

Stability and Genotype by Environment Interaction Analysis Based on AMMI Model for Seed Yield and Oil Content of Advanced Linseed Breeding Lines

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Abstract: The effect of genotype, environment and genotype by environment interaction on seed yield and oil content of 8 linseed genotypes was estimated based on AMMI Model. Analysis of AMMI variance for seed yield and oil content showed highly significant effects ($P \leq 0.001$) between genotypes, environments and genotype by environment interaction. Environmental effect accounted 63% and 75.43% of the total variation for seed yield and oil content respectively. The AMMI-I bi-plot analysis of seed yield, identified G2 and G7 the highest yielder but G1 is better stable and contributed little to genotype by environment interaction. Among the test environments S18, K19 and AD18 are the most discriminating locations. Oil content AMMI-I bi-plot analysis revealed genotypes G7, G4 and G6 were stable and less contribution to genotype by environment interaction and environment K19, B19 and AS9 identified as the most discriminating environments. The stability analysis based on AMMI stability indices for seed yield indicated G1 as stable and high yielder genotype; whereas for oil content G3 and G4 identified as stable genotypes. Generally based on the AMMI-I bi-plot and stability analysis G1, G3 and G4 genotypes are recommended as best genotypes for both seed yield and oil content.

Keywords: AMMI, Genotype X Environment, Linseed, Oil content, Seed yield, Stability

1. INTRODUCTION

Linseed (*Linum usitatissimum* L.) belongs to the genus *Linum* is one of the earliest crop cultivated for its seeds and fiber. Almost every part of the linseed plant is utilized commercially either directly or after processing. It has been under cultivation for its seed oil (linseed) or stem fibers (flax) or both (dual purpose) for 1000 years. The center of origin of cultivated flax is believed to be the Middle East, although secondary diversity centers were identified in the Mediterranean basin, Ethiopia, Central Asia, and India (Zohary and Hopf, 2000).

Nowadays, with the increased consciousness of consumers towards the strong relationship between food and health, linseed started to be considered a very promising functional food, due to the presence, in its seeds, of high amount of nutrients and biologically active compounds, such as fatty acids specifically α -linolenic acid (ALA), phytoestrogenic lignans (secoisolariciresinol diglycoside (SDG)), high quality proteins, dietary fibers and phenolic compounds (Xueming, 2010). Among these, ALA is one of the essential polyunsaturated fatty acids (PUFAs) representing the main functional component of linseed. It is clearly demonstrated that linseed represents the best ω -3 fatty acid source in vegetarian diets, having also the possibility to be used as additive in the preparation of many dietary products (Reta and Nigussie, 2017).

Ethiopia is the 5th major producer of linseed in the world after Canada, China, United States and India. It is the second most important oil crop in the highlands of Ethiopia in terms of area coverage and production (CSA, 2018). The crop performs best in altitudes ranging from 2200 to 2800 meters above sea level. Traditionally the seed is commonly roasted, ground and mixed with spices and some water to be served along with local breads. It also consumed in soups, soft drinks and with porridges or cooked potatoes. Its industrial use is higher than all other oil crops. It has traditionally been used for food and as a cash crop since ancient times.

The performance of any character is a combined result of the genotype (G) of the variety, the environment (E) and the interaction between genotype and environment (GE). To evaluate the

consistency of linseed seed yield and develop genotypes that respond optimally and consistently across years and geographic regions, it is necessary to research on yield stability and GE interactions. GE interactions exist when the responses of two genotypes to different levels of environmental stress are not consistent. GE interaction was quantified using several procedures based on evaluation of genotypes under multiple environments. These methods divided into univariate and multivariate stability statistics. Additive main effects and multiplicative interaction (AMMI) model is a popular and an alternative multivariate approach to the joint regression analysis in many breeding programs to study GE interaction (Annicchiarico 1997). This method extracts genotype and environment main effects and uses interaction principal components (IPCs) to explain patterns in the GE interaction or residual matrix, which provides a multiplicative model (Romagosa and Fox, 1993).

The AMMI model combines ANOVA for main effects of the genotype and environment with principal components analysis of GE interactions. Several AMMI parameters were introduced for studying the stability of genotypes across multi environments. AMMI stability value (ASV) (Purchase et al., 2000) is a reliable statistics for GE interaction description and simultaneous selection of yield and stability.

To achieve better economic benefits, identification of linseed varieties with wider adaptability and stability is important for variety recommendation from promising lines. The main objectives of the present study were to identify more high-yielding stable promising linseed lines and determine the areas where linseed genotypes would be adapted using AMMI model.

2. MATERIALS AND METHODS

2.1. Planting Materials and Testing Locations

Eight linseed genotypes developed by hybridization program were grown for two years (2018 and 2019) during the main cropping season (June–November) in six locations representing different linseed growing agro-ecological zones of Ethiopia. Each year and location was treated as a separate environment, making 12 test environments. Descriptions of the six test locations and the 8 test genotypes are presented in Tables 1.

2.2. Experimental Design and Layout

The experiments were laid out in Randomized Completely Block Design (RCBD) with four replications. The gross plot size was 3.6 m² (six rows spaced at 20 cm and 3 m long). A seed rate of 25 kg/ha and fertilizer rate 23/23 N/P₂O₅ was applied to each plot at planting. Agronomic practices were carried out as per the recommendations uniformly to the entire experimental area.

Table1. Description of the tested locations and genotypes used for evaluation of linseed genotypes during the 2018 and 2019 cropping season in Ethiopia.

Testing Environment			Tested Genotypes		
No.	Testing Environment	Environment code	No.	Pedigree	Genotype Code
1	Holetta2018	H18	1	CDC 1747 X CI 1652/SPS8	G1
2	Holetta 2019	H19	2	CI 1652 X CDC 1747/SPS2	G2
3	Kulumsa 2018	K18	3	Chilalo X R12-N27G/SPS1	G3
4	Kulumsa 2019	K19	4	CI 1652 X R12-D33C/SPS105	G4
5	Adet 2018	AD18	5	CI 1652 X R12-N27G/SPS5	G5
6	Adet 2019	AD19	6	R12-100 X CI 1525/SPS1	G6
7	Asasa 2018	AS18	7	Bekoji-14- SD	G7
8	Asasa 2019	AS19	8	LC-Check	G8
9	Bekoji 2018	B18			
10	Bekoji 2019	B19			
11	Sinana 2018	S18			

2.3. Statistical Data Analyses

AMMI Analysis

To evaluate the interaction effects, the data were subjected to stability analysis following the AMMI model. The AMMI model is a hybrid statistical model incorporating both ANOVA (for additive component) and PCA (for multiplicative component) for analyzing two way (genotype x environment interaction) data structures based on the following mathematical Model:

$$Y_{ij} = \mu + g_i + e_j + \sum_{k=1}^N \lambda_k \gamma_{ik} \alpha_{jk} + \epsilon_{ij}$$

Where;

Y_{ij} = yield of i^{th} genotype in the j^{th} environment.

μ = grand mean

g_i = genotype and environment deviations from the grand mean

λ_k = eigen value of the principal component analysis (PCA) axis k

γ_{ik} and α_{jk} = genotype and environment principal components scores for axis k

N = is the number of principal components in the AMMI model, and ϵ_{ij} = residual term.

AMMI Biplot Analysis

To show a clear insight into specific GEI combination and the general pattern of adaptation, a biplot of genotypes and environments were done. The AMMI biplot is developed by placing both genotype and environment values on the abscissa (X- axis) and the respective PCA axis, Eigen vector on the Y- axis.

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 and Tinker, 2005).

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).

AMMI Stability Analysis

The eigenvalue (EV) stability parameter of AMMI (Zobel et al, 1998) was calculated according to the expression:

$$EV = \sum_{n=1}^N \gamma_{in}^2 / n$$

In this formula, γ_{in} is the genotype eigenvector for axis n , and N is the number of IPCs that were retained in the AMMI procedure via different F -test.

The sum of IPCs scores (SIPC) parameter is expressed as (Sneller et al, 1997):

$$SIPC = \sum_{n=1}^N \lambda_n^{0.5} \gamma_{in}$$

Where, λ is the eigenvalue of the IPC analysis axis n . In this equation, $N = 1$ for $SIPC1$; and for $SIPCn$, N was the number of IPC that were retained in the AMMI model.

ASV was calculated as described by Purchase et al (2000) as follows:

$$ASV = \sqrt{\left[\frac{SSIPC1}{SSIPC2} (IPC1)^2 \right] + (IPC2)^2}$$

Where: *SSIPC1/SSIPC2* is the weight given to the *IPC1* value by dividing the *IPC1* sum of square by the *IPC2* sum of square. The larger *IPC* score, either negative or positive, the more specifically adapted a genotype is to certain environments. Smaller ASV scores indicate a more stable genotype across environments.

3. RESULT AND DISCUSSION

3.1. AMMI Analysis for Seed Yield and Oil Content

The result from the AMMI analysis of variance for seed yield and oil content revealed that the difference between genotypes, environments and genotype by environment interaction were highly significant effects ($P \leq 0.001$) for genotypes(G), environments(E) and genotype by environment interaction(GEI) (Table 2).

The results revealed that for seed yield 63% of the total variation was attributable to environmental effects, 16.1% genotype effects and 20.9 to GE interaction effects. For oil content 75.43% of the total variation was attributed to environmental effects, 5.31 and 19.26 % attributed to genotype effects and GE interaction effects respectively. Such a large sum and highly significant mean squares of environment indicated that the environments were diverse with large difference amount environmental means causing most of the variation on seed yield and oil content. This shows the overpowering influence that environments can have on the yield and oil performance of linseed. Similar results on the effect of environment and GEI were reported by Cherinet and Tadesse (2014); Jacobs et al., (2015); Tadesse, (2017) and Adane and Abebe, (2018) on linseed genotypes. The significant effects of GE interaction reflected on the differential response of genotypes in various environments demonstrated that it had remarkable effect on genotypic performance in different environments. Therefore, it was possible to proceed and calculate stability parameters. The application of AMMI model for partitioning the GE interaction effect showed that the first two terms of AMMI were significant based on Gollob's *F*-test (Gollob, 1968) for both seed yield and oil content. In this study, the proportion of *IPC1* (59.7 % for seed yield and 65.89 % for oil content) to the interaction sum of squares was far greater than that of *IPC2* (Table 2).

Table2. AMMI model ANOVA for Seed yield and oil content of 8 linseed genotypes over 12 tested environments.

Seed yield					Oil Content (%)			
Source of variation	df	SS	MS	Variance explained (%)	df	SS	MS	Variance explained (%)
Total	38	10445373	272725***		25	1806.	7.08***	
	3	7			5	3		
Treatments	95	68406771	720071***		63	1806.	28.67***	
						3		
Genotypes	7	10981800	1568829**	16.1	7	95.5	13.64***	5.31
			*					
Environments	11	43129152	3920832**	63.0	7	1364.	194.95**	75.43
			*			6	*	
Interactions	77	14295820	185660***	20.9	49	346.1	7.06***	19.26
Block	36	14813148	411476***		24	0.0	0.00***	
IPCA 1	17	8539943	502350***	59.7	13	229.3	17.64***	65.89
IPCA 2	15	2839373	189292*	19.9	11	42.2	3.83***	12.41
Residuals	5	63139	12628		1	0.3	0.28	
Error	25	21233818	84261		16	0.0	0.00	
	2				8			

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, df degree of freedom, SS sum of square and MS mean square

3.2. Biplot Analysis

The GEI was further explored through the genotype and (genotype x environment) bi-plot analysis. The first two principal components (PCs) of the bi-plot accounted for a total of 79.6% (IPC1 = 59.74%, IPC2 = 19.86%) of the variation for seed yield over 12 tested environments, while for oil content the PCs explained a total of 78.3% (IPC1 = 65.89%, IPC2 = 12.41%). In the biplot graphs, the quadrants represent: (Q-I & Q-II) higher mean, (Q-III & Q-IV) lower mean, (Q-I & Q-IV) +ve IPCA1 and (Q-II & Q-III) -ve IPCA1 scores.

Genotypes that are grouped together had similar adaptation while environments which were grouped together influence genotypes in the same way. The best adapted genotype can plot far from the environments. In AMMI-I bi-plot genotypes or an environment with IPCA-1 score closer to zero, it has small interaction effect and they are stable across the testing environments, whereas the larger scores depict more specific adaptation to environments with IPC1 scores of the same sign. However, if genotypes far from zero, it is highly responsive and does not perform consistently across the environments (Samonte et al., 2005). But genotypes with high mean performance and large IPCA-1 scores were considered as having specific adaptability to the environments. When a genotype and environment have the similar sign on IPCA-1 axis, their interaction is positive and this genotype is well adapted to this environment. But if they have opposite sign of IPCA-1 scores, their interaction is negative and the environment is not suitable to this genotype (Cross et al., 1990).

Based on the AMMI-I bi-plot analysis of seed yield, G1 and G8 have closer to zero IPCA-1 value therefore, they are better stable and contributed little to the G X E interaction. In addition environments k18 and AS18 contributing little to the G X E interaction (Fig 2a). Oil content analysis revealed genotypes G7, G4 and G6 were stable and less contribution to G X E interaction and environment B18 and AS18 had little contribution to G X E interaction (Fig 2b). When a genotype and environment have the same sign on the PCA axis, their interaction is positive and if different, their interaction is negative (Fig 2a and 2b).

However, G4, G2 and G7 attained values relatively close to 1, either for both or IPCA-1 with above average yield performance in specific locations and had large G X E interactions. Furthermore, S18, K19, AD18 and AD9 are the most discriminating locations of the test environments (Fig 1a). On the other hand, G2, G5 and G1 attained values relatively close to 1, either for both or IPCA-1 with above average yield performance in specific locations and had large G X E interactions. Furthermore, K19, B19 and AS9 are the most discriminating locations of the test environments (Fig 1b). Connecting vertex cultivars markers in all direction form a polygon, such that all genotypes are contained within the polygon and a set of straight lines that radiate from the biplot origin to intersect each of the polygon sides at right angles form sectors of genotypes and environments (Yan, 2011). Based on AMMI2, a biplot with five sections are formed depending upon signs of the genotypic and environmental IPC scores.

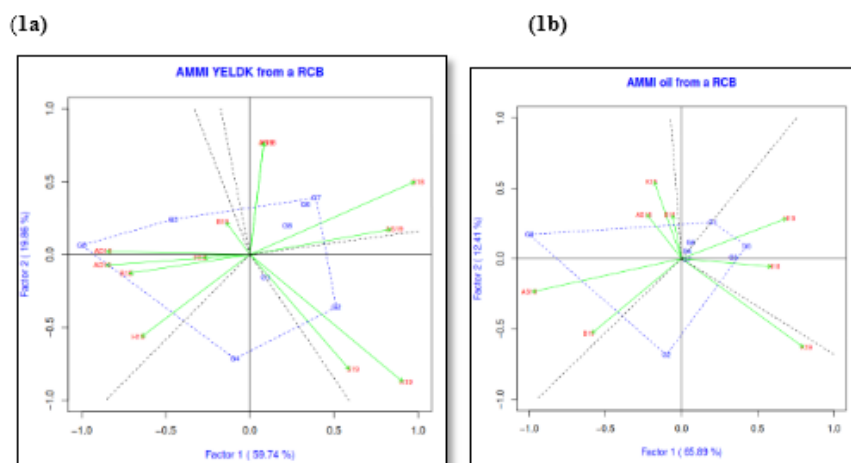


Figure 1 AMMI biplot analysis of the first (IPC1) and second (IPC2) interaction principal component (1a) for seed yield and (1b) for oil content

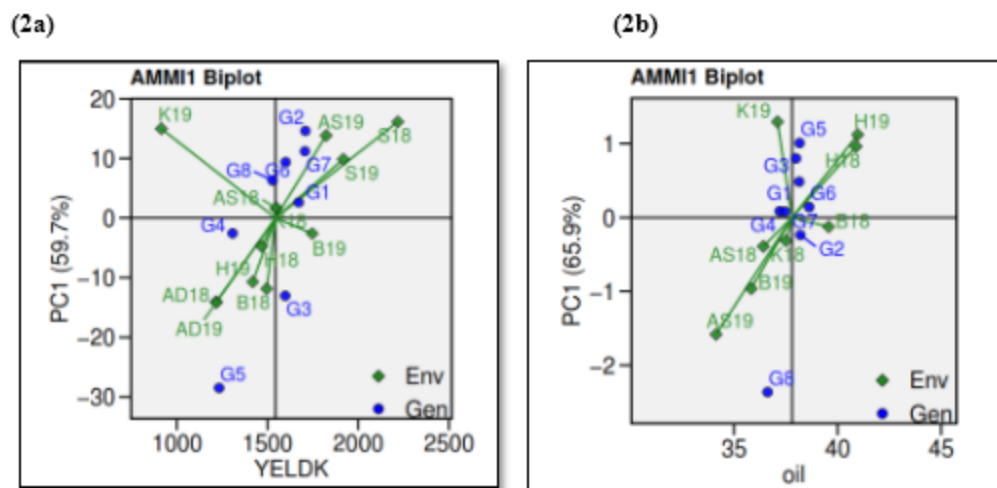


Figure 2 AMMI biplot Analysis of first interaction principal component axis (IPCA1) versus mean seed yields (kg/ha) (2a) and oil content (2b).

3.3. AMMI Stability Analysis

The AMMI model does not make provision for a quantitative stability measure, such a measure is essential in order to quantify and rank genotypes according to their yield stability. Therefore AMMI stability parameters are presented in (Table 3). According to the ASV stability statistic, a genotype with lower score value is considered to be more stable; therefore G1, G4 and G8 are stable but G2, G3 and G5 genotypes are unstable for seed yield. Based on ASV stability index for oil content G4, G6 and G7 are stable but G3, G5 and G8 are unstable genotypes. ASV is the distance from zero in a two dimensional scatter gram of IPCA1 (interaction principal component analysis axis 1) scores against IPCA2 scores. Since the IPCA1 score contributes more to GE sum of square.

According to SIPC G1, G6 & G8 were the most stable genotypes, whereas the most unstable genotypes were G2 & G5 for seed yield, whereas, G2, G3 & G4 were stable but G5 & G8 were unstable for oil content. According to EV value the most stable genotypes for seed yield were G1, G3 & G8 whereas, G4 & G5 are unstable genotypes. The EV score for oil content revealed that G2, G3 & G7 were the most stable but G1 and G6 are unstable genotypes.

Table3. Values of AMMI stability parameters for eight linseed genotypes tasted at 12 environments.

Geno type	Seed yield(Kg/ha)						Oil content (%)					
	Pooled mean	ASV	SIPC	EV	CV	R	Pooled mean	ASV	SIPC	EV	CV	R
G1	1671.7(3)	9.055(1)	7.074(1)	0.01 4(1)	25.82 5(5)	0.94 3(1)	38.2 (4)	2.641 (5)	3.20 0(7)	0.12 5(8)	8.01 (6)	0.90 (5)
G2	1708.4(1)	45.13 4(7)	24.90 6(7)	0.13 6(6)	24.49 5(3)	0.75 3(5)	38.2(2)	2.048 (4)	2.438(3)	0.125(2)	5.30(2)	0.79 (7)
G3	1597.6(5)	39.82 4(6)	19.92 5(4)	0.08 6(3)	23.62 4(1)	0.75 2(6)	38.0 (5)	4.251 (6)	2.113(1)	0.125(1)	8.16 (7)	0.95(3)
G4	1306.9(7)	21.802(3)	22.95 7(6)	0.24 9(7)	23.85 3(2)	0.72 0(7)	37.2 (7)	0.472(2)	2.337(2)	0.12 5(4)	7.31 (5)	0.96(2)
G5	1233.0(8)	85.70 0(8)	30.28 8(8)	0.28 0(8)	28.01 6(7)	0.27 9(8)	38.2(3)	5.358 (7)	3.14 1(6)	0.12 5(5)	9.25 (8)	0.97(1)

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G6	1599. 9(4)	29.87 5(4)	19.18 7(3)	0.08 7(4)	29.14 5(8)	0.92 5(2)	38.6 (1)	0.799 (3)	2.60 4(5)	0.12 5(7)	7.04 (3)	0.88 (6)
G7	1705. 8(2)	35.42 8(5)	22.26 3(5)	0.11 6(5)	27.01 0(6)	0.86 7(3)	37.5 (6)	0.427 (1)	2.48 7(4)	0.12 5(3)	7.16 (4)	0.94 (4)
G8	1527. 4(6)	19.70 6(2)	11.86 6(2)	0.03 2(2)	25.77 5(4)	0.86 6(4)	36.6 (8)	12.56 0(8)	3.28 6(8)	0.12 5(6)	5.16 (1)	0.14 (8)

ASV = AMMI stability Value; SIPC= sum of IPCs scores; EV = eigenvalue stability parameter; CV= Coefficient of variation

When we compare the coefficient of determination (CV) value G1, G8 and G3 considered as stable genotypes but G4 and G5 are unstable for seed yield whereas G2, G6 and G8 are stable and G3 and G5 are unstable genotypes for oil content. As a general rule CV values with lowest score are considered as stable genotypes across locations. The R value presented in table showed G1 and G6 as stable but G4 and G5 unstable genotypes for seed yield but for oil content G3, G6 and G8 are stable whereas G2 and G8 are unstable genotypes.

Generally based on the stability analysis for seed yield G1 is selected as stable genotype by most of the parameters; it is also among the top high yielder genotype and G5 is unstable low yielder genotype based on mean seed yield value. For oil content G3 and G4 is stable with SIPC, EV and CV parameters and moderately stable by ASV; this genotype is among the top high yielder and high oil content genotypes. G8 is a low oil value and the least stable genotype by most of the stability parameters.

4. CONCLUSION

Genotype-by-environment (GE) interaction has been an important and challenging issue among plant breeders, geneticists and agronomists engaged in performance testing. The genotype-environment interaction reduces association between phenotypic and genotypic values and leads to bias in the estimates of gene effects and combining ability for various characters sensitive to environmental fluctuations. Both high value of trait interest (i.e. yield and oil) and stability of performance should be considered simultaneously to reduce the effect of GE interaction and to make selection of genotypes more precise and refined. The results of this investigation showed there were desirable genotypes in terms of high oil content G3 and G4 and for seed yields G1 in terms of high mean yield and stability. Therefore, these genotypes will be used for further breeding program.

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