IPCA1 score of 1 and -0.759, respectively and therefore were specifically adapted.

Predictions from AMMI2 biplot

In the AMMI2 biplot for PDI (Fig. 4a), more responsive genotypes and environments are displayed away from the origin. E2 was the most differentiating environment followed by E4 and E6. Genotypes MT-9 (1), MT-25 (4), MT-45 (12), MT-55 (14), MT-60 (17), MT-67 (21), MT-89 (31), MT-126 (46), MT-140 (53), MT-156 (59) MT-184 (68), MT-185 (69), MT-187 (70) and MT-188 (71), were some of the most responsive lines as they were situated away from the origin while genotypes MT-74 (26), MT-77 (27), MT-84 (29), MT-90 (32), MT-101 (37), MT-129 (48), MT-136 (51) and MT-166 (62) situated in the vicinity of the origin were least responsive and hence more stable with respect to MLB disease reactions. The genotypes falling in same sector with a particular environment shows positive interaction with that environment while those falling in opposite sector showed opposite interaction with that environment. The genotypes falling in adjacent sector to a particular environment showed complex interactions. Genotype MT- 136 (51) and MT-166 (62) were situated closer to E2 while MT-35 (8), MT-69 (23), MT-93 (34) and MT-94 (35) were closer to E6 and therefore, displayed better adaptation in respective environments.

The AMMI2 biplot for grain yield (Fig. 4b) revealed that genotypes MT-41 (11), MT-45 (12), MT-72 (24), MT-176 (66) are away from the origin while genotypes MT-40 (10), MT-68 (22), MT-74 (26), MT-83 (28), MT-85 (30), MT-89 (31), MT-90 (32), MT-105 (38), MT-120 (45), MT-166 (62) are near the origin and thus they were respectively, more responsive and more stable for grain yield. The E6 was most differentiating environment followed by E4 and E2. Hence E6 can be used for screening of genotypes for grain yield. Genotypes MT-114 (42), MT-120 (45), MT-129 (48), MT-195 (73) were closer to E2; genotypes MT-32 (7), MT-37 (9), MT-63 (19), MT-68 (22), MT-74 (26), MT-93 (34), MT-101 (37), MT-107 (39) were closer to E4 and genotypes MT-9 (1), MT-61 (18), MT-95 (36), MT-137 (52), MT-144 (54), MT-151 (56), MT-161 (61), MT-172 (64) were closer to E6 indicating their specific adaptation to the respective environments and therefore varieties or hybrids derived from these genotypes can be recommended for cultivation in the respective environments.

GGE biplot analysis for PDI and grain yield

The magnitude of GEI was further studied by genotype and genotype x environment (GGE) biplot analysis. The first two principal components (PCs) of the GGE biplot accounted for 85.5% (PC1 = 54.5%, PC2 = 31.0%) of the total variation for PDI over three environments, while for grain yield the PCs explained a total of 99.2% (PC1 = 80.5%, PC2 = 18.7%) of the total variation.

The average environment coordination (AEC) view of GGE biplot for PDI (Fig. 5a) revealed that E6 was the most discriminating environment followed by E4 while E2 was least discriminating. The average environment axis (AEA) was used to show the ideal test environment for MLB. The E6 was placed closest to the “average environment” and was considered suitable for MLB resistance screening. However, E4 and E2 being situated away from the AEA were least representative of the ideal environment. For grain yield, E4 was identified as the most discriminating environment followed by E2 while E6 was least discriminating environment (Fig. 5b). E4 on account of being located closest to the AEA was also identified as the most representative of the ideal environment and was followed by E2.

The E6 was the least discriminative environment for grain yield. In Fig. 5a and Fig. 5b, the genotypes dispersed away from the origin and distanced from each other were different with respect to MLB resistance and grain yield, respectively. These genotypes were least stable and contributed to both G and GEI. While genotypes situated near to the origin were highly stable and contributed insignificantly to both G and GEI as discussed by Persaud et al. (2019) while evaluating rice genotypes in multiple environments for resistance to sheath blight.

An ideal test environment that can be recommended for testing of genotypes for a specific trait must have high discriminating ability for the genotypes as well as be true representative of all the environments (Yan and Kang 2002; Tekalign et al. 2017). In our study E6 had high discriminating ability for MLB resistance of genotypes and was also an ideal representative of all the environments as evident from the AEC view of GGE biplot for PDI. The E6 can therefore be recommended for selecting generally adapted genotypes for MLB resistance. For grain yield E4 could be used for selecting broadly adapted genotypes. The E4 was discriminating but was not an ideal representative of test environment this could therefore be used to select specifically adapted genotypes for MLB resistance as proposed by Yan and Tinker (2005).

Which-won-where biplot for PDI and grain yield

The which-won-where biplot confirmed that only two mega environments existed for PDI (Fig. 6a). The E2 constitutes one mega environment while E4 and E6 present in same sector constitute the second mega environment. Genotypes MT- 60 (17) and MT-67 (21) were the vertex genotypes in the sector that had environments E4 and E6. As these genotypes had high PC1 scores they were most susceptible to MLB in the environment they were located in. While MT-184 (68) was the vertex genotype for the sector containing environment E2 and had negative PC1 score indicating it to be the most resistant in E2. The other genotypes in the polygon view situated near the origin displayed low positive or negative PC1 scores signifying moderate resistance to moderate susceptibility and showed reduced responsiveness when compared with vertex genotypes (Tekalign et al. 2017) in a particular mega environment. For grain yield also two mega environments were observed (Fig. 6b). One mega environment consisted of E2 and E4 and MT-183 (67) was the vertex genotype while E6 was a part of second mega environment with MT-61 (18) and MT-72 (24) as vertex genotypes displaying highest grain yield in their respective environments. These genotypes can therefore be recommended as high yielding genotypes for their respective mega environments. Two mega environments for grain yield with different winning genotypes were also detected by Erdemci (2018) while evaluating chickpea genotypes in multi environment trials.

Stability of genotypes based on ASV for PDI and grain yield

ASV was calculated in order to estimate stability of genotypes with respect to MLB resistance and yield under three environments (Table 6). The genotypes having lower ASV were said to be more stable. For PDI, the genotype MT-90 (32) with lowest ASV of 0.193 was observed to be the most stable. Other genotypes with lower ASV scores were MT-148 (55), MT-77 (27), MT-151 (56), MT-120 (45), MT-172 (64) and MT-190 (72) having ASV scores of 0.210, 0.212, 0.214, 0.214, 0.214 and 0.214, respectively. These genotypes are assumed to exhibit broad adaptation for PDI while genotypes MT-184 (68), MT-9 (1), MT-89 (31), MT-60 (17), MT-188 (71) with relatively higher ASV scores of 1.934, 1.695, 1.483, 1.308 and 1.286 are expected to show specific adaptation and greater GEI for PDI. The genotypes identified to be promising were MT-83 (28), MT-40 (10), MT-85 (30), MT-105 (38) and MT-89 (31) with least ASV scores of 0.056, 0.395, 0.993, 1.117 and 1.210, respectively. High ASV scores of 25.700, 19.494, 15.979, 15.950 and 14.473 were noted with MT-176 (66), MT-72 (24), MT-185 (69), MT-151 (56) and MT-41 (11), respectively and opined that such situation leads to a tendency of specific adaptation of the genotypes. Gyawali et al. (2019) used the criteria of ASV in their investigation and identified 16 genotypes with stable spot form of net blotch disease resistance in barley.

Identification of superior genotypes

Genotypic selection index (GSI) for MLB resistance and grain yield and simultaneous selection index was calculated for 73 BILs (Table 7). Based on the GSI for PDI, five genotypes namely MT-148 (55), MT-190 (72), MT-19 (3), MT-26 (5) and MT-137 (52) which had the lower GSI_{PDI} score of 7, 11.5, 20.5, 21 and 32.5 amongst the evaluated BILs, exhibiting high stability and increased MLB resistance across environments were identified (Table 8). The genotypes MT-120 (45), MT-166 (62), MT-14 (2), MT-37 (9) and MT-57 (15) having lower GSI of 13, 17, 18, 25.5 and 31, respectively, for grain yield were identified. These genotypes possessed better adaptability and higher grain yield (Table 8). Summing up the GSI for PDI and GSI for grain yield, the genotype exhibiting the lowest score was considered to be the best as it combined stability and best trait mean for grain yield and resistance to MLB. Based on simultaneous selection index (SSI) (Table 8) ten genotypes namely, MT-120 (45), MT-14 (2), MT-166 (62), MT-148 (55), MT-190 (72), MT-37 (9), MT-19 (3), MT-114 (42), MT-77 (27) and MT-94 (35) with SSI of 54.5, 55, 57.5, 58, 58.5, 77.5, 81.5, 82, 88 and 88, respectively, were identified to be promising. Such genotypes could be potential germplasm for development of high yielding MLB resistant genotypes or can be used as parent in generating biparental mapping population.

Conclusion

The present investigation identified a total of 73 BILs displaying resistance to MLB in at least one of the environments. AMMI1 biplot identified MT-148 (55) as genotype having high degree of resistance and lower fluctuations to MLB while MT-172 (64) showed stable and susceptible disease response. The resistant genotypes can therefore be used as potential donor for stable disease resistance trait and the susceptible line can be used for producing pathogen inoculum for MLB screening notwithstanding the diverse environments. MT-120 (45) as revealed by AMMI1 biplot could serve as source of high and stable grain yield. The study was also able to classify different environments into various mega environment, as well as to identify the most discriminating ideal environments for assessing the variability with respect to MLB resistance and grain yield. For both PDI and grain yield two mega environments were identified. Mega environments are composed of environments falling in the same sector and consisting of same winning genotypes. Genotypes MT-60 (17) and MT-67 (21) were winning genotypes in the sector that had environments E4 and E6 for PDI. These genotypes showed highest MLB susceptible reaction in these environments. Therefore, if MLB screening is undertaken in E4 or E6 these genotypes can be used as susceptible checks. MT-184 (68) was the winning genotype for the sector containing environment E2 and showed resistant response. This genotype would hence be important MLB resistance donor for breeding resistant genotypes specifically adapted for E2. For development of high yielding varieties specifically for first mega environment consisting of E2 and E4, MT-183 (67) could be used as donor while for E6 MT-61 (18) and MT-72 (24) could be used as donor for high yield. The study ultimately identified ten genotypes namely, MT-120 (45), MT-14 (2), MT-166 (62), MT-148 (55), MT-190 (72), MT-37 (9), MT-19 (3), MT-114 (42), MT-77 (27) and MT-94 (35) as the best genotypes containing both high and stable MLB resistance and grain yield. These genotypes can serve as an important source of MLB resistance and grain yield in future breeding programmes and after analysis of combining ability can also serve as parent for development of hybrids or synthetic varieties characterized with resistance to MLB and possessing high yield. As the studied BILs fall into highly susceptible, susceptible, resistant and highly resistant categories it could be easily predicted that MLB resistance in these BILs were governed by both oligogenes and polygenes. However, this study did not aim to dissect the genes or the mechanisms accompanying MLB resistance. Hence further research is needed in this aspect to identify novel genes and QTLs governing MLB resistance so that the information can be helpful in development of reliable MLB specific molecular markers. The developed molecular marker will then be useful in reliable identification of resistant cultivars without the need to evaluate them in field trials which would ultimately speed up the process of resistance breeding and varietal development.

Declarations

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Conflict of interest

The authors declare no conflict of interest.

Availability of data and material

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Code availability

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Author's contribution

Conception or design of the work (AJ, NKS, RPS, JPJ, UP), Development of experimental material (NKS, AK, SA, AJ), Data collection (AJ, SA), Data analysis and interpretation (AJ, NKS), Drafting the article (AJ), Critical revision of the article (NKS).

Ethics approval

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Tables

Table 1: Description of the environments

Environment	Total rainfall during trial (mm)	Total temperature during trial (˚ Celsius)		Relative humidity during trial (%)		Sun shine hrs	Wind velocity (Km/hr)	Evaporation (mm)	Source of MLB infection
		Max	Min	Morning (07 hrs)	Afternoon (14 hrs)				
E2	12.3	31.4	22.7	89	72.9	5.0	4.1	3.6	Artificial inoculation
E4	9.5	30.7	20.6	90	70.0	5.4	3.8	3.2	Artificial inoculation
E6	9.5	30.7	20.6	90	70.0	5.4	3.8	3.2	Natural inoculation

Table 2: Scale used for assessing MLB resistance in maize*.

Rating scale	Degree of infection (% Diseased leaf area)	PDI	Disease reaction
1.0	Nil to very slight infection (≤10%).	≤11.11	Resistant (R) (Score: ≤ 3.0) (PDI: ≤ 33.33)
2.0	Slight infection, a few lesions scattered on two lower leaves (10.1-20%).	22.22	
3.0	Light infection, moderate number of lesions scattered on four lower leaves (20.1-30%).	33.33	
4.0	Light infection, moderate number of lesions scattered on lower leaves, a few lesions scattered on middle leaves below the cob (30.1-40%).	44.44	Moderately resistant (MR) (Score: 3.1–5.0) (PDI: 33.34-55.55)
5.0	Moderate infection, abundant number of lesions scattered on lower leaves, moderate number of lesions scattered on middle leaves below the cob (40.1-50%).	55.55	
6.0	Heavy infection, abundant number of lesions scattered on lower leaves, moderate infection on middle leaves and a few lesions on two leaves above the cob (50.1-60%).	66.66	Mod. susceptible (MS) (Score: 5.1-7.0) (PDI: 55.56-77.77)
7.0	Heavy infection, abundant number of lesions scattered on lower and middle leaves and moderate number of lesions on two to four leaves above the cob (60.1-70%).	77.77	
8.0	Very heavy infection, lesions abundant scattered on lower and middle leaves and spreading up to the flag leaf (70.1-80%).	88.88	Susceptible (S) (Score: >7.0) (PDI: >77.77)
9.0	Very heavy infection, lesions abundant scattered on almost all the leaves, plant prematurely dried and killed (>80%).	99.99	

*Hooda *et al*. (2018)

Table 3: Categorisation of BC_1F_5 lines using data across the environments in maize.

Groups	Disease reactions	Environments	No. of BILs	Genotypes
1	Resistant (R) (Score: ≤ 3.0) (PDI: ≤ 33.33)	E2	15	MT-14, MT-26, MT-35, MT-45, MT-57, MT-60, MT-95, MT-110, MT-130, MT-132, MT-144, MT-148, MT-158, MT-190, MT-195
		E4	4	MT-26, MT-95, MT-184, MT-195
		E6	11	MT-14, MT-26, MT-95, MT-110, MT-130, MT-132, MT-144, MT-148, MT-158, MT-190, MT-195
2	Moderately resistant (MR) (Score: 3.1 – 5.0) (PDI: 33.34-55.55)	E2	51	MT-19, MT-25, MT-32, MT-37, MT-40, MT-41, MT-53, MT-55, MT-58, MT-61, MT-63, MT-64,MT-67, MT-68, MT-69, MT-72, MT-73, MT-74, MT-77, MT-83, MT-84, MT-85, MT-90, MT-91, MT-93, MT-94, MT-101, MT-105, MT-111, MT-114, MT-115, MT-117, MT-120, MT-126, MT-128, MT-129, MT-136, MT-137, MT-140, MT-151, MT-152, MT-155, MT-156, MT-161, MT-166, MT-167, MT-172, MT-174, MT-176, MT-183, MT-185
		E4	10	MT-9, MT-27, MT-73, MT-89, MT-107, MT-115, MT-148, MT-187, MT-188, MT-190
		E6	33	MT-9, MT-19, MT-27, MT-35, MT-37, MT-73, MT-83, MT-89, MT-105, MT-107, MT-111, MT-114, MT-115, MT-117, MT-120, MT-126, MT-128, MT-129, MT-136, MT-137, MT-140, MT-151, MT-152, MT-155, MT-156, MT-161, MT-166, MT-167, MT-172, MT-174, MT-176, MT-183, MT-185
3	Moderately susceptible (MS) (Score: 5.1-7.0) (PDI: 55.56-77.77)	E2	77	MT-1, MT-2, MT-3, MT-6, MT-10, MT-13, MT-17, MT-18, MT-20, MT-22, MT-24, MT-27, MT-28, MT-29, MT-30, MT-33, MT-34, MT-39, MT-42, MT-44, MT-47, MT-50, MT-51, MT-52, MT-56, MT-59, MT-62, MT-65, MT-66, MT-70, MT-71, MT-78, MT-79, MT-81, MT-86, MT-87, MT-88, MT-92, MT-96, MT-97, MT-98, MT-100, MT-102, MT-103, MT-106, MT-107, MT-108, MT-109, MT-112, MT-116, MT-118, MT-121, MT-122, MT-123, MT-124, MT-125, MT-127, MT-131, MT-133, MT-135, MT-138, MT-139, MT-143, MT-147, MT-150, MT-153, MT-159, MT-160, MT-162, MT-164, MT-168, MT-169, MT-173, MT-182, MT-187, MT-188, MT-191
		E4	91	MT-1, MT-5, MT-6, MT-10, MT-11, MT-13, MT-14, MT-19, MT-20, MT-28, MT-29, MT-30, MT-33, MT-34, MT-35, MT-37, MT-39, MT-40, MT-42, MT-44, MT-47, MT-50, MT-51, MT-52, MT-53, MT-56, MT-57, MT-58, MT-64, MT-69, MT-70, MT-71, MT-72, MT-74, MT-77, MT-78, MT-79, MT-80, MT-81, MT-82, MT-83, MT-84, MT-88, MT-90, MT-92, MT-93, MT-94, MT-96, MT-97, MT-98, MT-99, MT-101, MT-103, MT-106, MT-109, MT-110, MT-111, MT-114, MT-116, MT-117, MT-120, MT-122, MT-123, MT-124, MT-130, MT-132, MT-135, MT-137, MT-138, MT-139, MT-144, MT-145, MT-146, MT-147, MT-151, MT-152, MT-155, MT-158, MT-160, MT-161, MT-166, MT-167, MT-168, MT-169, MT-170, MT-172, MT-173, MT-174, MT-175, MT-177, MT-182
		E6	63	MT-1, MT-2, MT-3, MT-5, MT-6, MT-10, MT-15, MT-18, MT-22, MT-28, MT-30, MT-33, MT-34, MT-40, MT-41, MT-47, MT-51, MT-53, MT-56, MT-57, MT-58, MT-59, MT-64, MT-69, MT-70, MT-72, MT-74, MT-77, MT-80, MT-81, MT-82, MT-84, MT-85, MT-90, MT-91, MT-92, MT-93, MT-94, MT-96, MT-97, MT-98, MT-100, MT-101, MT-111, MT-119, MT-121, MT-122, MT-123, MT-124, MT-125, MT-131, MT-135, MT-141, MT-143, MT-153, MT-160, MT-164, MT-169, MT-177, MT-179, MT-182, MT-187, MT-188
4	Susceptible (S)	E2	26	MT-5, MT-8, MT-9, MT-11, MT-12, MT-15, MT-16, MT-46, MT-80, MT-82, MT-89, MT-99, MT-119, MT-134, MT-141, MT-145, MT-

(Score: >7.0)			146, MT-149, MT-165, MT-170, MT-175, MT-177, MT-178, MT-179, MT-181, MT-184
(PDI: >77.77)	E4	64	MT-2, MT-3, MT-8, MT-12, MT-15, MT-16, MT-17, MT-18, MT-22, MT-24, MT-25, MT-32, MT-41, MT-45, MT-46, MT-55, MT-59, MT-60, MT-61, MT-62, MT-63, MT-65, MT-66, MT-67, MT-68, MT-85, MT-86, MT-87, MT-91, MT-100, MT-102, MT-105, MT-108, MT-112, MT-118, MT-119, MT-121, MT-125, MT-126, MT-127, MT-128, MT-129, MT-131, MT-133, MT-134, MT-136, MT-140, MT-141, MT-143, MT-149, MT-150, MT-153, MT-156, MT-159, MT-162, MT-164, MT-165, MT-176, MT-178, MT-179, MT-181, MT-183, MT-185, MT-191
	E6	62	MT-8, MT-11, MT-12, MT-13, MT-16, MT-17, MT-20, MT-24, MT-25, MT-29, MT-32, MT-39, MT-42, MT-44, MT-45, MT-46, MT-50, MT-52, MT-55, MT-60, MT-61, MT-62, MT-63, MT-65, MT-66, MT-67, MT-68, MT-71, MT-78, MT-79, MT-86, MT-87, MT-88, MT-99, MT-102, MT-103, MT-106, MT-108, MT-109, MT-116, MT-118, MT-127, MT-133, MT-134, MT-138, MT-139, MT-145, MT-146, MT-147, MT-149, MT-150, MT-159, MT-162, MT-165, MT-168, MT-170, MT-173, MT-175, MT-178, MT-181, MT-184, MT-191

Table 4: Combined Analysis of Variance for PDI and grain yield of 73 BILs in maize.

Source of variation	df	F value for PDI	F value for grain yield
Environment	2	2625.34**	410.63**
Genotype	72	133.92**	349.296**
G x E	144	79.48**	59.97**
Pooled error	216		

**Significant at 0.01 probability level.

Table 5. AMMI analysis of variance for PDI and grain yield in maize.

Source of variation	df	PDI			Grain yield per plant		
		SS	MS (**)	%SS explained	SS	MS (**)	%SS explained
Environment (E)	2	37087.46	18543.73**	26.59	34452.57	17226.29**	14.48
Genotype (G)	72	45826.38	636.48**	32.86	161886.47	2248.42**	68.02
G x E interaction	144	56560.77	392.78**	40.55	41657.96	289.29**	17.50
IPCA1	73	36247.95	496.55**	64.09	40097.79	549.28**	96.25
IPCA2	71	20312.82	286.1**	35.91	1560.17	21.97*	3.75
Residuals	219	2149.182	9.81		3342.11		

*, **Significant at 0.05 and 0.01 probability level, respectively.

Table 6: Mean PDI, mean PDI rank, ASV PDI, ASV PDI rank, mean yield, mean yield rank, ASV yield and ASV yield rank of evaluated 73 BILs of maize.

Genotype	Genotype code	Mean PDI	Mean PDI rank (A)	ASV PDI	ASV PDI rank (B)	Mean yield	Mean yield rank (C)	ASV yield	ASV yield rank (D)
MT-9	1	60.50	51	1.695	72	45.00	24	2.903	17
MT-14	2	36.23	4	0.439	33	55.89	12	1.276	6
MT-19	3	45.50	10.5	0.264	10	56.67	11	6.699	50
MT-25	4	76.54	72	0.923	62	21.45	58.5	8.110	58
MT-26	5	29.63	1	0.314	20	50.17	21	8.013	57
MT-27	6	50.61	19	1.142	67	52.11	16	4.971	39
MT-32	7	64.20	62	0.707	58	52.72	15	3.437	22.5
MT-35	8	47.96	15	0.326	25	53.61	14	6.481	48
MT-37	9	52.24	23	0.401	29	78.56	3	3.437	22.5
MT-40	10	55.56	36	0.499	43	20.94	61	0.395	2
MT-41	11	66.36	65	0.561	48	41.83	27	14.473	69
MT-45	12	64.49	63	1.138	66	26.33	48	2.433	14
MT-53	13	60.27	50	0.375	27	35.22	40	4.001	30
MT-55	14	73.11	71	0.948	64	26.46	47	5.559	43
MT-57	15	46.83	13	0.598	50	71.45	6	3.558	25
MT-58	16	51.03	22	0.536	46	46.11	22	5.003	40
MT-60	17	66.44	66	1.308	70	65.11	7	6.569	49
MT-61	18	69.06	68	0.759	59	99.22	2	9.834	61
MT-63	19	72.84	70	0.450	34	27.61	46	3.465	24
MT-64	20	56.17	39	0.602	51	50.22	20	2.683	15
MT-67	21	77.01	73	0.943	63	26.33	49	10.712	63
MT-68	22	69.33	69	0.366	26	25.78	54	2.056	11
MT-69	23	58.87	46	0.316	21	50.94	18	7.416	56
MT-72	24	59.13	47	0.648	53	57.94	10	19.494	72
MT-73	25	50.78	21	0.553	47	19.89	64	6.916	53
MT-74	26	54.28	32.5	0.274	15	22.45	58.5	1.868	9
MT-77	27	56.75	41	0.212	3	39.28	32	2.059	12
MT-83	28	46.84	14	0.519	45	19.89	63	0.056	1
MT-84	29	54.28	32.5	0.302	18.5	19.83	65	4.815	37
MT-85	30	62.33	58	0.793	61	40.55	30	0.993	3

MT-89	31	61.25	53	1.483	71	14.67	70	1.210	5
MT-90	32	56.59	40	0.193	1	25.95	52	1.993	10
MT-91	33	66.19	64	0.394	28	18.39	66	4.657	35
MT-93	34	64.12	61	0.317	22	41.61	29	2.995	18
MT-94	35	53.33	31	0.256	9	52.00	17	4.030	31
MT-95	36	29.87	2	0.658	54	22.56	57	14.069	68
MT-101	37	57.77	44	0.302	18.5	14.72	69	3.340	21
MT-105	38	56.79	42.5	0.491	41	15.33	68	1.117	4
MT-107	39	55.67	38	1.054	65	31.72	43	3.249	20
MT-110	40	41.98	8	0.491	41	43.17	25.5	13.750	67
MT-111	41	53.09	28	0.265	12.5	13.85	72	6.703	51
MT-114	42	53.08	25	0.253	8	39.17	33	2.835	16
MT-115	43	49.38	17	0.509	44	29.50	44	11.009	65
MT-117	44	56.79	42.5	0.265	12.5	26.04	51	4.480	33
MT-120	45	55.56	36	0.214	5.5	73.11	5	1.769	8
MT-126	46	62.96	59.5	0.678	55.5	34.00	42	3.849	29
MT-128	47	59.26	48.5	0.402	30	50.56	19	9.262	60
MT-129	48	62.96	59.5	0.678	55.5	14.39	71	3.808	27
MT-130	49	41.98	10.5	0.490	37.5	17.39	67	6.169	45
MT-132	50	41.98	10.5	0.490	37.5	24.50	56	4.810	36
MT-136	51	58.03	45	0.320	23.5	40.28	31	6.476	47
MT-137	52	48.76	16	0.298	16.5	37.00	37	4.503	34
MT-140	53	62.16	57	0.616	52	43.17	25.5	6.973	54
MT-144	54	38.27	7	0.490	37.5	37.11	35	12.253	66
MT-148	55	36.42	5	0.210	2	45.44	23	3.838	28
MT-151	56	55.56	36	0.214	5.5	25.89	53	15.950	70
MT-152	57	54.32	34	0.419	31.5	25.59	55	4.865	38
MT-155	58	53.09	28	0.265	12.5	20.89	62	5.123	41
MT-156	59	61.73	55.5	0.583	49	11.73	73	5.316	42
MT-158	60	41.98	10.5	0.491	41	21.41	60	7.188	55
MT-161	61	52.47	24	0.298	16.5	34.78	41	10.841	64
MT-166	62	53.09	28	0.265	12.5	73.94	4	2.239	13
MT-167	63	50.00	18	0.463	35	35.67	39	6.884	52

MT-172	64	59.26	48.5	0.214	5.5	59.00	9	4.315	32
MT-174	65	50.62	20	0.419	31.5	38.72	34	1.647	7
MT-176	66	53.09	28	0.490	37.5	54.33	13	25.700	73
MT-183	67	61.73	55.5	0.320	23.5	105.44	1	10.146	62
MT-184	68	61.11	52	1.934	73	61.06	8	9.261	59
MT-185	69	53.09	28	0.774	60	35.83	38	15.979	71
MT-187	70	66.66	67	1.186	68	26.05	50	6.069	44
MT-188	71	61.72	54	1.286	69	28.67	45	3.682	26
MT-190	72	37.04	6	0.214	5.5	41.83	28	3.205	19
MT-195	73	32.10	3	0.697	57	37.11	36	6.376	46

Table 7: $GSI_{MLB\ resistance}$, $GSI_{grain\ yield}$ and SSI for evaluated 73 BILs.

Genotype	Genotype code	$GSI_{MLB\ resistance}$ (A+B)	$GSI_{grain\ yield}$ (C+D)	SSI (A+B+C+D)
MT-9	1	123	41	164.0
MT-14	2	37	18	55.0
MT-19	3	20.5	61	81.5
MT-25	4	134	116.5	250.5
MT-26	5	21	78	99.0
MT-27	6	86	55	141.0
MT-32	7	120	37.5	157.5
MT-35	8	40	62	102.0
MT-37	9	52	25.5	77.5
MT-40	10	79	63	142.0
MT-41	11	113	96	209.0
MT-45	12	129	62	191.0
MT-53	13	77	70	147.0
MT-55	14	135	90	225.0
MT-57	15	63	31	94.0
MT-58	16	68	62	130.0
MT-60	17	136	56	192.0
MT-61	18	127	63	190.0
MT-63	19	104	70	174.0
MT-64	20	90	35	125.0
MT-67	21	136	112	248.0
MT-68	22	95	65	160.0
MT-69	23	67	74	141.0
MT-72	24	100	82	182.0
MT-73	25	68	117	185.0
MT-74	26	47.5	67.5	115.0
MT-77	27	44	44	88.0
MT-83	28	59	64	123.0
MT-84	29	51	102	153.0
MT-85	30	119	33	152.0
MT-89	31	124	75	199.0

MT-90	32	41	62	103.0
MT-91	33	92	101	193.0
MT-93	34	83	47	130.0
MT-94	35	40	48	88.0
MT-95	36	56	125	181.0
MT-101	37	62.5	90	152.5
MT-105	38	83.5	72	155.5
MT-107	39	103	63	166.0
MT-110	40	49	92.5	141.5
MT-111	41	40.5	123	163.5
MT-114	42	33	49	82.0
MT-115	43	61	109	170.0
MT-117	44	55	84	139.0
MT-120	45	41.5	13	54.5
MT-126	46	115	71	186.0
MT-128	47	78.5	79	157.5
MT-129	48	115	98	213.0
MT-130	49	48	112	160.0
MT-132	50	48	92	140.0
MT-136	51	68.5	78	146.5
MT-137	52	32.5	71	103.5
MT-140	53	109	79.5	188.5
MT-144	54	44.5	101	145.5
MT-148	55	7	51	58.0
MT-151	56	41.5	123	164.5
MT-152	57	65.5	93	158.5
MT-155	58	40.5	103	143.5
MT-156	59	104.5	115	219.5
MT-158	60	51.5	115	166.5
MT-161	61	40.5	105	145.5
MT-166	62	40.5	17	57.5
MT-167	63	53	91	144.0
MT-172	64	54	41	95.0

MT-174	65	51.5	41	92.5
MT-176	66	65.5	86	151.5
MT-183	67	79	63	142.0
MT-184	68	125	67	192.0
MT-185	69	88	109	197.0
MT-187	70	135	94	229.0
MT-188	71	123	71	194.0
MT-190	72	11.5	47	58.5
MT-195	73	60	82	142.0

Table 8: Top ten MLB resistant, high yielding and high yielding MLB resistant genotypes.

MLB Resistant Genotypes	$GSI_{MLB\ resistance}$	High yielding Genotypes	$GSI_{grain\ yield}$	MLB Resistant and high yielding Genotypes	Simultaneous selection Index
MT-148	7	MT-120	13	MT-120	54.5
MT-190	11.5	MT-166	17	MT-14	55
MT-19	20.5	MT-14	18	MT-166	57.5
MT-26	21	MT-37	25.5	MT-148	58
MT-137	32.5	MT-57	31	MT-190	58.5
MT-114	33	MT-85	33	MT-37	77.5
MT-14	37	MT-64	35	MT-19	81.5
MT-35	40	MT-32	37.5	MT-114	82
MT-94	40	MT-9	41	MT-77	88
MT-111	40.5	MT-172	41	MT-94	88

Figures

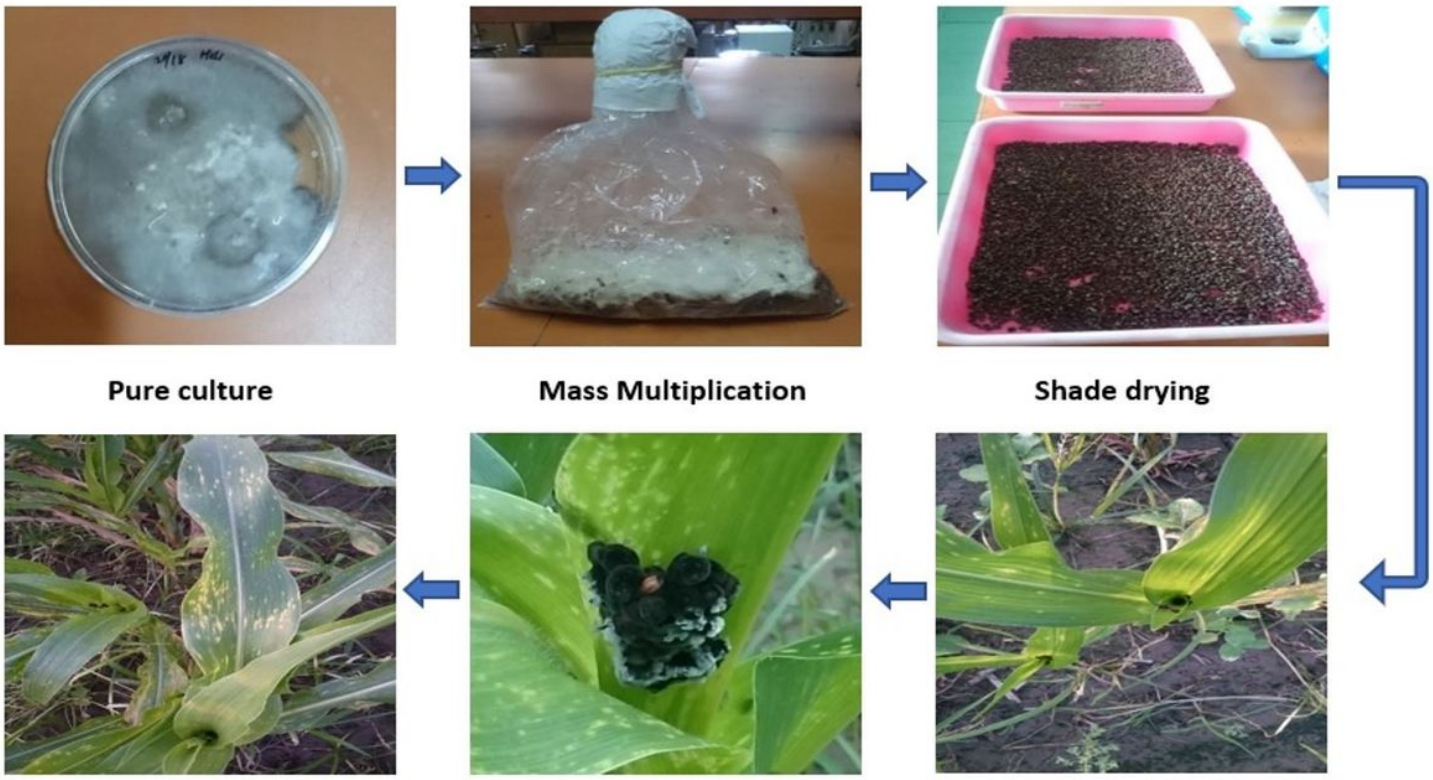

Figure 1

Mass multiplication and artificial inoculation of Bipolaris maydis in field.

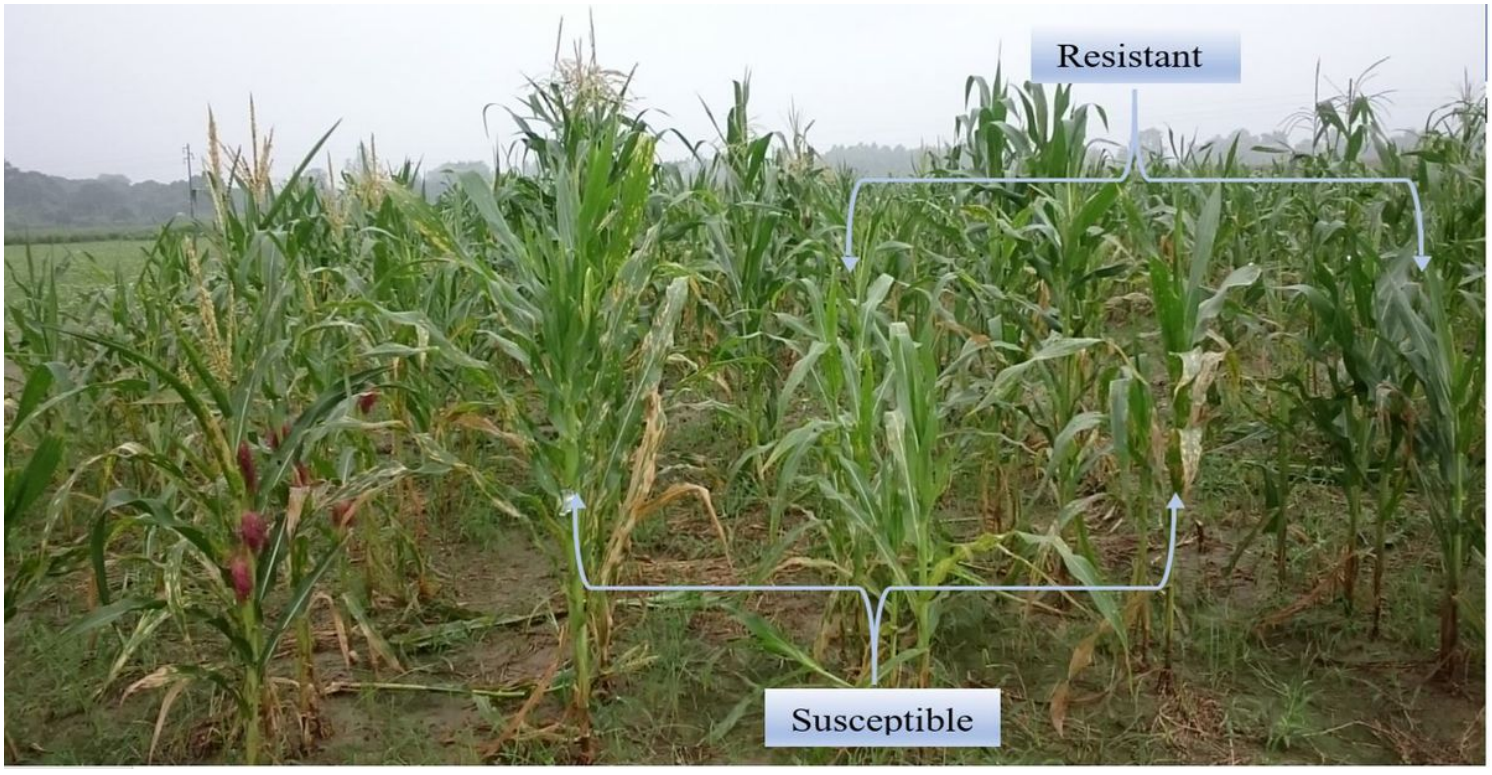

Figure 2

Differential response of BILs to Bipolaris maydis infection.

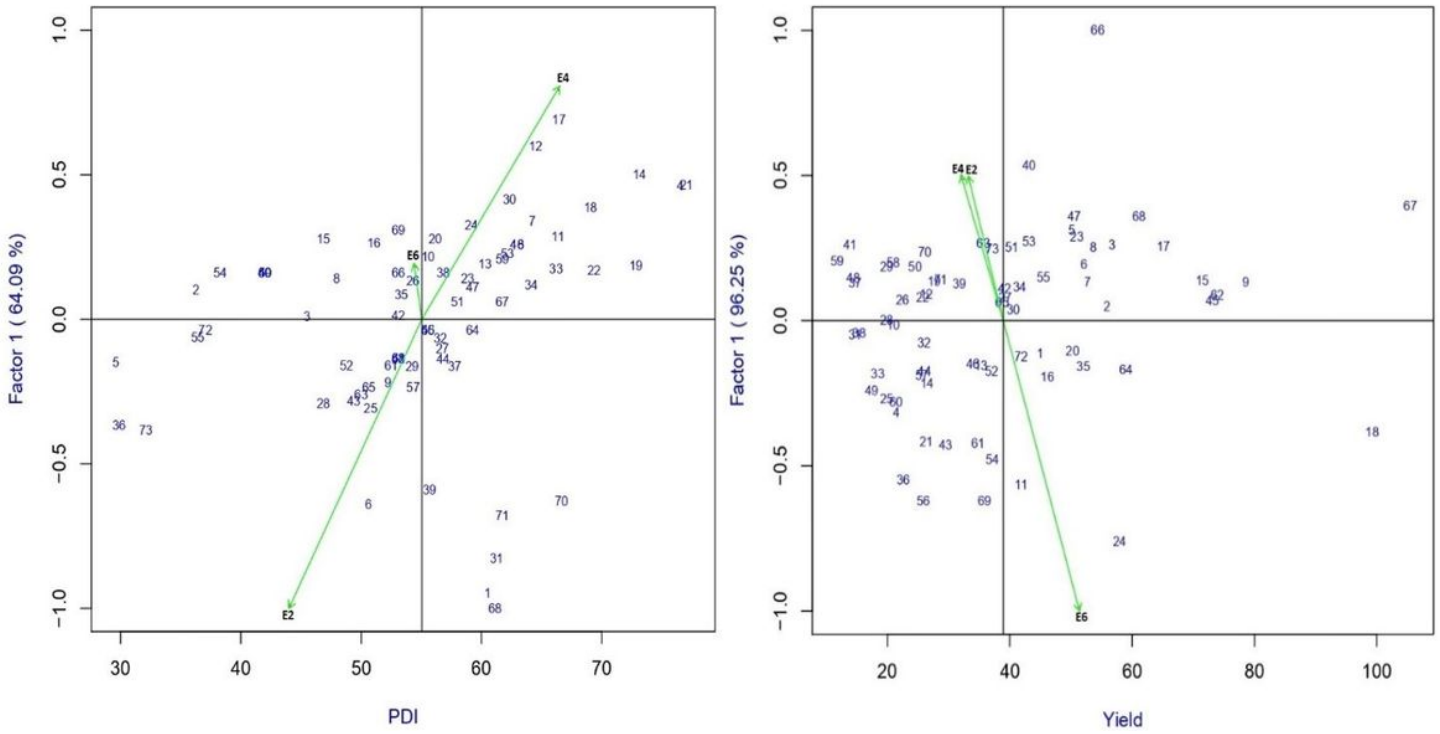

Figure 3

AMMI1 biplot for PDI (a) and grain yield (b).

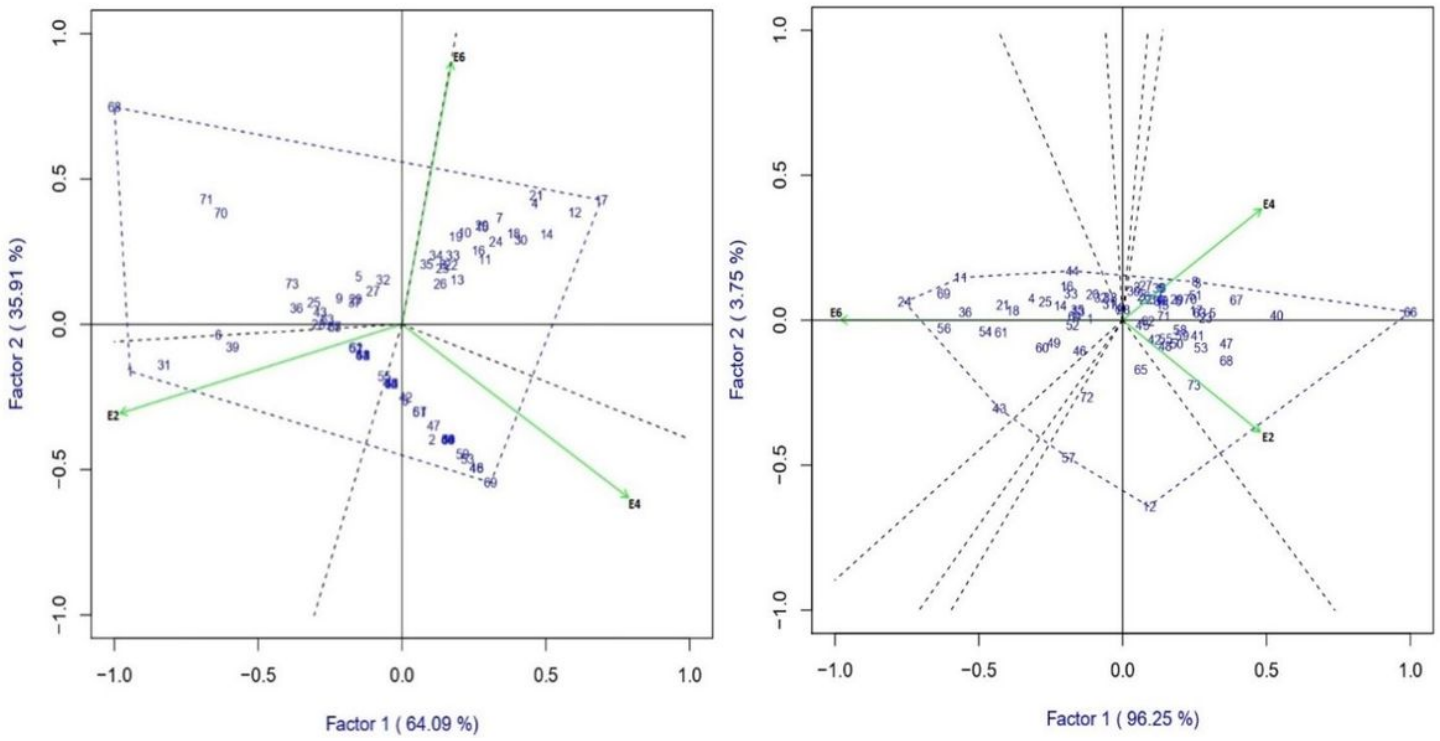

Figure 4

AMMI2 biplot for PDI (a) and grain yield (b)

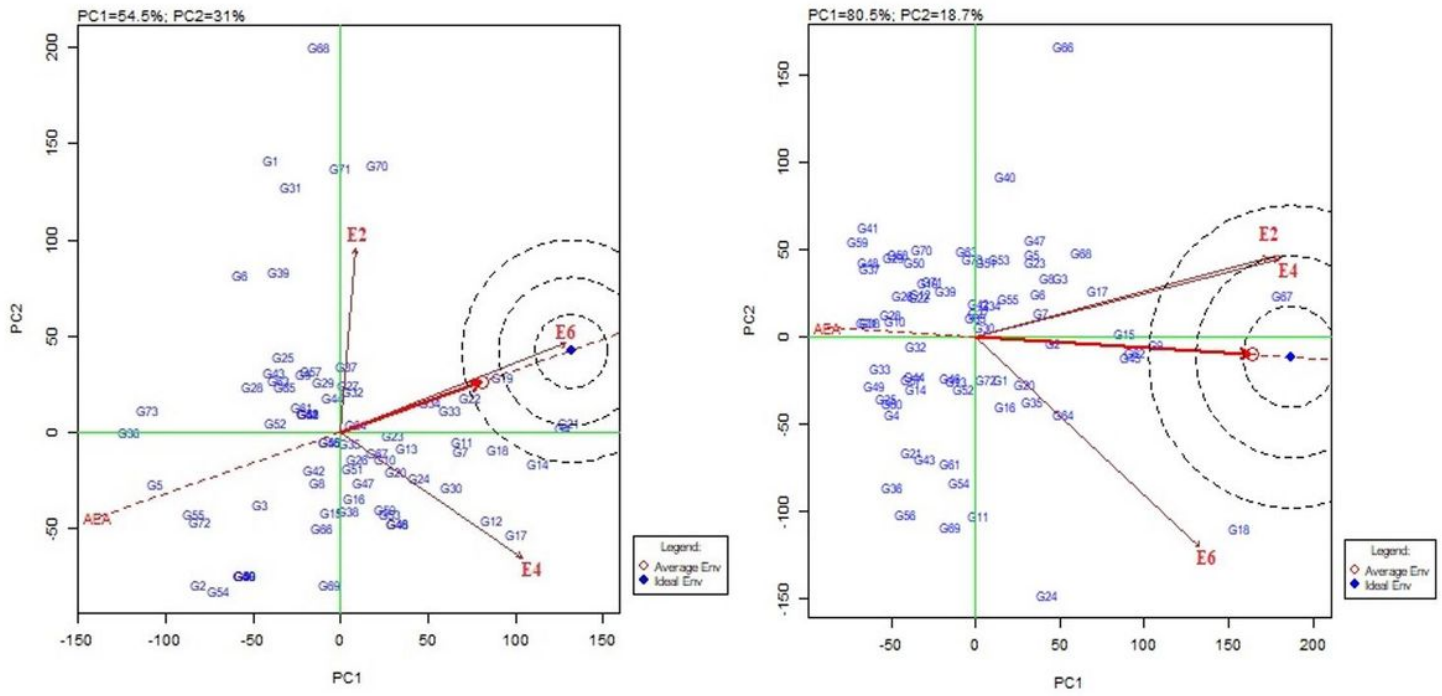

Figure 5

Average environment coordination (AEC) view of GGE for PDI (a) and grain yield (b)

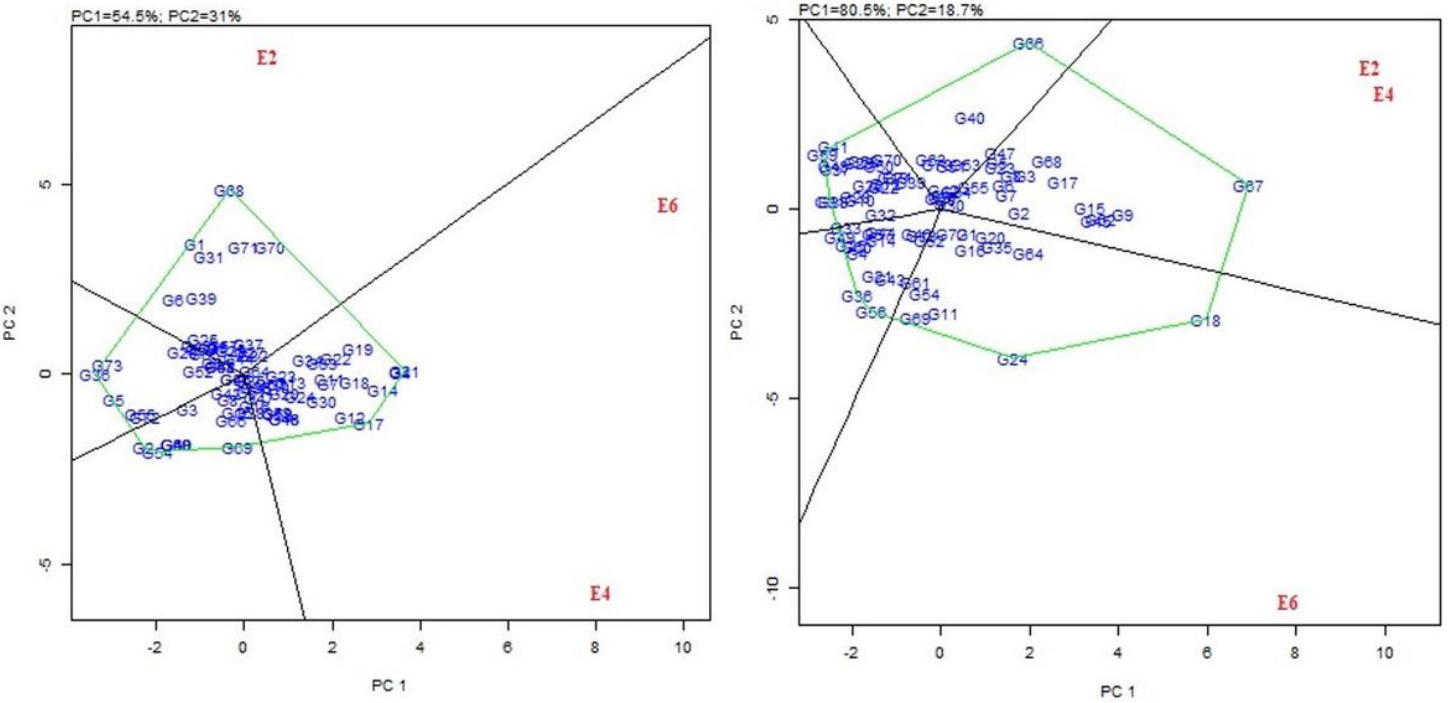

Figure 6

which-won-where view of GGE for PDI (a) and grain yield (b).