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AMMI Biplot Analysis for Yield Stability of Black Cumin (*Nigella Sativa* L.) Genotypes in East Shoa and West Arsi Zones

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ABSTRACT

Black cumin is an erect annual herb cultivated for its seed, growing on all kinds of soils. In Ethiopia black cumin is cultivated as rain fed crop in the highlands from 1500 to 2500meters above sea level and the weather makes a suitable environment for the growth of black cumin seed.AMMI model has found to be more effective in selection of stable crop genotypes and AMMI biplot analysis is possibly the most powerful interpretive tool for AMMI modelin order to have a clear observation in to specific genotype-environment combination and general pattern of adaptation. The objective of this study was to test, evaluate and identify the high yielding as well as the most stable and adaptable black cumin genotype and to determine the areas where black cumin would be adapted by using AMMI model along with its biplots.Fifteen black cumin genotypes were evaluated at six locations in randomized complete block design with three replications during 2018/2019 cropping season. The combined analysis of variance showed that the genotypes Gammachis and 90575-2 had the highest and the lowest seed yield, with their average seed yield of 1.35 ton ha-1and 0.78 ton ha-1 respectively. Generally, both AMMI-1 and AMMI-2 identified the genotypes 242834-1, Dirshaye and Soressaas the most stable genotypes. On the other hand, AMMI-1 and AMMI-2 identified the location Ali Woyyothat have shortest rib, and thus did not exert a strong interactive force indicating that this location was considered as the most suitable location for black cumin production.

Keywords: *Black cumin seed, Stability analysis, AMMI stability Values*

INTRODUCTION

Black cumin (*Nigella sativa* L.) is an erect annual herb cultivated for its seed, growing on all kinds of soils (Jansen, 1981). It is a medicinal annual plant belonging to *Ranunculacea* family grown naturally in Southwest Asia and the Mediterranean region (Toncer and Kizil, 2004). It originated in Egypt and East Mediterranean, but is widely cultivated in Iran, Japan, China and Turkey (Shewaye, 2011). Black cumin is cultivated in the subtropical belt extending from Morocco to Northern India and Bangladesh, East Africa and in the former Soviet Union. In Europe, North America and South-east Asia, it is cultivated on a minor scale, mainly for medicinal use (Akhtar and Saha, 1993).It is also cultivated in sub-Saharan Africa particularly in Niger, and Eastern Africa especially Ethiopia (Iqbal *et al*., 2010).

In Ethiopia black cumin is cultivated as rain fed crop in the highlands from 1500 to 2500 meters above sea level. It can be intercropped with barley and wheat (Ahmed and Haque, 1986). However improved production technology must be available (Ministry of Agriculture and Rural Development, 2003).

Black Cumin has a long history of uses for food flavors, perfumes and medicinal values. Studies have shown that *Nigella sativa* seeds have high nutritional values: proteins content ranging from 20% to 27%, carbohydrates ranging from 23.5% to 33.2%, moisture content ranging from 5.52% to 7.43% and ash content ranging from 3.77% to 4.92% (Al-Jassir, 1992; Nergiz and Otles, 1993).

Black cumin grows on a wide range of soils. Sandy loam soil rich in microbial activity is the most suitable for its cultivation. Areas with moderate rainfall and well drained soils with pH of 7-75 are quite suitable for black cumin production (Orgut, 2007). Some studies shown that black cumin is able to tolerate moderate levels of water stress and responds favorably to fertilizers (Mozzafari *et al*., 2000 and Bannayan *et al*., 2008).

Yield is the most complex and the most important character, as well as the most final result which is dependent on a number of other characters. It is highly influenced by many genetic factors as well as environmental fluctuations (such as soil PH: soil acidity and salinity, soil moisture content and/or humidity, temperature climax: both minimum and maximum, rain fall, etc).The genetic potential contained within the crop, the environmental effects and the interaction of the two have their own roles in determining the performance and stability of the crop to a given environment.

On the other hand, genotype x environment interaction (GEI) studies along with yield stability analysis are an important aspect of both plant breeding programs and introduction of new crop cultivars (McLaren CG. and Chaudhary C., 1994; Prasad KV. and Singh RL., 1990; Freeman GH., 1985). Therefore, genotype x environment interaction studies and yield stability analysis are the most imperative in assessing the crop varietal stability and suitability to a given sort of crop growing environments across the seasons.

Data from multi location trials are imprecise, rough and complex. The conventional method of partitioning the total variation in to components due to genotype, environment and the interaction of the two convey little information on the individual pattern of response (Zobel *et al*., 1988). To increase accuracy, additive main effect and multiplicative interaction (AMMI) is the first model of choice when both main effects and their interactions are important.

Additive Main Effects and Multiplicative Interaction (AMMI) model has found to be more effective in selection of stable crop genotypes (Ariyo and Ayo-Vaughan, 2000 and Taye *et al.*, 2000) and is used to analyze multi-location trials (Zobel*et al.*, 1988 and Crossa *et al.*, 1990). It integrates the analysis of variance and principal component analysis in to a unified approach (Bradu and Gabriel, 1978) and reveals a highly significant interaction component that has a clear agronomic meaning. AMMI uses ordinary ANOVA to analyze the main effects (additive part) and interaction principal component analysis (IPCAs) to analyze the nonadditive residual left over by the ANOVA (Gauch, 1993).

The results of AMMI analysis can be well presented graphically in the form of bi-plots (Gauch, 1988; Zobel *et al.*, 1988; Vargas *et al.*, 1999) in which the genotype and environment scores are represented by vectors in a space, with starting points at the origin and end points determined by the scores. AMMI biplot analysis is possibly the most powerful interpretive tool for AMMI models. Moreover, in order to have a clear observation in to specific genotype-environment combination and general pattern of adaptation, a bi-plot of genotypes and environments (Kempton, 1984) plays a great role. Therefore, the current study was conducted with the objective of testing, evaluating and identifying the high yielding as well as the most stable and adaptable black cumin genotype and determining the areas where black cumin would be adapted by using AMMI model along with its biplots.

MATERIALS AND METHODS

The study was conducted in East Shoa and West Arsi zones at six locations during the 2018/2019 main cropping season under rain fed conditions. Two sites [AdamiTullu Agricultural Research Center (ATARC) and BekeleGirrisa] are found in East Shoa zone while, the other four sites (Ali Woyyo, MakkoOda, Bute and Umbure) are found in West Arsi zone. The locations are the representative for the diverse agro-ecologies of spice crops growing environments in both East Shoa and West Arsi zones.

Table1. *Description of the test locations used in the study*

Locations	**Altitude (m.asl)**	**Average Annual RF (mm)**	**Soil Type**	**Global Positions**	
				Latitude	Longitude
ATARC	1650	760.9	Sandy, Clay and Silt (34%, 48% and 18%)	7^{o} 9' N	38^{o} 7'E
BekeleGirrisa	1600	700-800	Sandy and Clay	7^{o}58' N	38^{o}43' E
Ali Woyyo	1960	900	NA	7^{o} 23' N	38^{o} 43' E
MakkoOda	1980	920	NA	7^{o} 33' N	38^{o} 62' E
Bute	NA	980	Sandy	7^{o} 23' N	38^{o} 24' E
Umbure	NA	1057	Sandy	7^{o} 12' N	38^{o} 36' E

Key: *'NA' stands for not available and 'ATARC' for AdamiTullu Agricultural Research Center*

A total of fifteen black cumin genotypes (viz; AC-BC-4, AC-BC-9, AC-BC-10, AC-BC-19, MAB-042, MAB-057, 90575-2, 20750-1, 242834-1 and 244654-1) along with five standard checks (viz; Derbera, Dirshaye, Eden, Gammachis and Soressa) that were obtained from Sinana Agricultural Research Center were used in this study. The experiment was designed using Randomized Complete Block Design (RCBD)with three replications. The total plot area was 2.4m^{2}with the plot size of 1.2m x 2m (having4 rows; each 2m long). The spacing between rows, plots and blocks were 30cm, 50cm and 100cm, respectively.

Sowing was carried out in the first week of June 2018 up to the fourth week of June 2018. It was done by hand drilling and covered slightly with the soil. Fertilizer application of 46kg $P_2 0_5$ ha^{-1} along with 60kg N ha^{-1} was used as they enhance root development and seed yield in black cumin (Champawat and Pathak, 1982).All agronomic and other management practices were done as per requisition at each location.

Data on days to emergence, days to 50% flowering, days to maturity, plant height (cm), number of primary branches per plant, number of capsules per plant, seed yield per hectare (ton ha^{-1}) and thousand seed weight (g) were recorded during this study. Data was collected from the middle two harvestable rows for traits estimated from a plot.

All the recorded data were subjected to analysis of variance following the standard procedure for each location and combined analysis of variance over locations was computed using the Gen-Stat 18th Edition Statistical Computer Software Programs.

Bartlett's chi-square test was used to determine the validity of the combined analysis of variance and homogeneity of error variances among environments. Thereafter, the combined analysis of variance was carried out to estimate the additive effects of genotype, environment and their interactions.

AMMI stability analysis was used to analyze the main effects (additive part) and interaction principal component analysis (IPCAs) to analyze the non-additive residual left over by the analysis of variances. The results of AMMI analysis were presented graphically in the form of bi-plots in which both genotype and environment scores can be represented to have a clear observation in to specific genotype-environment combination and general pattern of adaptation to the specified environment.

Both AMMI biplots (viz; AMMI-1 biplotand AMMI-2 biplot were sketched and/orplotted). In AMMI-1 biplot, the main effects (genotype mean and environment mean) and IPCA-1 scores for both genotypes and environments were plotted against each other. Accordingly, the first IPCA was used as ordinate while, the main effect (means of genotypes and environments) was used as abscissa. On the other hand, in the AMMI-2 biplot, the scores for IPCA-1 and IPCA-2 are plotted against each other.

The AMMI model was given as:

$$\mathbf{Y_{ij} = \mu + G_i + E_j + \sum \lambda_k\, a_{ik}\, Y_{jK} + e_{ij}}$$

Where;

$\mathbf{Y_{ij}}$ is the yield of the ith genotype in the jth environment,

$\boldsymbol{\mu}$ is the grand mean,

$\mathbf{G_i}$ and $\mathbf{E_j}$ are the genotype and environment deviations from the grand mean respectively.

$\boldsymbol{\lambda_k}$ is the eigen value of the interaction principal component axis K

$\boldsymbol{\alpha_{ik}}$ and $\mathbf{Y_{jk}}$ are genotype and environment principal component scores for axis K

$\mathbf{e_{ij}}$ is the error term.

AMMI Stability Value (ASV), IPCA1 and IPCA2 were computed to identify the stable genotype with consistence yielding performance across the testing environments. The degrees of freedom for the IPCA axes were also calculated based on the following method (Zobel et al., 1988).

$$\mathbf{df = G + E - 1 - 2n}$$

Where;

df is degree of freedom

G is number of genotypes,

E is the number of environments and

n is the nth axis of IPCA

RESULTS AND DISCUSSIONS

The AMMI analysis revealed that the variances due to genotypes, environments and GEI showed a highly significant difference ($P < 0.01$). On the other hand, the presence of blocking and/or replicating within the testing environments could not influence the yield performance of the tested genotypes.

AMMI analysis of variance for seed yield (ton ha^{-1}) of fifteen black cumin genotypes that were tested and evaluated at six environments showed that most of the total sum square of the model (41.99%) was attributed to environmental effects while, the rest were attributed to the genotypic effects (31.96%) and the GEI (10.96%) as indicated in the Table -1.

Table2. *Additive main effects and multiplicative interaction (AMMI) analysis of variance for seed yield (ton ha^{-1}) of fifteen (15) black cumin genotypes across six environments*

Sources of variations	df	Mean Squares	% explained From TSS	% explained From GEI
Total	269	0.0714		
Genotype	14	0.4926**	31.96	
Locations	5	1.8122**	41.99	
Blocks (within locations)	12	0.0055 ^{ns}	0.31	
GEI	70	0.0338**	10.96	
IPCA$_1$	18	0.0824**	6.87	62.67
IPCA$_2$	16	0.0336**	2.49	22.71
Error	168	0.0049 ^{ns}	3.82	

Key: *** *stands for highly significant difference ($P \leq 0.01$), ns stands for non-significance difference, df for degree of freedom, MS for Mean Squares and TSS for Total Sum Squares*

The observed largest sum of squares along with highly significant mean of square for the environment showed that the test environments were highly diverse, with large differences among environmental means causing most of the variation to the total seed yield, which is in agreement with the findings of (Fufa, 2018).

The presence of genotype x environment interaction (GEI) was clearly demonstrated by the AMMI model, and the interaction was partitioned among the first two interaction principal component (IPC) axes (i. e.; IPCA-1 and IPCA-2). AMMI analysis have also revealed that the first and the second principal component axes (viz; IPCA-1 and IPCA-2) of the interaction contributed about 62.67% and 22.71% respectively to the total GEI sum of squares (Table 1).

The mean square for IPCA-1and IPCA-2were highly significant ($P \leq 0.01$) and cumulatively contributed about 85.38% to the total GEI sum square. Therefore, the AMMI model with only two interaction principal component axes was considered as the best predicting model for the interaction of fifteen black cumin genotypes and six environments.

The mean seed yield value of genotypes averaged over environments indicated that the genotypes Gammachis and 90575-2 had the highest and the lowest seed yield, with their average seed yield of 1.35 ton ha^{-1} and 0.78 ton ha^{-1}respectively.Different genotypes showed inconsistent performance across all environments. The environments mean seed yield ranged from 0.85 ton ha^{-1}for ATARC to 1.35 ton ha^{-1} for MakkoOda.

The AMMI analysis has plotted the summarized graphical representation of the information on the main effects (genotypes and environments) and interactions effects (GEI) both in AMMI-1 and AMMI-2 biplots.

AMMI-1 BIPLOT SKETCH

In the AMMI-1 bi-plot, the IPCA$_1$ scores of genotypes and environments are plotted against their respective means seed yield. The IPCA scores of a given genotype in the AMMI analysis indicate the stability and/or adaptability of that genotype over environments (Gauch and Zobel, 1996; Purchase, 1997 and Alberts, 2004).

The greater the IPCA scores (whether it is negative or positive), of a given genotype indicate specific adaptation of that genotype to certain growing environments. In contrast, the more the IPCA-1scores approximately closer to zero, the more stable or adaptable the genotype is over all sampled environments. Genotypes and environments on the right side of the midpoint of the axis have higher yields than those of left hand side. Consequently, the genotype Gammachis, Dirshaye, Soressa, Eden, Derbera and 244654 were generally exhibited the higher seed yield.

Genotypes that group together have similar adaptation while environments which group together influences the genotypes in the same way. If a genotype or an environment has a IPCA-1 score of nearly zero, it has small interaction effects and considered as stable.Accordingly, genotypes such as 242834-1, Dirshaye, AC-BC-10 and Soressa had high mean seed yield and had IPCA-1 score closer to zero values. Therefore, these genotypes were less influenced by the environmental fluctuations and considered as the most stable genotypes (Figure 1).

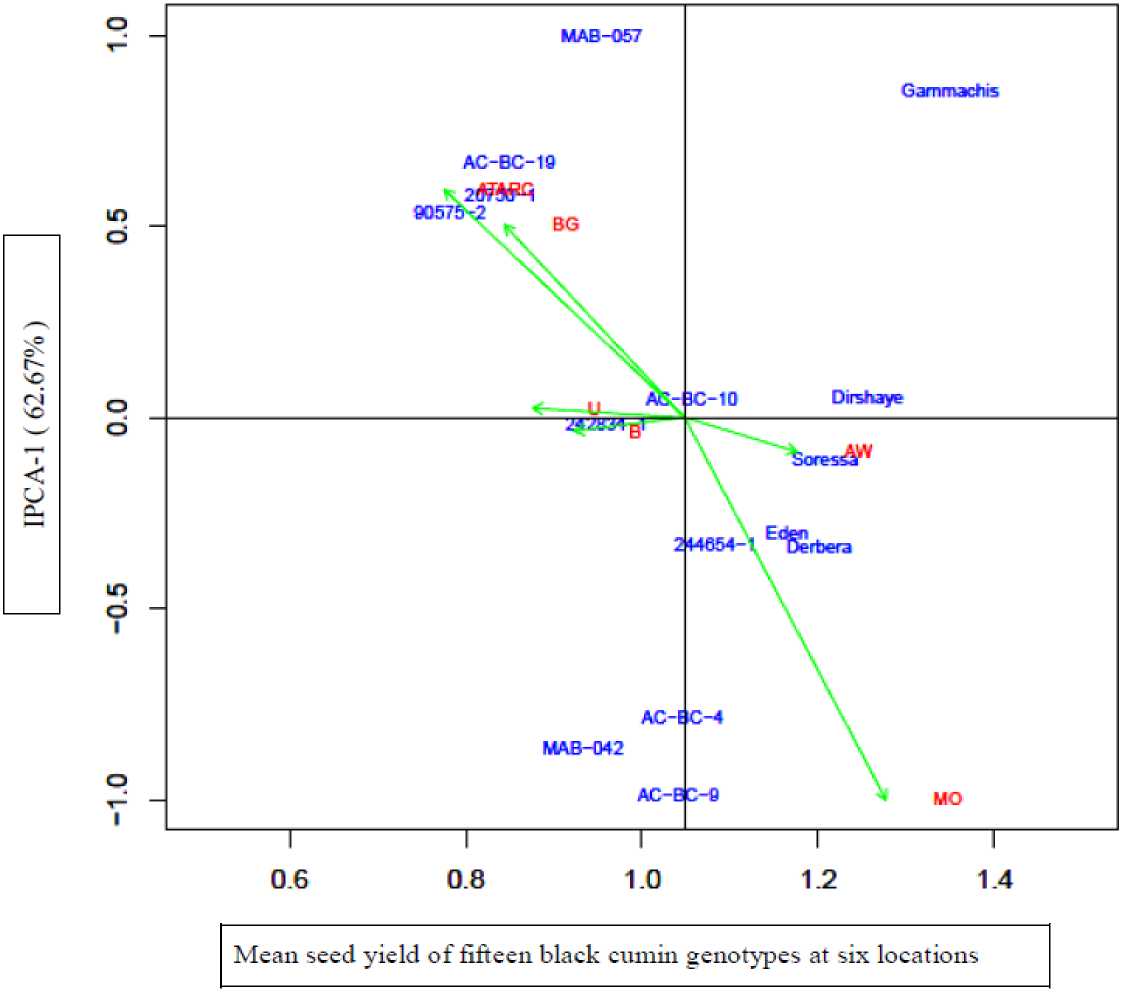

Figure1. *AMMI-1 bi-plot of IPCA-1scores against the mean seed yield of fifteen black cumin genotypes at six test environments*

On the other hand, the environments with positive IPCA-1 scores nearer to zero have small interaction effects indicating that all the genotypes performed well in those environments and thus these environments are considered as the favorable environments. Accordingly, the location Ali Woyyo was considered as the most favorable environment.

AMMI-2 BIPLOT SKETCH

IN THE CASE OF AMMI-2 biplot, the scores for IPCA-1 and IPCA-2 are plotted against each other. In AMMI-2 biplot, the environmental scores are joined to the origin by side lines. The environment with the shortest spoke do not exerts strong interactive forces while, the one with the longest spoke exerts strong interactive forces (Figure 2). Accordingly, the location Ali Woyyo had the shortest spoke, and thus did not exerta strong interactive force.

The genotypes occurring close together on the plot will tend to have similar seed yields across all test environments, while geno types far apart may differ in their mean seed yields, and therefore show a different pattern of response over the environments. For instance, the genotypes AC-BC-4 and MAB-042, Eden and Derbera, as well as AC-BC-19 and 90575-2 were plotted closer to each other on the AMMI-2 biplot and thus they had similar or nearly similar seed yields.

The genotypes found nearer the origin are not sensitive to environmental interaction and considered as the most stable genotypes, while those genotypes found distant fromthe origin are sensitive to the environmental interaction and could be considered as unstable genotypes. In view of that, the genotypes 242834-1, Dirshaye and Soressa were plotted closer to the origin and hence they were non sensitive to environmental interactive forces. Therefore these genotypes were considered as the most stable genotypes.

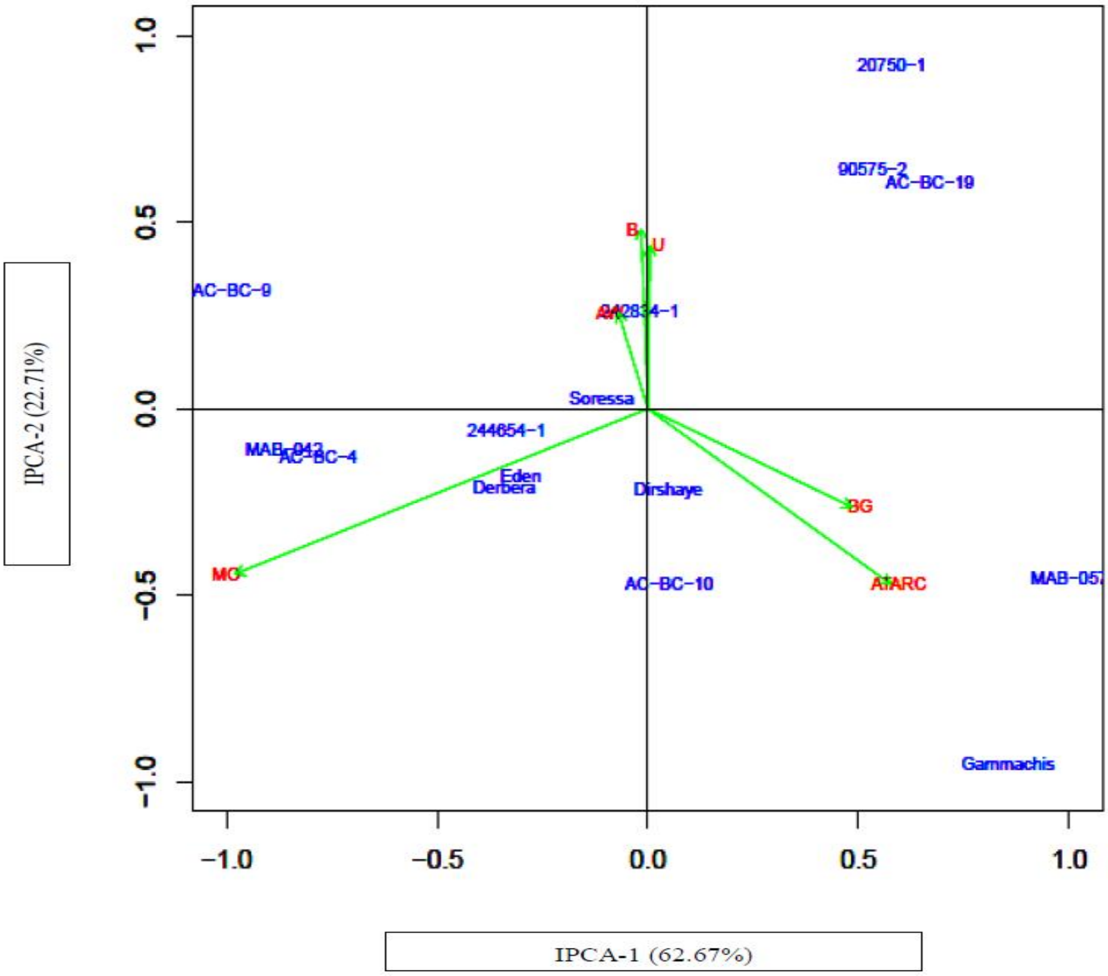

Figure2. *AMMI-2 bi-plot of IPCA-1 scores plotted against IPCA-2 scores*

SUMMARY AND CONCLUSION

Crop yield is a complex trait that can be influenced directly or indirectly by a number of both crop and environmental factors. The mean seed yield value of geno types averaged over environments indicated the variety Gammachis had the highest (1.35ton ha^{-1}) and the genotype 90575-2 had the lowest yield (0.782 ton ha^{-1}).Generally, the variety Gammachis as followed by Dirshaye, Soressa, Derbera, Eden and 244654 were generally exhibited the higher seed yield with their respective seed yields of(1.35ton ha^{-1}), (1.256 ton ha^{-1}), (1.208 ton ha^{-1}), (1.204 ton ha^{-1}), (1. 165 ton ha^{-1}) and (1.083 ton ha^{-1}).

AMMI analysis of variance for seed yield (ton ha^{-1}) of fifteen black cumin genotypes that were tested and evaluated at six locations showed that most of the total sum square of the model (41.99%) was attributed to environmental effects while, the rest were attributed to the genotypic effects (31.96%) and the GEI (10.96%).

Both AMMI-1 biplot and AMMI-2 biplot were sketched and/or plotted to display the results of AMMI analysis graphically in the form of bi-plots in which both genotype and environment scores can be well plotted/sketched to have a clear observation in to specific genotype-environment combination and general pattern of stability and adaptability to the specified environment.

Accordingly, AMMI-1 identified the genotypes Dirshaye, Soressa, AC-BC-10 and 242834-1 as the genotypes having the higher mean seed yield along with IPCA-1 score closer to zero values and therefore, they were considered as the most stable genotypes.In addition to AMMI-1 biplot, AMMI-2 biplot had identified the geno types Dirshaye, Soressa and 242834-1 as the most stable genotypes. On the other hand, the location Ali Woyyo was identified as the most favorable and/or suitable environment for black cumin production by both AMMI-1 and AMMI-2 biplots.

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