

Analysis of genotype \times environment interaction and stability for grain yield and chocolate spot (*Botrytis fabae*) disease resistance in faba bean (*Vicia faba*)

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Abstract

Chocolate spot disease, caused by *Botrytis fabae*, is a major constraint that limits productivity of faba bean (*Vicia faba*) in Ethiopia. This is mainly due to lack of disease resistant genotypes from the locally adapted varieties. Therefore, the development of resistant faba bean varieties that are adapted to different agro-ecologies are important as it improves selection efficiency and reduce breeding time and cost. The study was, therefore, conducted to evaluate the effect of the genotype \times environment interaction (GEI) for grain yield and chocolate spot disease resistance in 21 faba bean genotypes in six locations. A randomized complete block design with three replicates was used at each location. The additive main effects and multiplicative interaction (AMMI) and the genotype and (genotype \times environment) (GGE) biplot analyses resulted in highly significant differences amongst genotypes, environments and GEI. The influence of the environment was far larger (61.4% contribution to the total variation observed) than the contributions from the genotypes (20.9%) and GEI (17.7%). In contrast, genotypes had the largest contribution (73.4%) to the variability observed for chocolate spot resistance. The site Kulumsa (E3) provided the best discriminating ability for the genotypes, while both AMMI and GGE biplot analyses identified six most stable and productive genotypes, and four genotypes with low chocolate spot severity but moderate stability. Overall, G14 and G5 with high mean yield, stable and moderate level of resistance at all locations are recommended as the best genotypes.

Keywords: AMMI, *Botrytis fabae*; disease resistance stability; faba bean; GGE; genotype \times environment interaction.

Abbreviation: AMM I_Additive main effects and multiplicative interaction; AEC_Average Environment Coordinate; AEA_Average Environment Axis; GEI_Genotype by Environment Interaction; GGE_Genotype and Genotype \times Environment interaction; IPCA_Interaction Principal Component Axis; PC_Principal Components.

Introduction

Faba bean (*Vicia faba*) is globally the fourth most important food legume with great potential to alleviate malnutrition for the resource-poor farmers. It is the principal legume in the mid and highlands of Ethiopia grown to sustain the cropping systems and soil fertility. However, the average global yield of faba bean is low (1.8 t ha⁻¹), far below the potential yield (5-7 t ha⁻¹), due to a large number of biotic and abiotic stresses that affect the crop (FAOSTAT, 2014). Chocolate spot disease caused by *Botrytis fabae* is one of the most important constraint that contributes to the low productivity of faba bean (Stoddard et al., 2010). In addition, Ethiopia has diverse agro-ecological zones and faba bean varieties are bred for different zones. Consequently, the relative performance of cultivars often changes from one environment to another; thus extensive testing is required to identify genotypes with minimal interaction with environments. Thus, newly developed faba bean cultivars for release should exhibit great potential for yield

and disease resistance with average stability over different environmental conditions.

The genotype \times environment interaction (GEI) can reduce gains from selection and complicate identification of the best genotypes in breeding and cultivar recommendation. The presence of interactions indicates that the relative genotype performance in different sites depends essentially on the given environmental conditions. The phenotypic response of any genotype in relation to others could therefore be inconsistent, which is demonstrated by changes in the relative ranking of the genotypes from one environment to another. According to Yan et al. (2007), test environment and genotype evaluation are more meaningful when conducted within mega-environments. Mega-environment analysis simply involves investigating whether the covered growing region can be grouped into similar environments. Various analysis methods have been used to explore GEI and to identify superior genotypes with wide or

specific adaptation to different environments. Two frequently used methods are the additive main effects and multiplicative interaction (AMMI) model (Gauch et al., 2008), that combines a univariate method for the additive method for the multiplicative effect of GEI (Zobel et al., 1988) and the genotype and (genotype x environment) (GGE) biplot (Yan et al., 2001). The GGE biplot is a visual statistical tool for examining the performance of genotypes tested in different environments. The advantages and disadvantages of both the AMMI and GGE biplot analyses have been discussed in detail by Gauch (2006) and Yan et al. (2007). The GGE biplot model has been utilized to identify breeding lines and cultivars that are resistant to *Ascochyta fabae*, rust and chocolate spot diseases in faba bean (Villegas-Fernández et al., 2009; Villegas-Fernández et al., 2011; Rubiales et al., 2012). The use of AMMI in analysing the multi-environment disease data to identify stable sources of resistance has been reported for different crops (Mulema et al., 2008; Mukherjee et al., 2013). Evaluation of GEI in multi-environment trials is thus important in the development of disease resistant, high yielding and stable genotypes.

However, there is insufficient information on GEI and stability of faba bean genotypes for yield and chocolate spot resistance in Ethiopia. Therefore, the objectives of the study were: to (i) evaluate the influence of environments on disease resistance and yield of faba bean genotypes, (ii) identify stable genotypes for chocolate spot resistance and yield, (iii) determine whether locations belong to a single mega-environment, or a diverse set, and (iv) rank locations based on discriminating ability and representativeness.

Results

AMMI analysis for grain yield and chocolate spot disease severity

The AMMI analysis of variance showed highly significant effects ($P \leq 0.001$) for genotypes, environments and genotype by environment interaction (Table 1). The relative magnitude of the different sources of variation varied greatly as revealed by their sums of squares (Table 1). Environments contributed 61.4% to the total variation observed, while genotypes and genotype by environment interaction contributed 21% and 17.7%, respectively. The first and second interaction principal component axis (IPCA-1 and IPCA-2) contributed 82.6% to the total interaction (Table 1). The average grain yield for the genotypes across environments ranged from 2.1 t ha⁻¹ in Holetta (E1) to 5.6 t ha⁻¹ in Adadi (E2). Considering the best four performing genotypes for grain yield in each of the six environments, a crossover GEI was observed which resulted in different ranking of the genotypes for yield (Table 2). G8 ranked first at three environments, while G2, G11 and G15 were first at one environment each. G4 ranked second at four environments and G16 appeared in the top four in three environments. Quadrant I had genotypes with an IPCA score near the origin (zero) and high mean yield (ideal genotype) showing stability of the genotypes across the environments tested. These included genotypes G20, G11, G4 and G14 (Fig 1a).

However, genotypes with high mean performance but a large IPCA score are considered unstable across environments, but have specific adaptation to some environments. These included genotypes G8 which was better adapted to E6 (Kofele) and G21, G16, G9 and G12 with specific adaptation to E4 (Bekoji). In general, G18 was the most unstable genotype identified by AMMI model, with low grain yield and the least association with other environments, while G15 had the largest positive (0.99) interaction with environment with high grain yield and G3 had the largest negative interaction (-1.62) but low grain yield (Supplementary Table 1).

From the AMMI biplots, the markers for environment were more scattered than the markers for genotypes indicating that the variability due to environments was higher than that due to genotypes. From the AMMI 1 model, environments Adadi (E2), E4 (Bekoji) and E6 (Kofele) were classified as high yielding, while E1 (Holetta), E3 (Kulumsa) and E5 (Assasa) were low yielding environments. On the whole, Adadi (E2) was the most favourable and E1 (Holetta) the least favourable environment among the six environments included in the study for grain yield (Fig 1).

There were significant variations for the genotypes ($P \leq 0.001$) environments ($P \leq 0.001$) and genotype by environment interaction ($P \leq 0.05$) for chocolate spot disease indicating a differential response of genotypes across the environments (Table 1). Genotypes contributed 73.4% to the total variation observed, followed by GEI which explained 13.8%, while the environments accounted for 12.7% to the total variation for chocolate spot disease severity (Table 1). The first and second interaction principal component axis (IPCA-1 and IPCA-2) significantly ($P \leq 0.001$) contributed to 74.6% of the total interaction (Table 1).

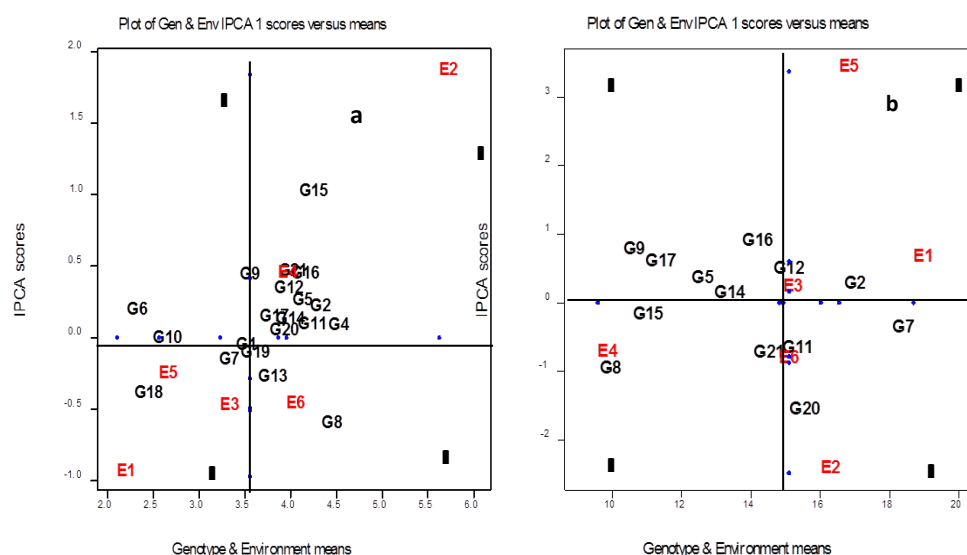
Environmental means for disease severity scores of the 21 faba bean genotypes over the six environments ranged from 9.6% in Bekoji (E4) to 18.7% in Holetta (E1). Thus, Holetta (E1) was the most favourable site for disease expression followed by Assasa (E5) (Table 2). The AMMI biplot indicated that of the 21 genotypes, G3 and G4 were highly resistant (quadrant III) and G19, G15, G17, G8, G16 and G9 were moderately resistant (quadrant II). On the other hand, G18, G6, and G10 were moderately susceptible (quadrant I) and G1 and G13 were highly susceptible (quadrant IV). The resistant check genotype G3 (ILB-4726) was resistant across all environments, while the susceptible genotype G13 (Kasa) was susceptible across all environment (Fig 1b).

Genotypes with IPCA 1 scores near zero had little interaction with the environment. Accordingly, the site Bekoji (E4) had a low environment score exhibiting little interaction with genotypes (quadrant III) and Adadi (E2) had a high environment score with a large negative interaction with genotypes (quadrant IV). Holetta (E1) and Assasa (E5) had high environment scores with a high positive interaction with genotypes (quadrant I). The genotypes G9, G15, G16, G17, and G19, were stable for low chocolate spot severity across the environments, while genotypes G3, G4 and G8 were resistant to chocolate spot but unstable across the environments. However, genotypes G6, G10 and G18 were susceptible for chocolate spot disease across all the environments (Fig 1b).

Table 1. AMMI analyses: grain yield and chocolate spot disease severity of 21 faba bean genotypes over six locations.

Source of variation	df	GY (t ha ⁻¹)			df	GDS (%)		
		SS	MS	Variance explained (%)		SS	MS GDS	Variance explained (%)
Total	377	949.9	2.52		377	29221	77.5	
Treatments	125	792.4	6.34***		125	22935	183.5***	
Genotypes	20	166.1	8.3***	20.96	20	16839	842***	73.43
Environments	5	486.3	97.26***	61.36	5	2920	584.1***	12.73
Block	12	24.4	2.04***		12	630	52.5**	
Interactions	100	140.1	1.4***	17.68	100	3175	31.8*	13.84
IPCA1	24	77.2	3.22***	63.49	24	1120	46.6***	40.96
IPCA2	22	23.2	1.06*	19.08	22	936	42.6*	33.96
IPCA3	20	21.2	1.06*	17.43	20	700	35	25.39
Residuals	34	18.4	0.54		54	1119	20.7	
Error	240	133	0.55		240	5656	23.6	

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively; GDS: general disease severity score for Chocolate spot (%); GY: grain yield (t ha⁻¹).

**Fig 1 a and b.** AMMI biplot of the first interaction principal component axis (IPCA1) versus mean grain yields (t ha⁻¹) (a) and mean chocolate spot disease severity (%) of faba bean genotypes (G1 – G21 full name Supplementary Table 2) and environments (E1: Holetta; E2: Adadi; E3: Kulumsa; E4: Bekoji; E5: Assasa; E6: Kofele).**Table 2.** The first four AMMI selections for grain yield per environment and mean for disease severity score.

Environment ID	Environment	Mean GY (t ha ⁻¹)	1 st	2 nd	3 rd	4 th	Mean GDS (%)
E1	Holetta	2.10	G8	G4	G3	G17	18.69
E2	Adadi	5.62	G15	G4	G21	G16	16.01
E3	Kulumsa	3.23	G8	G4	G14	G2	14.93
E4	Bekoji	3.86	G11	G16	G15	G20	9.58
E5	Assasa	2.56	G2	G4	G16	G8	16.54
E6	Kofele	3.95	G8	G21	G11	G13	14.82

GY: Grain yield (t ha⁻¹); GDS: General disease severity score; 1st – 4th indicates rank of genotype for yield across different environments & b 'Which-won-where or which is best at what': for grain yield (a) and polygon view of GGE biplot, showing which genotype is resistant for chocolate spot (b) based on a genotype x environment of 21 faba bean genotypes evaluated in six environments.

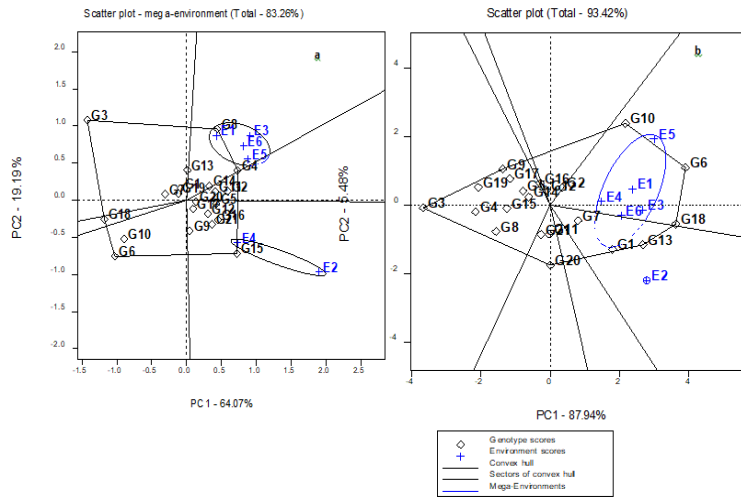


Fig 2a and b ‘Which-won-where or which is best at what’: for grain yield (a) and polygon view of GGE biplot, showing which genotype is resistant for chocolate spot (b) based on a genotype x environment of 21 faba bean genotypes evaluated in six environments.

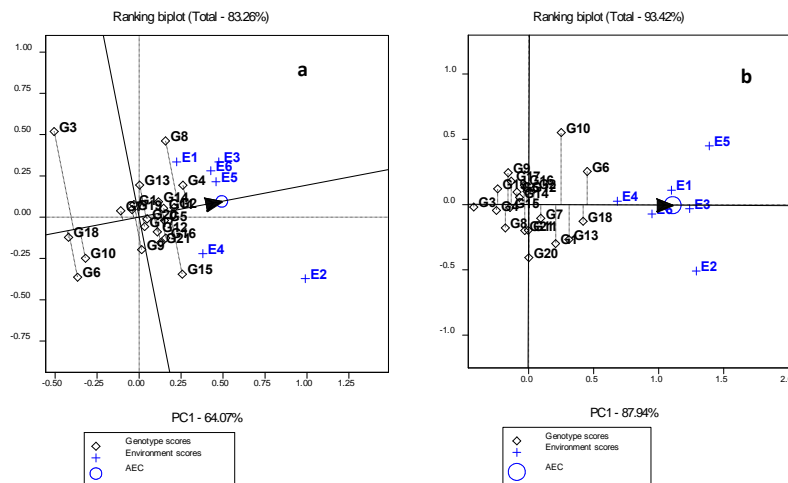


Fig 3a and b Average environment coordination (AEC) views of the GGE biplot on environment-focused scaling for the grain yield means (a) and for chocolate spot severity (b) performance and stability of genotypes.

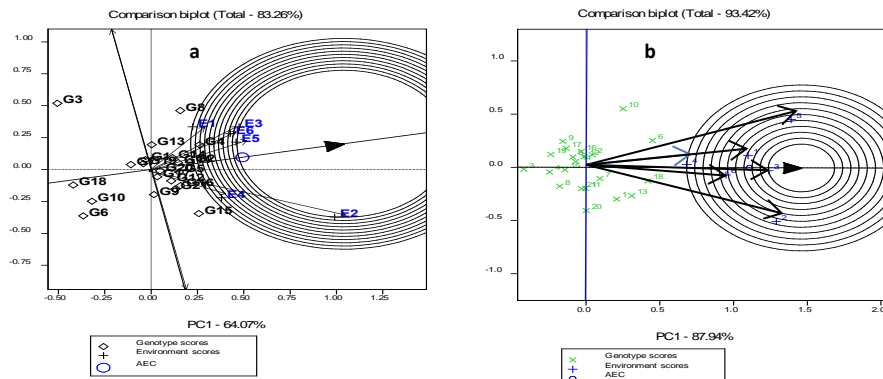


Fig 4a and b .The vector view of GGE biplot shows interrelationships among the test environment and comparison of environment with ‘the ideal environment’ based on a genotype x environment for yield (a) and for chocolate spot disease (b) of 21 faba bean genotypes.

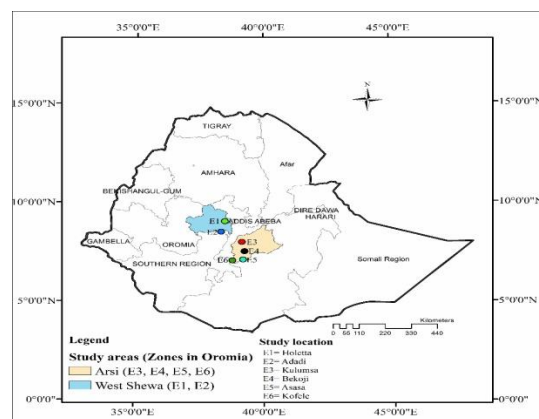


Fig 5. Geographical map major faba bean growing areas in Ethiopia where the study was conducted: E1, Holetta; E2, Adadi; E3, Kulumsa; E4, Bekoji; E5, Asasa; E6, Kofele.

GGE biplot analysis for grain yield and chocolate spot disease severity

The GEI was further explored through the genotype and (genotype x environment) (GGE) biplot analysis. The first two principal components (PCs) of the GGE biplot accounted for a total of 83.26% (PC1 = 64.07%, PC2 = 19.19%) of the variation for grain yield over six locations, while for chocolate spot disease severity the PCs explained a total of 93.42% (PC1 = 87.94%, PC2 = 5.48%) (Fig 2a & b).

The ‘which –won–where’ pattern and mega environments

A polygon view of the GGE biplot for grain yield resulted in six vertex genotypes with both positive (high yielding) and negative (low yielding) PC1 scores (Fig 2a). These genotypes included G8 and G4 which were vertex genotypes in the sector where environments E1, E3, E6 and E5 sites fell. Genotype G15 was the vertex genotype in E2 and E4. The other three genotypes (G3, G18 and G6) fell in sectors with no environment markers. Four environments (E1 (Holetta), E3 (Kulumsa), E5 (Assasa), and E6 (Kofele)) fell in one sector thus comprising one large mega-environment, and environments (E4 (Bekoji) and E2 (Adadi) were grouped into the other mega-environment (Fig 2a).

Fig 2b shows the polygon view of the GGE biplot of 21 faba bean genotypes for chocolate spot disease severity. In the biplot view, genotypes G6 and G10 were the vertex genotypes in the sector that had environments E1, E4, and E6, while G18 was the vertex genotype for E6 and E3. Genotype G13 was the vertex genotype in the sector with environment E2 (Fig 2b). Based on the disease rating scale used, these genotypes were the most susceptible to chocolate spot disease in the environments they were located in as indicated by the high PC1 scores. G3, on the other hand, was the most resistant genotype to chocolate spot disease as indicated by the high negative PC1 score. The other genotypes within the polygon view and near the origin had low positive or negative PC1 scores indicating moderate resistance to moderate susceptibility and were less responsive than the vertex genotypes. Although the test environments fell in three sectors of the polygon view, they

were grouped into two mega-environments with E1, E3, E4, E5 and E6 as one mega-environment and E2 the second mega-environments for chocolate spot disease.

Mean performance and stability of the genotypes

The GGE biplot analysis, for mean performance and stability of genotypes for grain yield, based on an average-environment coordinate (AEC) is presented in Fig 3a. The single-arrow on the AEC points to higher mean yield. G4 had the highest yield, followed by G8. The double-arrowed line is the AEC ordinate that points in either direction to greater variability (least stability). Genotypes G2, G11, G5, G14, G21 and G20 were the most stable with above average performance while genotypes, G8 and G15 were the least stable but high yielding (Fig 3a).

Similarly, based on the average-environment coordinate (AEC) (Fig 3b) genotypes G3, G4, G8, G9, G15, G16 and G19 had low chocolate spot disease severity (negative low PC1 scores) with the least to moderate stability across the environments. Although G18 was stable it was one of the most susceptible genotypes. Genotypes G13, G6 and G10 were susceptible and less stable to chocolate spot (Fig 3a).

Discriminating power and representativeness of the test environments

The GGE biplots depicting the discriminating ability and representativeness of the test environments are presented in Fig 4a and b. In this figure the average environment is represented by the small circle at the end of the arrow and contains the average coordinates of all test environments (Yan and Tinker, 2005). The biplot contains the “Average-Environment Axis” [AEA, or average-tester-axis, Yan (2001)] which is the line that passes through the average environment and the biplot origin. Environment E5 had the smallest angle with the AEA indicating it was more representative of the test environments while E1 and E4 were the least representative. The concentric circles aid in the visualisation of the length of the environment vectors. Environment E2 had the longest vector from the biplot origin indicating it was the most discriminating (informative) of

the environments. E5, E6 and E3 had moderate vector lengths. E1 and E4 had the shortest vector lengths.

In terms of correlation, none of the environments were negatively correlated as there was no obtuse angle observed between any of the environments (Fig 4a). All the environments had acute angles ($< 90^\circ$) with each other, with some of the environments like, E3 and E6; E4 and E2 having even smaller angles between them indicating more positive correlations between the environments. Further, the angles between E1, E3, E6, and E5 were all smaller (acute). For chocolate spot disease severity, environments E3 and E4 had the least angles to the AEA line indicating that there were the most representative of the test environments (Fig 4b). In addition, the environment vector for E3 was relatively long indicating both discriminating ability and representativeness. E2 and E5 had the longest vectors and high positive PC1 scores, suggesting that they were more discriminating of the genotypes than the other environments (Fig 4b). Environment E4 had the shortest environment vector and PC2 close to zero, suggesting less discriminating ability. Further the angles between all the six environments were acute ($< 90^\circ$) indicating positive correlations among them (Fig 4b).

Discussion

The AMMI analysis revealed highly significant effects for genotypes (G), environments (E), and genotype \times environment interaction (GEI). Grain yield performances for the tested faba bean genotypes were influenced highly by the environments (61.4% contribution to total variation), followed by GEI reflecting that the genotypes were highly variable in their responses to different environmental changes. These results are in agreement with the findings of Fikere et al. (2008) who observed high contribution (88.5% to the total variation) of the environments to grain yield variability in AMMI analysis of some faba bean accessions in Ethiopia. Other studies have reported significantly different GEI for grain yield in faba bean (Abebe et al., 2015; Karadavut et al., 2010). The significant genotype and GEI for grain yield suggested the presence of differentially adapted faba bean genotypes. Therefore, different faba bean genotypes could be selected for the different environments as reported for other crops (Derera et al., 2008; Sibiyi et al., 2013; Zhang et al., 2006). AMMI biplot revealed that genotypes G20, G11, G4, and G14 were the most stable and adapted to all environments. However, G8 had specific adaptation in E6 (Kofele). Similarly, G21, G16, G9 and G12 were adapted to E4 (Bekoji). Environment Adadi (E2) had the highest yielding genotypes, thus representing a high potential environment while Holetta (E1) was the lowest yielding environment. This can be explained by the neutral nature of the soils at Adadi, but extremely acidic soils at Holetta. Acidic soils have been reported to be one of the biotic factors that reduce nodulation and yield of faba bean (Zerihun and Abera, 2014).

AMMI analysis also showed that genotypes were the main source of variation for chocolate spot disease expression, followed by GEI. This suggests differential responses of the genotypes. Equal contributions of the genotypes and GEI to disease expression were also reported for faba bean genotypes evaluated in different environments (Villegas-Fernández et al., 2011). In this present study, Holetta (E1) was the most favourable site for disease expression followed by Assasa (E5).

In contrast E4 (Bekoji) was the least discriminating environment for disease expression. Genotypes G3, G4, G19, G15, G17, G8, G16 and G9 were identified as resistant to moderately resistant with G3, G4 and G19 as the best. In contrast, G13, G18, G6, G1 and G10 were susceptible with G6 and G13 as the most susceptible genotypes. Genotypes G19, G15, G17, G9 and G16 were resistant and stable across the environments. Genotype G3, G4 and G8 were unstable but resistant. Considering the additive gene action mode of inheritance for chocolate spot resistance (Beyene et al., 2015), the unstable yet resistant faba bean genotypes could be exploited as a genetic source of different breeding strategy. G6, G10 and G18 were stable but susceptible.

The GGE biplot analysis provided a visual depiction of the relationship among the genotypes and test environments. The polygon view of the GGE biplot indicated the presence of a crossover GEI as the environments fell in different sectors of the polygon view and had different high yielding genotypes (Yan and Kang, 2002). Genotypes G8 and G4 were the highest yielding in environments E1, E3, E6 and E5, and genotype G15 was the highest yielding genotype at E2 and E4. Based on the average-environment coordinate (AEC) in the GGE biplot, genotypes G2, G11, G14, G5, G21 and G20 were the most stable with an above average performance (Yan et al., 2007).

In this study, the environments fell in two mega-environments. Adadi (E2) was the most discriminating environment for grain yield followed by Kulumsa (E3), Kofele (E6) and Assasa (E5). In contrast, Bekoji (E4) and Holetta (E1) were the least discriminating environments. However, Adadi (E2) although discriminating of the genotypes, it was the least representative of the test environments. On the other hand, E5 (Assasa) was the most representative of the environments for grain yield, followed by Kulumsa (E3). An ideal test environment should effectively discriminate genotypes and represent the environments (Yan and Kang, 2002). According to Yan and Tinker (2005), environments that give little information on genotypes (Non-discriminating) should not be used as test environments. Thus, in this study among all the six environments, Kulumsa (E3) represented the ideal testing environment with high discriminating ability of the genotypes and moderate in representativeness of the test environment for faba bean grain yield. This environment can be used for selecting generally adapted genotypes. Environments such as Adadi (E2) which was discriminating but non-representative are recommended for selecting specifically adapted genotypes (Yan and Tinker, 2005). The angles between all the six environments were acute ($< 90^\circ$) indicating positive correlations among them for both grain yield and chocolate spot disease severity. This suggests that the same information could be obtained about the genotypes from these environments which are closely associated, thus fewer test environments could be used to reduce costs.

Stability of resistance to chocolate spot is crucial for the success of breeding programme. In the present study, the polygon view of the GGE biplot revealed that genotypes G3, G4, G15, G14, G16, G19, G8, and G9 had low chocolate spot severity, but were the least stable genotypes. On the other hand, G13, G6 and G10 had high disease severity but least stable. G18 was the most stable genotype with high diseases severity. The test environments fell in two sectors of the polygon forming two mega-environments, E1, E3, E4, E5 and E6 as one mega-environment and E2 the second mega-environments. G3

was a desirable genotype for its low disease severity. Whereas G18, was most susceptible of all tested genotypes followed by genotypes G6, G13 and G10. Genotypes were thus ranked as: $G3 < G4 = G19 = G8 < G9 = G15 = G17 < G5 = G14 = G16 = G21 < G12 = G11 = G20$ for stability to chocolate spot disease resistance. Genotypes G5, G14, G16, G21 and G20 were selected for their lower disease severity with moderate stability. Variation in stability of faba bean genotypes for chocolate spot disease resistance was observed in different locations (Villegas-Fernández et al., 2009).

Environments Adadi (E2) and Assasa (E5) had more discriminating ability of the genotypes for chocolate spot disease than the other environments. In contrast, environment E4 had less discriminating ability for the genotypes. However, environment E3 (Kulumsa) was the most discriminating and representative environment for chocolate spot disease followed by Holetta (E1). This indicated these environments are the most efficient for evaluating the potential of genotypes for chocolate spot resistance. Bekoji (E4) had less discriminating ability for the genotypes for chocolate spot disease. An ideal test environment should effectively discriminate genotypes and represent the environments (Yan and Kang, 2002). Therefore, among the six environments Kulumsa (E3) represented the ideal testing environment for chocolate spot disease and would be appropriate for selecting best faba bean genotypes resistant to chocolate spot.

Materials and methods

Plant materials and environments

Twenty one faba bean genotypes consisting of nineteen genotypes previously recommended as moderately resistant to chocolate spot disease (*Botrytis fabae*) along with two standard checks Kassa (susceptible) and ILB 4726 (resistant) to chocolate spot disease were tested over six locations (Supplementary Table 2 & Fig 5). The sites represented the principal faba bean growing areas in Ethiopia and substantially differed in terms of geographic locations, temperature, rainfall and soil pH. The trials in all the environments were conducted under rain fed conditions.

Experimental design and management

The 21 faba bean genotypes were planted in a randomized complete block design with three replications in all the locations. Diammonium phosphate (DAP) fertilizer was applied at 100 kg ha⁻¹, that is, 20 kg ha⁻¹ phosphorus and 18 kg ha⁻¹ nitrogen, at planting. The fields were managed following recommendations for the specific locations. For better evaluation of the genotypes, natural disease infestation for chocolate spot disease was supplemented with artificial inoculation of *Botrytis fabae* isolate at a spore concentration of 5 x 10⁵ ml⁻¹, one month after planting using a knap sac sprayer (Mohammed et al., 1994).

Disease severity scores were recorded from the whole plot once at 88 days after planting. The severity of chocolate spot was recorded as a percentage of leaf area infected as follows: 1%-no disease symptoms or very small specks (highly resistant); 3%-few small disease lesions (resistant); 6%-small coalesced lesions, with some defoliation (moderately resistant); 12%-large coalesced sporulating lesions, 20% defoliation

(moderately susceptible); 25%-large coalesced sporulating lesions, 50% defoliation and some dead plants (susceptible) and 50%-extensive, heavy sporulation, stem girdling, blackening and death of more than 80% of the plants (highly susceptible) (Bernier et al., 1993; Bernard et al., 2006). The data for grain yield and other agronomic traits were taken following the standard practice for faba bean trial used. Grain yield was taken as weight of seeds from the middle two rows per plot. Grain yield adjustment was made based on oven dried seeds and adjusted to constant moisture level of 10%. The total grain yield was recorded on a plot basis and converted to t ha⁻¹ for statistical analysis.

Statistical data analyses

Both additive main effects and multiplicative interaction (AMMI) and the genotype and (genotype x environment) (GGE) biplot methods were used to investigate the genotype, environment and genotype x environment interaction effects on grain yield and chocolate spot disease severity of faba bean genotype as described by Yan (2002). The AMMI model, which combines the standard analysis of variance with principal component analysis, was used to investigate the nature of genotype x environment interaction (Zobel et al., 1988). A total of six test environments were used for the analysis. Additive Main Effects and Multiplicative Analysis was performed using the AMMI macros in GenStat 14th (Payne et al., 2012). The following AMMI model was used for the 21 germplasm and 6 test environments (Gauch, 1992).

$$Y_{ij} = \mu + g_i + e_j + \sum_{n=1}^{n'} \lambda_n \alpha_{in} \gamma_{jn} + \theta_{ij}$$

$\theta_{ij} \sim N(0, \sigma^2)$; $i = 1, 2, \dots, 21$; $j = 1, 2, \dots, 6$

Where: Y_{ij} = yield mean of i^{th} genotype in j environment

μ = grand mean; g_i = main effect of genotypes; e_j = main effects of environments;

λ_n = Eigen values for PCA axis n ; α_{in} and γ_{jn} = the i^{th} genotype j^{th} environment PCA scores for the PCA axis n ; θ_{ij} is the residual; n' is the number of PCA axes retained in the model GGE biplot analysis was also done using GenStat software version 14 (Payne et al., 2012). To visualize the performance of the genotypes in each environment and groups of environments, a polygon view was drawn by connecting genotypes that were furthest from the biplot origin such that all genotypes were enclosed within the polygon (Yan, 2002). The biplot was also used to explore the interrelationships among environments by constructing lines (environment vectors) from the biplot origin to markers for the environments. The cosine of the angle between environments corresponds to the degree of correlation between environments. The length of the vectors was used to determine the discriminating ability of each of the test environments, with a shorter vector implying that the environment was not well represented by PC1 and PC2 (Yan et al., 2007).

Conclusion

Yield performance of the newly selected faba bean genotypes in this study were highly influenced by environment and genotype x environment interaction, in contrast to the chocolate spot disease which was influenced largely by genotypic differences. Both the GGE biplot and AMMI analysis provided almost

similar results in terms of stability and performance of the genotypes. Among the genotypes FBColl-0012 (G2), FBColl-0034 (G11), FBColl-0055 (G14), FBColl-0025 (G5), FBColl-0049 (G21) and FBColl-0036 (G20) all from the landrace collection were the highest yielding and most stable genotypes across the six environments, while for chocolate spot disease resistance genotypes G5, G14, G16 and G21 had moderate stability and resistance to chocolate spot disease. In addition, G20, G4, G19, G8, G9, G15 and G17 were also low in chocolate spot severity. The less stable yet resistant faba bean genotypes could be exploited as a source of resistance for different breeding strategies. Overall, genotypes FBColl-0055 (G14) and FBColl-0025 (G5) were the best in terms of yield, stability and disease resistance and could thus be recommended over the test environments. The GGE biplot clustered the six environments into two mega environments and Kulumsa (E3) was found to be the best in terms of discriminating ability and representative of the test location to evaluate faba bean for yield potential and chocolate spot resistance.

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References

- Abebe T, Yemane N, Muez M, Adhiena M, Assefa W, Hadas B (2015) Genotype by environment interaction of some faba bean genotypes under diverse broomrape environments of Tigray, Ethiopia. *J Plant Breeding Crop Sci*. 7: 79-86
- Beyene A, John D, Sibiyaj, Fikre A (2016) Gene action determining grain yield and chocolate spot (*Botrytis fabae*) resistance in a faba bean. *Euphytica*. 207: 293-304.
- Bernard T, Alain B, Carmen MA, Sabine B, Martin B, Weidong C, Jenny D, Kurt L, Mohammed K, Diego R, Mohamed S, Josefina CS, Mark S, Fred JM (2006) Screening techniques and sources of resistance to foliar diseases caused by major necrotrophic fungi in grain legumes. *Euphytica*. 147:223-253.
- Bernier CC, Hanounik SB, Hussein MM, Mohamed HA (1993) Field manual of common faba bean diseases in the Nile Valley. International Centre for Agricultural Research in the Dry Areas (ICARDA). Information Bulletin No. 3.
- Derera J, Tongoona P, Pixley KV, Vivek B, Laing MD and Rij NC (2008) Gene action controlling grey leaf spot resistance in Southern African maize germplasm. *Crop Sci*. 48: 93-98.
- FAOSTAT (2014) Data base. Available at: <http://faostat3.fao.org/faostat-gateway>.
- Fikere M, Suso MJ, Tadesse T, Legesse T (2008) Analysis of multi-environment yield performance of faba bean (*Vicia faba* L.) genotypes using AMMI model. *J Genet Breed*. 62:25-30.
- Gauch HG (1992) Statistical analysis of regional yield trials. AMMI analysis of factorial designs. Elsevier, Amsterdam.
- Gauch HG (2006) Statistical analysis of yield trials by AMMI and GGE. *Crop Sci*. 46:1488-1500.
- Gauch HG, Piepho HP, Annicchiarico P (2008) Statistical analysis of yield trials by AMMI and GGE: further considerations. *Crop Sci*. 48:866-889.
- Karadavut U, Palta Ç, Kavurmaci Z, Bölek Y (2010) Some grain yield parameters of multi-environmental trials in faba bean (*Vicia faba*) genotypes. *Int J Agric Biol*. 12: 217-220
- Mohammed HA, Aly HA, and Wadia FH (1994) The antagonistic effect of faba bean phytoplane to *Botrytis fabae* Sard. *Egypt J Agric Res*. 72:645-654.
- Mukherjee AK, Mohapatra NK, Bose LK, Jambhulkar NN, Nayak P (2013) Additive main effects and multiplicative interaction (AMMI) analysis of GxE interactions in rice-blast pathosystem to identify stable resistant genotypes. *Afric J Agric Res* 8:5492-5507.
- Mulema JMK, Adipala E, Olanya OM, Wagoire W (2008) Yield stability analysis of late blight resistant potato selection. *Exp Agr*. 44:145-155.
- Payne GA, Harding SA, Murray DA, Soutar DM, Baird DB, Glaser AI (2012) GenStat for Windows Introduction. VSN International, Hemel Hempstead. 14 ed., UK.
- Rubiales D, Avila CM, Sillero JC, Hybl M, Narits L, Sass O, Flores F (2012) Identification and multi-environment validation of resistance to *Ascochyta fabae* in faba bean (*Vicia faba*). *Field Crops Res*. 126:165-170.
- Sibiya J, Tongoona P, Derera J (2013) Combining ability and GGE biplot analyses for resistance to northern blight in tropical and subtropical elite maize inbred lines. *Euphytica* 191:245-257.
- Stoddard FL, Nicholas AH, Rubiales D, Thomas J, Villegas-Fernández AM (2010) Integrated pest management in faba bean. *Field Crops Res*. 115:308-318.
- Villegas-Fernández AM, Sillero JC, Emeran AA, Winkler J, Raffiot B, Tay J, Flores F, Rubiales D (2009) Identification and multi-environment validation of resistance to *Botrytis fabae* in *Vicia faba*. *Field Crops Res*. 114:84-90.
- Villegas-Fernández AM, Sillero JC, Emeran AA, Flores F, Rubiales D (2011) Multiple-disease resistance in *Vicia faba*: Multi-environment field testing for identification of combined resistance to rust and chocolate spot. *Field Crops Res*. 124:59-65.
- Yan W (2002) Singular-value partitioning in biplot analysis of multi-environmental trial data. *Agron J*. 94:990-996.
- Yan W, Cornelius PL, Crossa J, Hunt LA (2001) Two types of GGE biplots for analyzing multi-environment trial data. *Crop Sci*. 41:656-663.
- Yan W, Kang MS (2002) A graphical tool for breeders, geneticists, and agronomists. CRC Press, UK.
- Yan W, Kang MS, Ma B, Woods S, Cornelius PL (2007) GGE biplot vs. AMMI analysis of genotype-by environment data. *Crop Sci*. 47: 643-655.
- Yan W, Tinker NA (2005) An integrated biplot analysis system for displaying, interpreting, and exploring genotype × environment interaction. *Crop Sci*. 45:1004-1016.
- Zerihun A, Abera T (2014) Yield response of faba bean to fertilizer rate, rhizobium inoculation and lime rate at Gedo highland, western Ethiopia. *Global Sci Res J*. 2:134-139.
- Zhang Y, He Z, Zhang A, van Ginkel M, Ye G (2006) Pattern analysis of grain yield performance of Chinese and CIMMYT spring wheat cultivars sown in China and CIMMYT. *Euphytica*. 147:409-420.
- Zobel RW, Wright MS, Gauch HG (1988) Statistical analysis of a yield trial. *Agron J*. 80:388-393.