Assessment of genotype x environment interactions and stability for seed yield of selected safflower (*Carthamus tinctorius* L.) genotypes in central and northern Sudan

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ABSTRACT

Safflower is an important oilseed crop worldwide. In Sudan, safflower is only cultivated in the Northern State along the River Nile. The success of safflower improvement and production activities can be enhanced with scientific information generated from the study of genotypes, environments and genotype by environment interactions (GEI). In this study, 15 safflower genotypes were evaluated during two consecutive winter seasons (2013/14 and 2014/15) at three locations *viz* Gezira, El- Suki and Hudeiba Research Station Farms of the Agricultural Research Corporation, Sudan. The objectives of this study were to estimate G x E interactions and identify the highest yielding and stable genotypes under different environments. A wide range of genetic variability was observed among the genotypes for most of the studied traits. Combined analyses of variance revealed highly significant environment, genotype and genotype x environment (GE) component of interaction and indicated wide differences among the environments and differential genotypic behavior to the tested environments. Additive main effects and multiplicative interactions (AMMI) analyses have higher efficiency in partitioning and analyzing stability studies compared to regression analysis. First and second principal component axis (PCA 1 and PCA 2) in AMMI explained 64.6% and 25.3% of the interaction sum of squares, respectively, and together they accounted for 89.9 % of the G X E sum of squares, while the regression model accounted only for 13.8% of the G X E sum of squares. Hence, AMMI analysis was superior to regression techniques in accounting for the large part of the G X E sum of squares and more effective in partitioning the interaction sum of squares. In conclusion, genotypes Saff 1, Saff 12 and Saff 14 were high yielding (582.4, 507.9 and 572.8, kg/ha, respectively) and stable under all environments. Therefore, they are recommended to be grown under winter irrigated conditions of central and northern Sudan.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is one of the oldest oilseed crops and has been grown on a relatively small scale in parts of North Africa and Middle East. It is cultivated mainly for its seed, which was used as edible oil and birdseed (Dordas and Sioulas, 2008, Istanbulluoglu, 2009).

Safflower has been grown for centuries in India for the orange – red dye (carthamin) extracted from its brilliantly colored flowers and for its high quality oil which is rich in polyunsaturated fatty acids (linoleic acid).

Safflower can be grazed or stored as hay or silage, its forage is palatable, and its feed value and yields are similar to or better than those of oat or alfalfa. Safflower has high adaptability to low moisture conditions. Therefore, its production is mainly confined to areas with scanty rainfall. Research and development on different aspects of safflower, despite its adaptability to varied growing conditions with very high yield potential and diversified uses of different plant parts, have not received much attention. This probably is the main reason for its status as a minor crop in terms of area and production, compared to the other oilseed crops.

Conventional methods of partitioning total variation into components due to variety, environment and variety-environment interaction conveyed little information on individual patterns of response (Kempton, 1984). Other methods used include regression analysis to partition genotype x environment interaction and multivariate analysis (Westcoff, 1987).Thus, information on varietal stability with high yield to varied environments in safflower may be helpful in selecting the promising genotype(s). Different yield stability statistics proposed to characterize GE interactions and several methods have been proposed to evaluate stability. These methods could be in the form of a linear regression (Eberhart and Russell, 1966), clustering procedures (Lin and Butler, 1990) and multiplicative approaches such as additive main effects and multiplicative interaction (AMMI) (Zobel *et al.,* 1988).

Due to climate change, development of any crop with high yielding and desirable quality for different environments is one of the research goals. So the introduction of a new crop to a regional cropping system requires information concerning its performance under local environmental conditions.

Engels (1991) mentioned that Sudan has to be a worldwide partner in safflower research activity. Thus, adaptation of the crop to different ecological zones of the Sudan should be included in the strategy of research on safflower. Therefore, there is a pressing need for identifying stable genotypes of safflower with high seed yield potential under different production systems in Sudan. Hence, the objective of this study was to estimate G x E interactions of safflower genotypes grown under different environments and the stability of some safflower genotypes for seed yield using Eberhart and Russell (1966) and AMMI stability methods.

MATERIALS AND METHODS

Experimental sites

The experiments were conducted during two consecutive winter seasons (2013/14 and 2014/15), at three locations, *viz.* Gezira (GRS), El-Suki (SRS) and Hudeiba (HRS) Research Stations Farms,

of the Agricultural Research Corporation (ARC), Sudan. The Gezira Research Station farm is located in Wad Medani, latitude 14° 24′N and longitude 33° 29′E. It is characterized by heavy cracking clays (58%), calcareous alkaline soil, with a pH of 8.3 and low organic matter content (0.02%). El-Suki Research Station farm is located in Sinnar State, latitude 13° 20′ N, longitude 33° 50 E, is characterized by heavy clay soil, with a pH of 8.0. Hudeiba Research Station farm is located (on high terrace soil) near EL- Damer in River Nile State, latitude 17° 34′N and longitude 33° 56′E. The high terrace soil is characterized by clay loam with high calcium carbonate content.

Plant material and experimental design

Fifteen genotypes of safflower introduced from different countries were used in this study (Table 1). The experiments were arranged in a randomized complete block design with four replicates in all locations and seasons.

Cultural practices

Across all growing seasons and locations, the land was plowed, harrowed, leveled and ridged. In the first season, sowing date was at the second week of December across different locations and at the second week of November for the second season. The plot size was 6.0 * 3.2 m, with four ridges. Inter and intra- row spacing was 0.8 and 0.2m, respectively. Three seeds per hill were sown and thinned to one plant per hill after three weeks. Weeding was done manually. The crop was irrigated every two weeks or whenever necessary and irrigation was held three weeks before harvest. No pest or diseases were observed during the growing seasons. Data were collected on days to 50% flowering, days to maturity, plant height (cm), number of branches per plant, number of capitula per plant, number of seeds per capitula, 100 seed weight (g), seed yield (kg/ha) and oil content.

Statistical analysis

Data were analyzed using the analysis of variance procedure to examine the differences among the genotypes for all measured traits. Analysis of variance procedure was used for each season to test the significant differences among the evaluated genotypes. Combined analysis of variance was carried out for testing the effect of environments, genotypes and their interactions.

Stability analysis

The combined analysis of data generated from different environments were used to estimate the yield stability using Eberhart and Russel (1966) and AMMI models. Genotype means from individual environments were regressed on the environmental means. Genotypes which had regression coefficient larger than one were regarded to be more adapted to favorable environments, and those which had regression coefficient less than one were regarded to be more adapted to unfavorable environments (stable). Genotypes which showed small values of deviation from regression ($_s^2$ d) were claimed to have high yield stability (Gauch and Zobel, 1988; Nachit *et.al.,* 1992*)*

Table 1. Safflower genotypes grown at Gezira, El-Suki and Hudeiba Research Station farms in seasons 2013/ 14 and 2014 / 15.

enotype	Pedigree	Origin	Description
aff 1	Saff 89	Pakistan	Non-spiny
aff 2	Saff 151	Iran	Spiny
aff 3	Saff 171	China	Spiny
aff 4	Saff 150	Uzbekistan	Spiny
aff 5	Saff 69	Morocco	Non-spiny
aff 6	Saff 79	Afghanistan	Spiny
aff 7	Saff 78	Afghanistan	Spiny
aff 8	Saff 153	Thailand	Spiny
aff 9	Saff 155	Libya	Non-spiny
aff 10	Saff 75	Iran	Non-spiny
aff 11	Saff 152	Syria	Spiny
aff 12	Saff 146	Ukraine	Spiny
aff 13	Saff 135	India	Spiny
aff 14	Saff 156	Bangladesh	Spiny
aff 15	Saff 131	Egypt	Spiny

RESULTS AND DISCUSSION

Genotype x environment interaction (G x E)

The combined analysis of variance showed highly significant differences among seasons for all the studied traits (Table 2). Among locations, there were highly significant differences for all studied traits with the exception of days to maturity. Also, among genotypes there were significant differences for all measured traits. The interaction effects of genotype x location were highly significant for most of the studied traits with the exception of days to maturity, number of branches per plant and number of capitula/plant.

The significance of genotype x environment interaction indicated that the genotypes responded differently to the environment and some were environment specific. Similar results were reported by Beena *et al.,* (2006). Also, this finding indicated the importance of these components in affecting the phenotypic performance of the evaluated safflower genotypes.

Genotypes significantly interacted with seasons for most of the studied traits with the exception of days to maturity, 100-seed weight and seed yield. However, the significant interactions of genotype with seasons which were shown by most of the studied traits reflect their instability over seasons. On the other hand, the traits which had nonsignificant interaction with seasons had stable performance over seasons.

As different genotypes reacted differently to varying seasons as indicated by the high significant G× E interaction, hence, environmental effects are important in understanding plant growth and

should be considered in safflower breeding programs. It is advisable to test new genotypes in the environments of intended use before release to farmers. It is essential to identify genotypes which have relatively low G× E interactions with stable yield in tested environments.

The interaction of season x location x genotype was significant for all studied traits with the exception of number of capitulum/plant. Generally, these results are in agreement with the findings of Omidi *et al.,* (2009), who reported that the year x location and also year x location x genotypes interactions were highly significant in safflower crop. The current findings indicated wide range of genetic variability among the tested genotypes, which could be attributed to both genetic and environmental factors and their interaction effects. Therefore, it can be concluded that safflower genotypes responded to G× E interaction over the environments. Similar results were observed by Singh *et al.*, (2004).

Seed yield stability

Evaluation of genotypes in any breeding program aims at identifying genotypes that consistently produce stable yields over a range of diverse environments. In the present study, the mean seed yield ranged between 478.5 to 627.5 kg/ha, with an average mean of 561.51 kg/ha. Ten genotypes out of 15 gave higher seed yield than the grand mean. These were Saff 1, Saff 2, Saff 6, Saff 7, Saff 8, Saff 9, Saff 10, Saff 11, Saff 14 and Saff 15. The genotype Saff 6 out-yielded all the genotypes (Table 3).

Stability of seed yield over various environments is the most desirable property of genotype to be released as a variety for a wide range of environments. Estimates of stability parameters should be measured only if the variance due to G x E is significant. In the current study, the mean square of seed yield for genotypes x locations was significantly different (Table 2), therefore, two models of stability that had been used.

Eberhart and Russell's stability model (1966)

The deviation from regression is used to assess unpredictable part of variability of any genotype with respect to environment that could not be predicted by the regression. Eberhart and Russell (1966) defined the stable genotype as one with $b_i = 1$, $S^2d = 0$ and higher than the overall mean seed yield. From Table 3, the results showed clear differences in slopes of the regression lines between tested genotypes. Some regression coefficients (b_i) exceeded unity while others were close to or less than one. The values of regression coefficient (slope) ranged from 0.38 for Saff 10 to 1.72 for Saff 6 (Table 3). From this study, ten genotypes, Saff 1, Saff 2, Saff 6, Saff 7, Saff 8, Saff 9, Saff 10, Saff 11, Saff 14 and Saff 15 gave seed yield higher than the general mean of the trials.

From Table 3, the genotypes with (b_i) greater than one and mean seed yield greater than the general mean, were Saff 2, Saff 6, Saff 11 and Saff 15, indicating that they were more responsive to environmental changes and therefore suitable for favorable environments. Genotypes with (b_i) close to 1.0 but low yielding (below the general mean), were Saff 4 and Saff 13 (526.3 and 478.5kg/ha, respectively). These findings indicate that those genotypes had better response under unfavorable environments but inconsistent.

Genotypes with ($b_i = 1$), S^2d close to zero and yield below general mean are represented by genotype Saff 12 (507.9 kg/ha). These findings indicate that this genotype is more responsive under all environments and stable. From these findings, Saff 14 ranks first among the most stable genotypes with small deviation followed by the genotypes Saff 12 and Saff 1, respectively. When the mean yield, regression coefficient and the deviation from regression were considered together, it can be concluded that genotypes Saff 1 and Saff 14 are the most stable of the 15 tested genotypes. These findings are consistent with those of Rudra *et al.* (2005) who evaluated ten promising safflower lines

in three different locations of India and reported that on the basis of stability parameters and overall mean, lines 98-29, BIP-2, 98-51 and A1 were identified as stable genotypes.

The additive main effects and multiplicative interactions (AMMI) analysis model

The parametric approach gives only the individual aspect of stability but cannot provide an overall picture of the responses, consequently, non-parametric approach (multivariate) has been proposed to overcome problems associated with parametric approach (Lin *et al,,* 1986).

The combined analysis of variance according to the AMMI model is presented in Table 4. The AMMI analysis of variance on seed yield showed that 47.2% of the total sum of squares was attributable to environmental effects, 29.7% to genotypic effects, and 13.8% to GEI effects. Results of the AMMI analysis also indicated that the two multiplicative terms (IPCA1, and IPCA2) were significant and the first PC axis (IPCA1) of the interaction showed 64.6% of the interaction sum of squares. Also, the second PC axis (IPCA2) explained a further 25.3% of the GEI sum of squares. Together they accounted for 89.9% of GE interaction sum of squares.

However, most of variation was explained by the first principal components (IPCA1), according to Crossa *et al.,* (1990). In the present study, a large amount of the total sum of squares (13.8%) of data combined over environments was due to GEI. Mozaffari and Asadi (2006) stated that 87% of total variation for the seed yield could be explained by the four first components.

Table 3. Stability parameters for seed yield of 15 safflower genotypes tested at Gezira, El-Suki, and Hudeiba during growing winter seasons 2013/14 and 2014/15.

Genotype (Saff).	Mean (kg/ha)	Bi	S²d
1	582.4	0.90	0.5
2	567.7	1.12	0.6
3	508.7	0.72	0.6
4	526.3	1.02	0.8
5	554.7	1.23	0.2
6	627.5	1.72	1.4
7	571.3	0.63	0.7
8	561.9	1.12	0.9
9	618.2	0.78	1.0
10	562.0	0.38	1.3
11	601.7	1.27	0.5
12	507.9	0.95	0.3
13	478.5	0.87	1.2
14	572.8	1.03	0.2
15	581.0	1.27	1.4

Mean	561.51

Bi = regression coefficients and S²d = deviation from regression

Table 4. The additive main effect and multiplicative interaction (AMMI) analysis of variance of the significant effects of genotypes (G), environment (E) and genotype X environment interaction (GE) on seed yield and the partitioning of the GE into AMMI scores.

Source of variation	DF	SS	MS	F	Efficiency (%)
Environment (E)	5	9223623	1844725	13.45**	47.2
Genotypes (G)	14	578805	41343	2.28**	29.7
GE I	70	2683689	38338	2.11**	13.8
PCA 1	18	1733956	96331	5.31**	64.6
PCA 2	16	678183	42386	2.34**	25.3
PCA 3	14	185329	13238	0.73	6.9
Residual	22	86222	3919	0.22	

** Significant at P = 0.01 level of probability.
PCA 1 = first principal component.
PCA 2 = second principal component.
PCA 3 = third principal component.

Variation of the studied genotypes for seed yield and their interaction to the environments are presented in Table 5. The highest average yield was obtained in E-3 (El-Suki, 2013/14) followed by the E-1 (Gezira, 2013/14), whereas E-6 (Hudeiba, 2014/15) obtained the lowest seed yield. The E-3(El-Suki, 2013/14) exhibited the largest absolute PCA1 score (had the highest interaction effect), whereas the smallest score was shown by the E-1(Gezira, 2013/14), (had the least interaction effects). Based on AMMI biplot, G and E having IPCA values close to zero had small interaction effects, whereas those having large positive or negative IPCA absolute values had large interaction effect. Hence, E-3 (El-Suki, 2013/14) was the most interactive, while E-1 (Gezira, 2013/14) was the least interactive among the six environments.

Table 5. IPCA 1 and IPCA 2 scores for the six growing environments of safflower genotypes.

Environment	E-mean	IPCAe[1]	IPCAe[2]
E1	655.8	1.56	-4.48
E2	485.4	-1.85	-13.26
E3	825.0	-21.77	4.56
E4	488.0	5.87	13.86

E5	603.3	10.63	0.82
E6	311.6	5.56	-1.49

IPCA 1 = first PC axis

IPCA 2 = second PC axis

E1(Gezira, 2013/14), E2 (Gezira, 2014/15) , E3 (El-Suki, 2013/14),

E4 (El-Suki, 2014/15), E5 (Hudeiba, 2013/14), E6(Hudeiba, 2014/15) environment.

AMMI across sites analysis

   To characterize GE interaction and adaptation graphically, AMMI biplot analysis was used with the IPCA1 scores plotted against the mean yield (main effect) to assess the relationships between genotypes and environments and facilitate a visual description of which the best genotype(s) in each location(s). The AMMI across sites analysis of the mean yield explained large proportion of the treatment sum of squares. The high IPCA scores, negative or positive, are for a more specific or adaptive genotype to certain environments. The IPCA score close to zero is for a more stable genotype over all environments. Accordingly, the genotypes Saff 14 and Saff 1 revealed good stability across environments and also high seed yields, while the genotypes Saff 2, Saff 4, Saff 6, Saff 8, Saff 9, Saff 11, Saff 12 and Saff 15 were adapted for specific environments. Genotype Saff 6 exhibited high seed yield in environment 3 (El-Suki environment, 2013/14),   indicating that this genotype was adapted to specific environments (Fig 1).

AMMI bi-plot of the first two principal component axes (PCA1 and PCA2)

   AMMI biplot analysis is the interpretive tool for AMMI models. Therefore, to further explain the GE interaction and to understand the relationships between particular genotypes and environments, IPCA1 scores were plotted against IPCA2 scores of the AMMI analysis (Fig. 2). This analysis represents stability of the genotypes across environments in terms of principal component analysis. It is used to identify broadly adapted genotypes that offer stable performance across environments, as well as genotypes that perform well under specific conditions. In this study, the first two principal component axes (IPCA1 and IPCA2) in bi-plot analysis explained a large proportion of the variation: 89.9% of the total GE sum of squares (Table 4).

   On this AMMI bi-plot, genotypes and environments having IPCA values close to zero (near the origin) have small interaction effects, whereas those having large positive or negative IPCA values (distant from zero) largely contribute to GE interaction (Yau, 1995). Hence, the genotypes Saff 6, Saff 15, Saff 10, Saff 13, Saff 7 and Saff 8 were the most interactive, while genotypes Saff 1, Saff 14 and Saff 12 were the least interactive. On the other hand, E3 and E4 (El-Suki environment) appeared far distant from the origin (large IPCA score), hence they had large interaction effects, whereas E1, E 5 and E 6 (Gezira and Hudeiba environments), had small interaction effects (Fig 2).

   Genotypes Saff 1, Saff 12 and Saff 14 were more stable and responsive for good environments (3 and 4), while the genotypes Saff 2, Saff 4, Saff 8 and Saff 11 were responsive and suitable for environment E- 2 (Fig 2). Hence, in this investigation, visual observations of AMMI bi-plot analysis

enable us to identify genotypes and testing environments that exhibited major sources of GE interaction as well as those that were stable. Similar results were reported by Sneller *et al.* (1997).

AMMI model is more effective in partitioning interaction SS than the linear regression techniques, resulting in increased precision equivalent to the number of components by a factor of two to five. Such gain may be used to reduce cost by reducing the number of replications, to include more treatments in the experiment or to improve efficiency in selecting the best genotypes. In this study, comparing the effectiveness of joint regression and AMMI analysis for analyzing GE interaction, it was found that IPCA1 in AMMI accounted for the GE sum of squares by 89.9%, while regression analysis accounted for GE sum of squares by 13.8%. Hence, AMMI analysis was superior to regression techniques in accounting for GE sum of squares and more effective in partitioning the interaction sum of squares. From this study, the genotypes Saff 1 and Saff 14 were more stable and high yielding genotypes across the six environments. These findings were in agreement with those of Eberhart and Russell's stability model (1966) in this study.
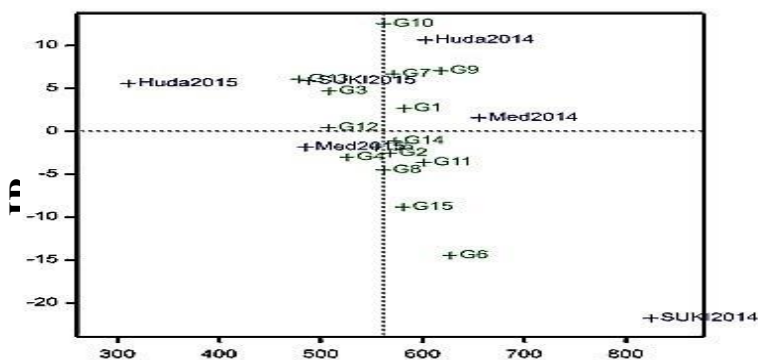
Mean seed yield *vs* IPCA1: AMMI plot

Fig.1. The AMMI bi plot of the main and the PCAI effects of both genotypes and environment on seed yield of 15 safflower genotypes grown in six environments. Genotypes are indicated by +G.
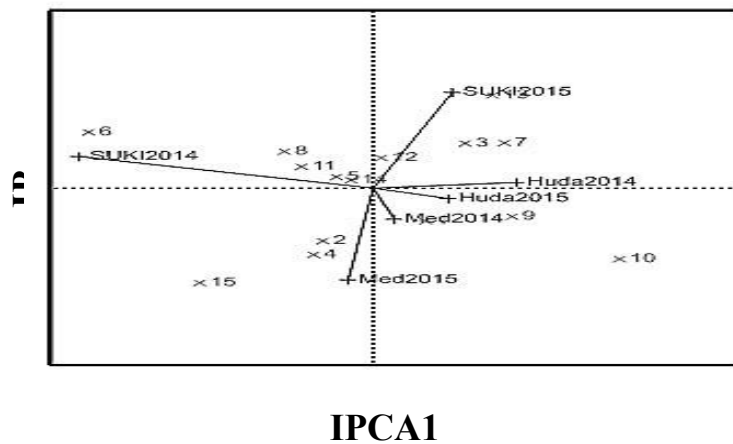
Seed yield: AMMI biplot

IPCA1

Fig.2. The AMMI biplot of the PCAI and PCA2 axes

CONCLUSIONS

From this study it could be concluded that, the significant environment, genotype, and genotype x environment component of interaction indicated wide differences between the environments and differential genotypic behavior under the test environments. Additive main effects and multiplicative interactions (AMMI) and pattern analysis have higher efficiency for stability studies compared to regression analysis. First and second principal component axis (PCA 1 and PCA 2) in AMMI accounted for the GE sum of squares by 64.6%, 25.3%, and together they accounted for 89.9%, while regression model accounted for the GE sum of squares by 13.8%. Hence, AMMI analysis was superior to regression techniques in accounting for GE sum of squares and more effective in partitioning the interaction sum of squares.

From the stability analysis using both Eberhart and Russell's (1966) and AMMI, it could be recommended that genotypes Saff 1, Saff 12 and Saff 14 (stable and high yielding) be grown successfully under winter irrigation condition of central and northern Sudan.

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تقدير التفاعل الوراثي البيئ وثبات إنتاجية الحبوب لطرز وراثية مختارة من القرطم (*Carthamus tinctorius* L.) في وسط وشمال السودان

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الخلاصة

يعتبر القرطم من المحاصيل الزيتية المهمة في العالم و يزرع في السودان فقط في الولاية الشمالية في المساحة الواقعة علي طول النيل. إن نجاح تحسين محصول القرطم ونشاطاتِ إنتاجِه يُمْكِنُ أَنْ يُحَسّنا بالمعلومات العلمية التي تنتج مِنْ دراسة التراكيب الوراثية وتفاعلاتِ البيئة والتفاعل بين التراكيب الوراثية والبيئة. قُيّمَت في هذه الدراسةِ خمسة عشرَ تركيباً وراثياً من القرطمَ علي مدي فصلي شتاء متتاليينِ (15/2014-14/2013) في ثلاثة مواقع هي محطات بحوث الجزيرة و السوكي والحديبة التابعة لهيئة البحوث الزراعية بالسودان. هدفت الدراسة إلي تُقييم هذه التراكيب الوراثية من حيث الانتاجية ومكوّناتِها و دراسة التفاعل بين البيئة و التراكيب الوراثية المختلفة وتمييز التراكيب الوراثية الأعلى إنتاجية و المستقرة في البيئاتِ المختلفة. أوضحت النتائج وجود مدي واسع من التباين الوراثي بين التراكيب الوراثية لمعظم الصفات المدروسة. أيضاً أظهرت النتائج وجود فروقات معنوية كبيرة بين التراكيب الوراثية و البيئة والتفاعل بينهما. أوضحت الدراسة أنه في طريقة الأثر التجميعي الرئيسي والتفاعل المتراكم لتحليل التباين (AMMI) الناتج عن التفاعل بين التركيب الوراثي والبيئة أن محور المكون الأول والثاني قد فسرا حوالي 64.6 % و25.3 %، علي التوالي من هذا التباين، بينما وجد في طريقة معامل الارتداد (1966) Eberhart and Russell's أنه قد فُسَر حوالي 13.8 % منه. عليه فإن طريقة الأثر التجميعي الرئيسي والتفاعل المتراكم أعلي كفاءةً من طريقة معامل الارتداد الخطي في تقسيم التباين الناتج عن التفاعل بين التراكيب الوراثية والبيئة. خلصت الدراسة إلي أن التراكيب الوراثية Saff 1 وSaff 12 و Saff 14 كانت عالية الإنتاجيةً ولها ثباتاً وراثياً في كل بيئات التقييم لذلك يوصى بزراعتها بنجاح تحت ظروف الري الشتوي بأواسط وشمال السودان.