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RESEARCH ARTICLE

Application of multivariate analysis for assessment of stability in winter safflower (*Carthamus tinctorius* L.) genotypes under rainfed conditions

Khosro Zerehgar*, Seyyed Saeid Pourdad[1], Reza Aziznezhad and Ali Reza Etminan

Abstract

Assessment of genotype x environment interaction (GEI) is an important constituent of cultivar selection process in multi-environment traits (MET). A set of 15 genotypes of winter safflower (*Carthamus tinctorius* L.) were evaluated for grain yield, compatibility, yield stability and GEI in diverse environments under rainfed conditions. The analysis of variance conducted through AMMI method showed significant variation in grain yield with respect to mean squares of environments, genotypes, and GEI. The two main components, IPCA1 and IPCA2, accounted for 85.29% of the variation and GEI, indicating almost high biplot validity. The polygon biplot analysis identified six superior and stable genotypes and two mega-environments.

Keywords: AMMI method, GGE biplot, genotype x environment interaction, stability, safflower

Introduction

Safflower (*Carthamus tinctorius* L.) belongs to the Casserian or Asteraceae family (Weiss 1999), which originated from the Eastern Mediterranean region and Asia and is widely distributed across the world. Safflower seeds contain between 13 and 46% oil, 90% of which are unsaturated fatty acids; this makes them one of the most important members of oilseed crops. In addition, it should be noted that safflower is a multipurpose crop that can be used in several industries, such as food, pharmaceuticals, and dye. Plant breeders continue to search for suitable genotypes of different crops for various stresses such as drought, heat and flooding that coexist in rainfed ecologies (Bin Rahman and Zhang 2022). Safflower is heat and drought-tolerant, suitable for arid and semi-arid climates as well as irrigation (Hussain et al. 2015). Bassil and Kaffka (2002) stated that in addition to the drought, it is also tolerating salinity. Due to its long and deep roots, it is known as a drought-tolerant crop. Therefore, it is suitable for dry land cropping systems (Hussain et al. 2015). Safflower is adapted to arid and semi-arid conditions; hence, it may be considered an alternative crop under climate change. The main objective of plant breeding centres worldwide is to develop varieties with high compatibility, stability and tolerance to abiotic stresses (Crossa 1990). If a genotype has compatibility or stability, it would have a high average performance in different environments (Ashraf et al. 2001). Compatibility of a genotype is reflected through hereditary changes in the structure and nature that increase the ability to survive and reproduce in a given environment. Similarly, the consistent performance of a genotype under different environments and over the years is called stability (Fernandez 1992). Different responses in different environments often cause reactions from different genotypes. Reciprocal actions between genotypes and impacts of the environment are called interactions between genotypes and the environment (Weber et al. 1996). Genotype-environment interaction (GEI) is a genotypic response to environmental change (Roozeboom et al.

Department of Plant Breeding, Science and Research Branch, Islamic Azad University, Tehran, Iran.

[1]Agricultural Biotechnology Research Institute of Iran (ABRII), Tehran-Iran.

***Corresponding Author:** Khosro Zerehgar, Department of Plant Breeding, Science and Research Branch, Islamic Azad University, Tehran, Iran, E-Mail: khosrozerehgar@yahoo.com

2008), thus GEI has great significance for plant breeders and is one of the most complex factors and crucial for the dynamic breeding programs for producing high-yielding and stable genotypes (Cornelius and Crossa 1999; Gauch 2006; Yan et al. 2010; Ramburan 2014). Therefore, the analysis of GEI in multi-environment trials (METs) is essential for the assessment, selection of genotypes adapted to the target environments and comparison of GEI (Kamila et al. 2016) and finally the recommendation of cultivars (Regis et al. 2018). Using multivariate methods to study GEI is a powerful and useful strategy that also explains the complexity and multidimensionality of the interaction.

The MET procedure involves an array of tests. It is useful if plant breeders combine multiple attributes in one genotype. Wide use of MET data from such studies is to evaluate the relative performance of genotype, help to understand the corresponding aims and consideration of the development of analytical methods and finally recommend outcomes with suggestions. Therefore, the best representative environment and superior genotype with high performance and stability will be determined. Biplot analysis can use MET reconnaissance data graphically, efficiently and conveniently. For the evaluation and selection of stable genotypes, several statistical models have been used by the breeders but among all statistical packages, the Additive Main effect and Multiplicative Interaction Model (AMMI) is widely used due to its ability to extract genotype x environment interaction and additive effects using analysis of variance (ANOVA) and multiplicative effects through principal component analysis (PCA) (Gauch 1992; Gauch 2006; Ajay et al. 2021).

Finally, genotypes and environment on the biplot are localized and viewed due to the amount, size, and sign of principal component scores. The AMMI method can describe the pattern and the relationship between genotype and environment. Also, yield estimation is performed more accurately as interactions increase (Gauch 1990). Yan et al. (2000) proposed a technique known as the GGE biplot for the graphical display of GEI pattern MET data with several advantages. The GGE biplot method enables a simultaneous and graphical examination of the GEI (Lin et al. 1986). This technique also facilitates the study of environmental relationships and the search for target environments in a breeding program (Lin 1982). The efficiency of the process of selecting the best safflower genotype can be increased by combining graphical methods and statistical analysis. These statistical procedures have been applied earlier in several crops to identify stable genotypes in various crops such as peanut (Lal et al. 2021), pearl millet (Reddy et al. 2022), sesame (Baraki and Gebremariam 2018), rapeseed (Kamila et al. 2016) and bread wheat (Purchase et al. 2000; Naroui Rad et al. 2013) etc. However, very few reports are available in the literature on the study of genotype-environment

interaction (GEI) for the multi-environment traits in safflower (Moghadam and Pourdad 2009; Ebrahimi et al. 2016). Therefore, the present study aimed to identify ideal, high yielding and stable genotypes through MET application using the AMMI and GGE biplot analysis method in safflower for rainfed conditions.

Materials and methods

Materials used and the experimentation
Grain yield of 15 safflower genotypes comprising of 11 synthetic lines and four cultivars (Table 1) were evaluated in a Randomized Complete Block Design (RCBD) with three replications under rainfed conditions across test areas namely, Kermanshah (Sararood), Khorramabad and Ilam for three consecutive cropping seasons in Iran. The combination of year and locations formed an environment (E1 to E6). The climatic specifications of the locations were relatively similar, with a maximum temperature of 16.5 to 20.7°C and a minimum temperature of 3.8-9.4°C during the growing season. The average precipitation in the test area was 450 mm per year. Each genotype was seeded in the experimental plots comprising of five rows of four-meter length with row to row spacing of 30 cm. The application of fertilizers 60 Kg N and 60 Kg of P was made in each plot. The main soil texture in the test areas was loamy, with a pH reaction of 7.5 to 7.8 and an average organic carbon content of 1.1%. Common agricultural practices were followed to raise the crop. Only 3 middle rows were harvested after removing a 25 cm area on both sides to record data on grain yield.

Statistical analysis
AMMI model (Gauch1996; Najafyyan et al. 2010) and GGE biplot analyses were carried out. A graphical representation was depicted to show the distribution of genotypes and environments per the procedure of Gabriel (1971). Purchase et al. (2000) developed a quantitative stability value to rank genotypes through the AMMI model, namely the AMMI Stability Value (ASV) and to compute the IPCA1 and IPCA2 values which were performed using the SAS software. The GGE biplot technique was divided into two areas, the biplot part (Gabriel 1971) and the GGE part (Yan et al. 2000) was used to visually verify the MET data. This technique uses a biplot to show the G and GE factors; they are sources of variation in GEI analysis of METs data and crucial in genotype assessment (Yan et al. 2000; Yan 2001). The average yield was adjusted as a two-way matrix for drawing biplot charts via StatGraph software.

Results and discussion

AMMI analysis
The analysis of variance (ANOVA) showed highly significant (p < 0.01) differences among the genotypes (G), environment (E) and genotype x environment interaction (GEI) suggesting

Table 1. Codes and mean yield of safflower genotypes, G1- G15 are the codes of the studied genotypes

Genotype code	Genotype	Description	Yield (kg ha⁻¹)
G1	Local Kurdistan	Cultivar	816.12
G2	FARAMAN (411)	Cultivar	881.84
G3	386	Synthetic line	886.12
G4	376	Synthetic line	670.89
G5	44	Synthetic line	905.45
G6	PI-258417	Synthetic line	800
G7	27-N-825	Synthetic line	782.28
G8	62	Synthetic line	1038.78
G9	27-41/1	Synthetic line	928
G10	357/S6/697	Synthetic line	596.17
G11	PI-592391/Sunset	Synthetic line	649.67
G12	366/S6-697	Synthetic line	640.05
G13	324-S6-697	Synthetic line	719.78
G14	SINA (check)	Cultivar	1114
G15	ZARGHAN – 279 (check)	Cultivar	705.72

the presence of genotypic variations (Table 2) in terms of genotypic response to environments. The combined ANOVA showed that the environment significantly affected safflower grain yields. Gauch and Zobel (1996) stated that the best situation happens when only the first two principal components axes (PCA) are significantly different and others have minor variance. The existence of GEI required a selection process since it reduces the usefulness of genotypes by interfering with their yield performance due to the collaboration between genotypic and phenotypic values (Comstock and Moll 1963). Since the GEI is significant, the phenotypic stability could be calculated. Genotype, environment, and GEI express 19.31, 43.96, and 36.73% of the sum of the square. The study indicated significant differences in the performance of the tested genotypes according to compatibility and different response to the tested environments. Although the GEI is not the primary source of variation the dual effects of GEI indicate its relative importance. Tarakanovas and Ruzgas (2006) proposed the AMMI method as an effective way to study GEI and argued that the graphical results obtained can be defined as suitable cultivars for cultivation under certain environmental conditions. Thus, the present study showed that the genotypic screening by the AMMI method may be useful. Stable genotypes and their GEI can also be classified using AMMI analysis, and compatible genotypes can be displayed graphically. Table 3 reflected SIPC1 (Sum of the value of IPC) scores, ASV (AMMI stability values), and other stability criteria. Note that genotypes with lower ASV, SIPC1,

and other stability criteria are the most stable. Therefore, G2, G12 and G3 showed the lowest scores in the SIPC1, and G7 showed the highest. The ASV ranked G2 > G12 > G3 as the most stable and G7 > G5 as the most unstable but the SIPCf ranked the genotypes as G10 > G9 > G3 as the most stable and G14 > G5 as the least stable. Sums of the absolute value of the IPC Scores (SIPC) based on the AMMI model via F tests were also used to rank the genotypes of soybean (Sneller et al. 1997). As per the ASV ranking, Philanim et al. (2022) also identified the most stable genotypes for seed yield and its component traits in *Vigna umbellata* as determined by the lowest ASV value and also conferred as the most unstable genotypes based on the stability score.

The closer the SIPC scores are to zero, the more stable the genotypes are across test environments. The D*i* ranked G10 > G12 > G3 as the most stable and G7 as the least stable. The Eigen Value (EV) ranked G10 > G12 > G3 as the most stable and G7 > G5 as the least stable. The Sum Across Environments of GEI Modeled by AMMI (AMGE) ranked G3 > G14 > G12 as the most stable and G7 > G5 as the least stable. The ranking of the genotypes according to the above criteria can be summarized as follows: G3 > G12 is the most stable, and G7 is the least stable of the other understudied genotypes. Rad et al. (2013) applied AMMI model to calculate stability value (ASV) that revealed that a wheat F₁ hybrid 14 was stable, whereas GGE-biplot models showed that the six environments used for the study belonged to two mega-environments and the study resulted in the identification of most stable hybrid.

AMMI1 biplot
AMMI model generates biplots that are useful for simultaneously analyzing the genotypes and environment. The horizontal axis (X-axis) represents the additive main effect or average grain yield (kg ha⁻¹) and the vertical axis (Y-axis) shows multiplication interactions which are the

Table 2. Analysis of variance results based AMMI method for grain yield of safflower genotypes in six test environments

SOV	df	SS	MS	F
Total	289	34571774	128520	7.60
Treatments	89	26399864	296628	9.32
Genotypes (G)	14	5097509	364108	17.28
Environments (E)	05	11605523	2321105	3.44
Block	12	1611927	134327	3.55
Interaction (GEI)	70	9696831	138526	9.46
IPC1	18	6645709	369206	2.24
IPC2	16	1398845	87428	2.00
IPC3	14	1094638	78188	0.65
Residual	22	557638	25347	
Error	168	6559984	39048	

Table 3. Mean grain yield, AMMI stability value (ASV) and other stability criteria of safflower genotypes in six test environments

| Genotype	Yield in kg ha^{-1}	ASV	SIPC1	SIPCf	Di	EV	AMGE
G1	816.12	14.99	2.83	23.79	15.74	82.58	-0.10
G2	881.84	10.59	3.31	21.18	13.17	57.85	-0.13
G3	886.12	12.48	5.52	14.41	8.52	24.25	0.00
G4	670.89	27.82	12.62	18.57	13.40	59.87	-0.14
G5	905.45	35.54	-15.94	27.31	18.03	108.43	0.19
G6	800.00	16.52	7.15	25.02	15.33	78.42	0.05
G7	782.28	41.77	-19.14	24.66	19.56	127.56	0.22
G8	1038.78	15.16	5.17	20.34	12.13	49.06	-0.11
G9	928.00	19.33	-8.82	13.13	9.34	29.13	0.11
G10	596.17	16.13	7.36	9.92	7.59	19.20	-0.08
G11	649.67	25.26	11.46	21.13	13.44	60.26	-0.05
G12	640.05	11.54	4.72	12.99	7.66	19.58	-0.01
G13	719.78	18.26	6.30	21.19	13.88	64.23	-0.09
G14	1114.00	24.64	-10.97	27.97	16.69	92.91	0.00
G15	705.72	25.46	-11.60	17.98	12.45	51.69	0.15

values of the first principal component that is known as factor coefficients (IPCA1) in the mentioned biplot (Fig.1). Two pairs of data are displayed on the axes. This biplot explained 85.29% of the variability relating to GEI (Fig.1) and is consistent with the results of Mattos et al. (2013) and Regis et al. (2018). The first pair corresponds to the mean yield of each genotype (X-axis) and the values of the IPCA1 of the same genotype (Y-axis) and the second pair corresponds to the mean yield of each environment (X-axis) and IPCA1 values of the same environment (Y-axis. The genotypes which are positioned at the centre of the biplot (Fig.1) have almost no interaction, which means that they are stable overall. The yield of G8 has been above average, and its IPCA1 value was too low, hence it is the most stable. G2 and G5 have weak interactions and their average yields were higher than the overall average, making them the most stable genotypes. G1, G15, and to some extent, G6, G9, and G14 are closest to the coordinate source and have the least interaction with the environment, so they are unstable. The IPCA1 scores of E2 and E4 are close to zero, so they are not suitable for distinguishing genotypes. The IPCA1 score of E5 is remarkable and can be considered an absolute indicator of genotype recognition and separation. G7 is close to the origin; therefore, it does not respond to the environment as required and is insensitive to environmental changes. G14, G9, and G8 are the genotypes farthest from the centre of the biplot and, therefore, have strong interactions and the greatest environmental impact. Genotypes with a high yield value (X-axis) and a low IPCA value (Y-axis) will be more desirable because this symbolizes high yielding and stability. Genotypes or environments that were placed on

Y-axis have the same performance; likewise, genotypes or environments placed on X-axis showed the same IPCA. The procedure is consistent with the operation of (Baraki and Gebremariam 2018).
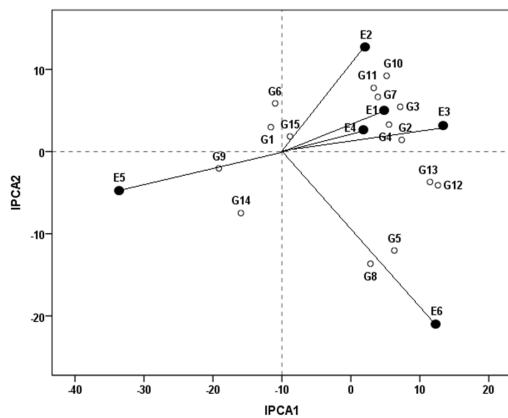
AMMI2 biplot
To understand the stability of safflower genotypes and their interaction effects of G x E, AMMI 2 biplots are depicted through IPCA 1 and IPCA 2 scores for grain yield and stability. This biplot showed that the IPCA1 and IPCA2 accounted for 59.03 and 26.26% of variance, respectively (Fig. 2). From the IPCA point of view, if the genotype sign and environment sign are the same, they have a positive interaction. In contrast, if their signs are not the same, they have a negative interaction. A significant IPCA for a genotype indicates the genotype's specific compatibility with a particular environment (Sanni et al. 2009). The environments can be grouped by areas of the diagram Fig. 2. Every trial environment in each section falls into a private group (Crossa et al. 1991). The first section includes E1, E2, E3, and E4; the second section includes E6; the third section contains E5. Likewise, the groups of genotypes with similar responses are as follows: The first group has G1 and G6, the 2nd group has G2, G3, G4, G7, G10, and G11. The 3rd group has G5, G8, G12, and G13, the 4th group has G9 and G14. According to the biplot, the IPC1 and IPC2 of G1 and G15 have the shortest distance from the coordinate's origin, comparatively stable genotypes. G12 and G13 are further away than the coordinate's origin and self-identify as unstable genotypes. Genotypes close to a given environment as a specific location have specific compatibility with that environment, and when close to the
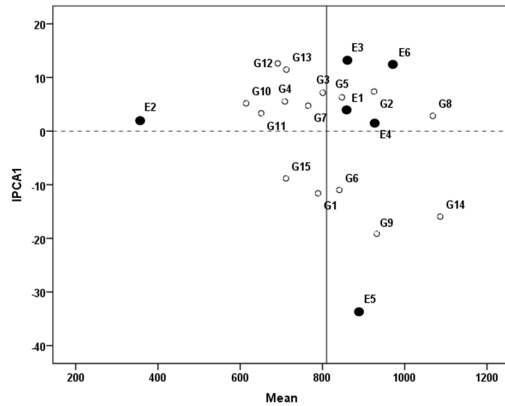
Fig. 1. AMMI1 biplot mean grain yield of safflower genotypes against IPCA1 values in 15 genotypes of safflower over six environments in three provinces viz., Kermanshah, Ilam and Khoramabad of Iran
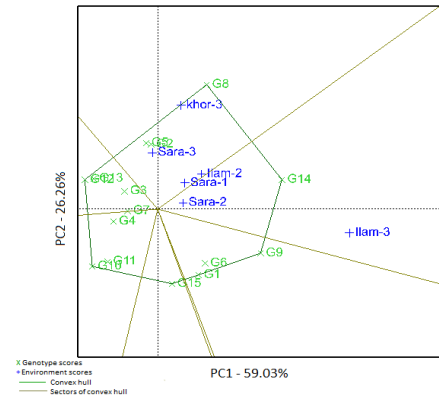

Fig. 3. Polygon outlook determine superior or inferior safflower genotypes over six test environments across the three provinces in Iran

Fig. 2. AMMI2 biplot IPCA1 against IPC2 for grain yield and stability of safflower genotypes over six test environments across the three provinces in Iran

Fig. 4. Ranking biplot showing mean yield and stability performance of safflower genotypes for both yield and stability performance over six test environments across the three provinces in Iran
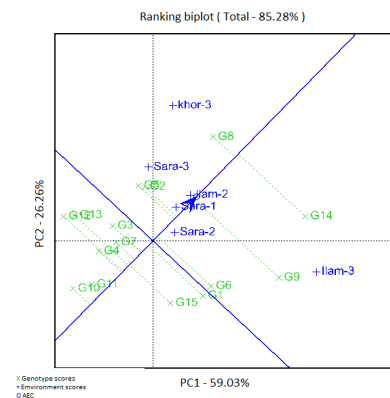
IPCA they have general compatibility. The results showed that G9 and G14 have specific compatibility with E5 and also G5, G8, G12, and G13 showed specific compatibility with E6. The same is also observed for G2, G3, G4, G7, G10, G11, and G15 with E1, E2, E3, and E4, while G1 and G6 have not specific compatibility with either environment. In addition, the general compatible genotypes are found to be as follows G1, G4, G6, G9, and G15. The undersized yield and IPCA-positive genotypes are suitable for cultivation in poor areas, in other words, they have a positive interaction as has been observed earlier in bread wheat (Farshadfar 2008). Results also indicated that G4, G10, G11, and G12 with E2 are in a similar situation. AMMI2 is also useful for describing mega-environments; these environments contain identical, high-yielding genotypes (Hongyu et al. 2014). The biplot of AMMI 2 analysis has also been useful in identifying sugarcane genotypes with stable performance in specific environments and across the environments; it is also useful in finding out the mega environments (Elayaraja et al. 2022).

GGE biplot analysis
The performance of a genotype in an environment consists

of the environment (E) and genotype (G) main effect as well genotype x environment interaction (GEI). Yan (2000) stated that the E effects were the main contributor to total yield variation and the G and GEI effects are less significant than the E effects. However, these two effects were involved in genotype evaluation experiments; therefore, they should be considered for the selection of superior genotypes. Moghadam and Pourdad (2009) conducted an experiment on safflower cultivars and found that over 80% of the variability was due to environmental influences. In the GGE biplot, the effects of G and GEI are also not separated. In most cases, the E effect is very important but not exploitable, so it is essential to ignore and focus on the G and GEI effects (Gauch et al. 1996). The study of polygon plots was first applied by Yan (2001), who described how this method is used to find better genotypes in different environments and how to find mega-environments. GGE biplot through G x E graph helps breeders check the stability of genotypes and corresponding performance traits in different environments with this method. For a better analysis of a biplot, the following points should be noted (Kang 1993): (I) The biplot centre represents the mean for each environment
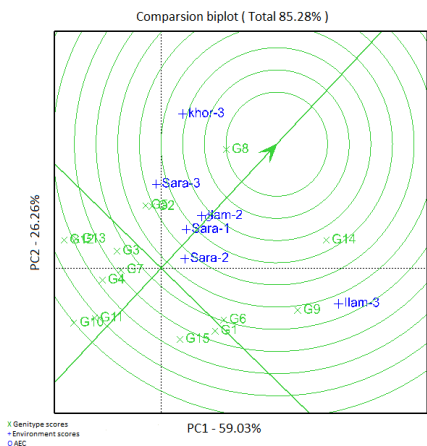
Fig. 5. Comparison biplot of safflower genotypes with ideal yield and stability genotypes over six test environments in three provinces,Kermanshah, Ilam and Lorestan of Iran.

or genotype (II) The genotype (or environment) has been positioned away from the centre of the biplot and has a strong interaction with at least one genotype (or environment) (III) The angle between a genotype and an environment indicates whether the interaction is positive or negative. The most significant advantage of the GGE biplot is GEI visual assessment. With the GGE biplot, two-dimensional plots are drawn to better analyze data and facilitate discussion of results. Therefore, it is a suitable method for stability analysis and selection, considering performance and stability at the same time. According to (Kizilgecietal 2019), this method is a graphical tool that helps plant breeders and geneticists find high yielding and stable genotypes in multiple locations. Results-based GGE biplot analysis allows systematic analysis of the variability present in MET data by GEI.

GGE biplot polygon

The graphical representation through polygon determines the superior or inferior genotype in an environment or group of them. Each polygon vertex shows the genotype that is farthest from the biplot centre. They are connected by straight lines, resulting in a polygon. As can be seen in Fig 3 drawing perpendicular rays from the coordinates origin to the sides of polygon sectors, the so-called mega-environments can be created. Also, the perspective of the polygon showed that G8, G9, G10, G12, G14, and G15 were placed at the top of the polygon and thus identified as a better or worse genotype of that environment. According to the MET data query, the polygon is divided into seven sectors as mega-environments, and six test environments fall into two of them. Gauch and Zoble (1997) found that mega-environments have two characteristics: there are different superior genotypes in various mega-environments, and the variance between mega-environments is much larger than the variance within mega-environments. The 1st

mega-environment contains E1 (Sararood 1), E3 (Ilam 2), E4 (Sararood 3), and E6 (Khorramabad 3), which encompasses G2, G5, and G8, with G8 being at the top of the polygon. The 2nd contains E2 (Sararood 2) and E5 (Ilam 3) where only G14 was observed at the apex of the polygon. No experimental environments can be seen in the sections where the G9, G10, G12 and G15 are located at the apices, meaning they didn't perform very well everywhere and were the weakest in most of the test environments. The same goes for G1, G3, G4, G6, G7, G11, and G13, which belong to Mega Environments 3, 4, 5, and 6. Also, the genotype was placed in the biplot centre, which is called weak and had the same response to most environments. The G7 has this feature.

Ranking biplot

To simultaneously assess the stability and performance of genotypes, the mean environment, coordinate (AEC) plot (Yan and Kang 2002), also called the mean versus stability plot is used (Yan et al. 2007). In this diagram, the horizontal axis (X-axis) or PC1, which has an arrow, goes through a small circle representing the mean of the environment and the coordinate system's origin. The appearance of genotypes on this axis is an estimate of the genotype's yield. This axis is also known as the abscissa axis or the main axis of the environment. The vertical axis (Y-axis) or PC2 that has two arrows and passes through the origin of coordinates is perpendicular to the mean axis of the environment. This axis indicates the interaction of the genotype with the environment and determines the stability of genotypes also known as the ordinate axis or measure of stability. AEC is the line that goes through the origin of the biplot. Genotypes to the right of the AEC on the Y-axis showed above-average yield. The same is true for genotypes on the left side, but their yields are below average. As visualized in Fig. 4 G9, G14 and G6 have the highest yield and G5 and G2 rank after them. Also, G10 and G11 were placed at the end of the AEC ordinate, showing the lowest yield. Yan (2000) found that the presence of genotypes in the AEC provides a correct estimate of their performance. The line rises or falls directly on the AEC ordinate and passes through the origin, expressing the GEI and determining the stability of the genotypes. The most unstable genotype would have a taller copy of the AEC abscissa apart from its orientation (Yan 2001; Yan and Kang 2002). Although G9 (928 kg ha^{-1}), G14 (1114 kg ha^{-1}) and G6 (800kg ha^{-1}) have a higher yield they are unstable. Instead G11 (649.67 kg ha^{-1}), G10 (596.17 kg ha^{-1}) after G7 (782.28 kg ha^{-1}) and G4 (670.86 kg ha^{-1}) with low yields, they are considered the most stable genotypes.

Comparison biplot of genotypes

The ideal genotype is the hypothetical genotype that has the highest yield and stability at the centre of the biplot's concentric circles (Yan 2001). A comparison biplot is drawn based on determining the distance from the assumed ideal

genotype, which is characterized by the highest stability and productivity. The degree of desirability of genotypes depends on their distance from the ideal genotype (Yan and Kang 2002). Such a genotype is defined by the least role in the interaction and the most length on the average high-yielding vector. The ideal genotype is placed within concentric circles in the comparison chart and indicated by the medium high-yielding arrow. Concentric circles are drawn on the biplot to use the ideal genotype as an evaluation framework, which graphically represents the distance between the tested genotypes and the ideal genotype. Any genotype within the concentric circle or closest to the assumed ideal genotype is considered a superior genotype with high yield and stability, as shown in Fig. 5. Accordingly, the genotype is more comfortable and closer to the ideal genotype. The ideal genotype should be considered as it has the highest average yield and is perfectly stable. Therefore, it can be used as a reference for genotype assessment. Comparing genotypes with ideal genotypes showed that G8 (1038.78 kg ha-1), and G14 (1114 kg ha-1) came closest to the ideal genotype, followed by G5 and G2. The ranking of genotypes based on the ideal genotype was: G8 > G14 > G5 > G2. G10, G11 and G12 were also furthest from the ideal genotype and they are lower yielding, hence unfavorable.

Authors' contribution

Conceptualization of research (KZ, SSP, RA, ARE); Designing of the experiments (KZ, SSP, RA, ARE); Contribution of experimental materials (KZ, SSP); Execution of field/lab experiments and data collection (KZ, SSP); Analysis of data and interpretation (KZ, SSP, RA, ARE); Preparation of the manuscript (KZ, SSP, RA, ARE).

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