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Assessment of genotype-by-environment interactions for seed yield and yield-related traits in oilseed sunflower genotypes under normal and salinity stress conditions

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Abstract

Context Sunflower, as a significant source of edible oil worldwide, requires the development of stable and high-yield genotypes, particularly under salinity and adverse environmental conditions.

Objectives This research was conducted with the aim of assessing the stability of seed yield and yield-related traits in 61 sunflower genotypes under normal and salinity stress conditions across two cropping years (2015–2016).

Methods For a more accurate assessment of genotype × environment interactions (GEI) in sunflower, robust statistical methods including mixed model analysis of variance, AMMI and GGE models, weighted average of absolute scores (WAASB), Multi-Trait Stability Index (MTSI), and parametric and non-parametric stability indices were used and addressed.

Results Mixed modeling ANOVA revealed the significant effect of environment, genotype, as well as GEI for seed yield and associated traits, demonstrating different responses of genotypes across environments. The outcomes of various statistical methods showed a high level of consistency. Genotypes 4, 6, 44, 22, and 59 demonstrated high stability and yield. The MTSI identified genotypes 4, 61, 25, 50, 55, 26, 32, 40, and 34 as the most desirable with maximum stability across various traits.

Conclusion Genotype 4 (270-ENSAT) is introduced as a promising candidate for creating salt-resistant hybrid varieties.

Significance Different stability assessment methods helped in the discovery of superior genotypes that can be used for developing salt-resistant and stable hybrids, thereby establish opportunity for exploiting salt-affected soils for crop production.

Keywords Adaptability Analysis, Multi-Environment Trials, Stability, Sunflower, Yield

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Introduction

Sunflower (*Helianthus annuus* L.) is cultivated globally, primarily for oil extraction, and its high-fiber seed meal holds a special place in animal feeding [52]. Sunflower seeds are a rich source of nutrients and various phytochemicals including antioxidants, flavonoids, phenolic acids, procyanidins, phytosterols, amino acids, fiber, potassium, arginine, and unsaturated fatty acids [1]. Sunflower oil contains beneficial compounds such as linoleic acid, oleic acid, palmitic acid, stearic acid, omega-9, and omega-6, which have significant impacts on human health [8, 70].

Saline soils expanding at a rate of 10 percent per year, pose a serious threat to the adequate provision of food, globally, and it is predicted by 2050 more than 50 percent of cultivated lands will become saline [49, 57]. Salinity is a determining factor in crop productivity, particularly in arid and semi-arid regions. Salt accumulation in the soil limits water and nutrient uptake and causes osmotic stress, ionic toxicity, and nutrient imbalance [87]. These factors lead to metabolic disorders, reduced growth, and a significant decline in crop yields [87]. Sunflower is classified as a moderately salt-tolerant crop [31]. Reduced root proliferation and browning of root tips, leaf necrosis, reduced head diameter, delayed seed formation [30], reduced chlorophyll content [3, 19], decreased seed yield due to reduced pollen viability and stigma receptivity [55], decreased triacylglycerol (TAG) storage [30], reduced leaf area, dry matter, number of seeds per head, seed yield per plant, seed yield, and oil content are major morphological disorders in response to salinity stress in sunflower [40, 67]. Therefore, achieving high-yielding and stable genotypes is a primary objective of breeding programs. Climatic and ecological factors, as the main constituents of an environment, have positive or negative impacts on the quality and performance of genotypes. Consequently, developing stable genotypes that can grow under diverse environmental conditions is essential [33].

Genotypes do not always respond in the same manner to environmental conditions, a phenomenon known as genotype $\times$ environment (GE) interaction [34]. GE interaction appears in two types of multi-environment trials in plant breeding activities: (1) a change in the rank of genotypes across different environments, which is referred to as the severe type in multi-environment trials, and (2) a change in the absolute differences between genotypes without a change in ranking [12]. Yield, as an end product of the plant, is a complex quantitative trait that is highly affected by genotype-by-environment interaction [59]. Yield stability can be enhanced by combining agronomic traits that exhibit high stability [5].

Hühn [38] proposed various statistical models and approaches for analyzing and interpreting the GE interaction effects, which can be subdivided into two major

groups: parametric and non-parametric approaches. In the non-parametric approach, based on trait mean values, the ranks of genotypes are estimated [65, 78]. In the examination and interpretation of genotype $\times$ environment interactions, these approaches can complement each other, leading to a coherent and logical picture of genotype-environment interactions [16]. Additionally, researchers use multivariate methods such as additive main effects and multiplicative interaction (AMMI) and genotype plus genotype-by-environment (GGE) biplot for effectively analyzing and interpreting the structure of multi-environment trial data in plant breeding programs [83]. Recently, a new approach called WAASB, which combines the AMMI and BLUP features, has been introduced by Olivoto et al. [60] that can facilitate identifying high-yield and quality as well as stable cultivars well-adapted to the region's climatic and ecological conditions. These cultivars can significantly increase production in unit area [33].

Some of the above-mentioned methods have been used to evaluate the GEI pattern and select stable genotypes in sunflower in terms of seed yield [7, 11, 13, 15, 32, 53, 54, 71, 77, 80] however, there is limited information on selecting stable sunflower genotypes by simultaneously considering various traits. Therefore, the present study aims to analyze the G $\times$ E interaction in 61 sunflower genotypes based on seed yield and yield-related traits using a set of stability analysis methods under normal and saline stress conditions.

Materials and methods

Plant materials and experimental design

Sixty-one sunflower genotypes from an international collection (Table 1) were evaluated for yield and yield-related traits under both normal and 8 dS/m saline conditions over two growing seasons (2014–2015) (four environments). The experiment was conducted at the Department of Crop Production and Genetics, Urmia University, Nazloo, Iran (37°32'N, 45°5'E, 1313 m above sea level) in a randomized complete block design (RCBD) with three replications. Seeds of each genotype were sown in 24 $\times$ 24 cm pots containing filed soil. Based on the initial soil salinity, 12 g of NaCl was dissolved in 500 ml of water and applied to each pot when plants reached the eight-leaf stage. Soil salinity in the pots was monitored throughout the experiment using an EC meter. Harvesting was carried out when the back of the sunflower head turned yellowish-brown. The measured traits included plant height (PH, centimeters), 100-seed weight (FSW), head diameter (HD), and grain yield per plant (GY).

It should be noted that the sunflower genotypes used in this study were obtained from the germplasm collection of the Department of Plant Production and Genetics,

Table 1 Name, code and origin of 61 oilseed sunflower genotypes investigated in the present study

Code	Genotype name	Name of the research center	Country name	Code	Genotype name	Name of the research center	Country name
1	H209A/LC1064	ASGROW	France	32	RT948	RUSTICA	France
2	AS5306	ENSAT	France	33	283-ENSAT	-	-
3	AS5304	ASGROW	France	34	QHP-1	INRAMONT	France
4	270-ENSAT	ENSAT	France	35	HA337B	USDA	America
5	AS613	ASGROW	France	36	H100B	ASGROW	France
6	A-F1POPA	NOVARTIS	France	37	B454/03	-	Hungary
7	OES	INRAMONT	France	38	HA304	USDA	America
8	H100A/LC1064	ASGROW	France	39	RT931	RUSTICA	France
9	H157A/LC1064	ASGROW	France	40	HA335B	USDA	America
10	5DES20QR	BRN	France	41	NS-B5	NOVARTIS	France
11	12ASB3	ASGROW	France	42	SDB3	USDA	America
12	ASB28	ASGROW	France	43	LC1064C	ASGROW	France
13	H049 + FSB	-	France	44	H156A/RHA274	ASGROW	France
14	SSD-580	ASGROW	France	45	SDB1	USDA	America
15	5AS-F1/A2*R2	ASGROW	France	46	HAR-4	USDA	America
16	7CR16=PRH6	C.F	France	47	AS5305	ASGROW	France
17	ENSAT699	ENSAT	France	48	RHA274	USDA	America
18	SSD-581	ASGROW	France	49	H100A/RHA274	ASGROW	France
19	110	SPIL	Iran	50	ASO-1-POP-A	ENSAT	France
20	H603R	INRAMONT	France	51	B-F1POPB	NOVARTIS	France
21	4	Iran	SPIL	52	36	Iran	SPIL
22	NSF1-A4*R5	NOVARTIS	France	53	SDB2	INRAMONT	France
23	28	Iran	SPIL	54	H158A/LC1064	ASGROW	France
24	30	Iran	SPIL	55	H156A/H543R	ASGROW	France
25	LP-CSYB	ENSAT	France	56	HR543	-	France
26	1,009,370–1(100 K)	ENSAT	France	57	15,031	ASGROW	France
27	CSWW2X	Caussade semences	France	58	SF076	ENSAT	France
28	1,009,370–3(100 K)	ENSAT	France	59	SF092	ENSAT	France
29	H158A/H543R	ASGROW	France	60	HC91	Iran	SPIL
30	15,031	ASGROW	France	61	SF105	ENSAT	France
31	RHA265	USDA	America				

Urmia University, Iran. All plant materials were cultivated breeding lines or accessions of *Helianthus annuus* L. No wild populations were sampled, and no protected or endangered species were involved. Therefore, no specific permits or licenses were required for plant material collection. The study complied with institutional, national, and international guidelines governing plant research.

Stability analyses

Parametric and non-parametric approaches

A combined analysis of variance was performed for yield and yield-related traits over four experimental environments. According to significance of genotype-environment interaction, genotypic stability was evaluated for grain yield using both parametric [Regression coefficient [25], Deviation mean square [22], Wricke's ecovalence [81], Shukla's stability variance [75], Environmental coefficient of variation (S2) [27], mean–variance component

[64], and Genotype × environment (GE) variance component [63]] and non-parametric [Mean absolute deviation (S1) and Variance or standard deviation (S2) [58], Sum of absolute deviations (S3), Sum of ranks squares for each genotype relative to the mean ranks (S6) [37], NP(i) statistic [76], and Kang's yield stability [43]] methods.

AMMI analysis

AMMI analysis of grain yield data was performed according to the following model [86]:

$$\gamma_{ger} = \mu + \alpha_g + \beta_e + \sum_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + K_{r(e)} + \varepsilon_{ger} \quad (1)$$

where, γ_{ger} represents the performance of genotype g in environment e in replication r , μ is the overall mean, α_g is the deviation of genotype g from μ , β_e is the deviation of environment e from μ , λ_n is the singular value for the n^{th} principal component (IPC), γ_{gn} and δ_{en} are the eigenvector values for genotype g and environment e , respectively,

for the n^{th} axis, ρ_{ge} is the residual, $K_{r(e)}$ is the replication r in environment j , and ε_{ger} is the error term.

GGE biplot analysis

Genotype plus genotype-by-environment (GGE) biplot analysis of grain yield data over four experimental environments was conducted using the following model:

$$\hat{Y}_{ij} - u - \beta_j = \lambda_1 \xi_{I1} \eta_{1J} + \lambda_2 \xi_{i2} \eta_{2j} + \varepsilon_{ij} \quad (2)$$

where $\hat{Y}_{ij}$ is the mean of genotype i in environment j , u is the overall mean, β_j is the main effect of the j th environment, λ_1 and λ_2 are the singular values for the first and second components, ξ_{I1} and ξ_{i2} are the genotypes i eigenvectors, η_{1J} and η_{2j} are the environment eigenvectors for the first and second components, and ε_{ij} is the residual for genotype i in environment j .

Genetic stability based on Best Linear Unbiased Prediction (BLUP)

To integrate BLUP and AMMI features for identifying stable genotypes, the WAASBg index was calculated for each genotype according to [60] as follows:

$$\text{WAASBg} = \frac{\sum_{n=1}^p |\text{IPCA}_{gn} \times EP_n|}{\sum_{n=1}^p EP_n} \quad (3)$$

where WAASBg represents the weighted average of the absolute score of genotype g , IPCA_{gn} is the score of genotype g in the n^{th} principal component of interaction (IPCA), and EP_n is the amount of variance explained by the n^{th} IPCA. The genotype with the lowest WAASBg value is considered the most stable genotype, indicating the least deviation from the average performance across environments [60].

WAASB is calculated from the singular value decomposition of the best linear unbiased prediction (BLUP) matrix of GEI effects derived from the linear mixed model. Furthermore, to simultaneously select for high-yielding and stability, the WAASBY index was calculated as follows:

$$\text{WAASBY}_g = \frac{(rG_g + \theta_Y) + (rW_g + \theta_S)}{\theta_Y + \theta_S} \quad (4)$$

where WAASBY is the superiority index with varying weights between yield and stability for genotype g , θ_Y and θ_S are the weights for yield and stability, and rG_g and rW_g are the rescaled values of genotype g for yield and WAASB, respectively.

Multi-traits stability index (MTSI)

The stability analysis based on MTSI was calculated using the following Eq. (5) [60]:

$$\text{MTSLi} = \left[\sum_{j=1}^f (F_{ij} - F_j)^2 \right]^{0.5} \quad (5)$$

In this formula, MTSI is the multivariate stability index for the i^{th} genotype, F_{ij} is the score of the j^{th} factor for the i^{th} genotype, and F_j is the score of the j^{th} ideal genotype (ideotype). The scores were derived from factor analysis of the two-way table of WAASBY_g values for genotypes and traits. The genotype with the lowest MTSI is closest to the ideal genotype and thus has the highest average performance and stability for all studied traits.

Statistical software

Variance and AMMI analysis were performed using the agricolae package [56] in R software. Parametric and non-parametric univariate stability indices were calculated using the online program STABILITYSOFT [66]. GGE biplots were generated using the GEA-R program [62], and WAASBY and MTSI analyses were conducted using the metan package [60] in R.

Results

Combined analysis of variance revealed the significant effects ($P < 0.001$) of environment, genotype, and genotype $\times$ environment interaction on yield and yield related-traits (Table 2). The significant interaction effect indicates that the responses of sunflower genotypes regarding yield and agronomic traits vary from one environment to another (Table 2). The overall means across the four environments were 84.480 cm for plant height (PH), 206.0 g for 100-seed weight (FSW), 7.872 cm for head diameter (HD), and 22.25 g for grain yield (GY) (Table 2). Genotypes 4, 46, 6, 59, 22, 35, 38, 26, 44, and 47 were identified as the top-performing genotypes for grain yield across all environments.

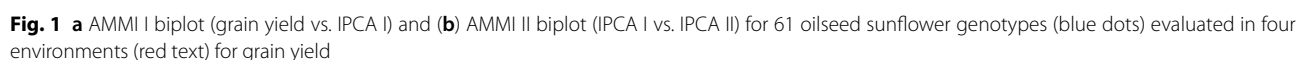
Parametric and non-parametric stability statistics

Overall, the genotypes 50, 59, 22, 24, 4, 6, 44, 31, 25, and 12 were identified as the most stable based on the results of parametric and non-parametric methods. Among these, genotypes 4, 6, 44, 22, and 59 also exhibited high grain yield, suggesting they are desirable. Conversely, Genotypes 24, 31, and 50 performed near the overall average, while genotypes 12 and 25 had the lowest grain yield. Thus, despite exhibiting high stability, genotypes 12 and 25 lacked high grain yield, a key desirability criterion (Supplementary Table 1).

AMMI biplot

The potential of sunflower genotypes in terms of grain yield, the correlation between studied environments, and genotype stability is presented in Fig. 1. Figure 1a shows the distribution of genotypes and environments based on IPC1 scores versus grain yield. Genotypes 4, 22, 46,

Source	PH		FSW		HD		MS	
	SS	MS	SS	MS	SS	MS	SS	MS
ENV	71,289	23,763.1 ^a	0.653	0.218 ^a	797.77	265.925 ^a	71,289	23,763.1 ^a
REP: ENV	14,606	1217.2 ^a	0.088	0.007 ^a	223.18	18.599 ^a	14,606	1217.2 ^{ns}
GEN	76,862	1281 ^a	2.811	0.047 ^a	671.38	11.19 ^a	76,862	1281 ^{***}
ENV: GEN	61,838	343.5 ^a	1.708	0.009 ^a	1180.11	6.556 ^a	61,838	343.5 ^{***}
Residuals	100,339	139.4	1.729	0.002	1751.84	2.433	100,339	139.4 ^{***}
Mean	84,480		0.206		7.872		22.25	

^{ns}non-significant, respectively

Genotypes 22 and 24 are promising candidates, exhibiting high yield (above the total average) and favorable stability. Based on the biplot graph in Fig. 1a, the environments exhibited more excellent dispersion than the genotypes. The saline environment in 2016, which is closer to the origin and has the shortest environmental vector, shows the least environmental variability, while the normal environments in 2016 and the saline environment in 2015, with longer vectors from the origin, demonstrated more significant variability. Based on AMMI analysis, the two first and second principal components

explained 69.5 and 20.9 percent of genotype-by-environment interaction variation, respectively. Figure 1b shows the AMMI2 biplot that depicts the position of genotypes and environments based on the first two IPCs. The origin of the biplot shows a value of zero for both components. An IPC score of zero signifies maximum stability and minimal contribution to GEI; Therefore, genotypes with scores further from zero contribute more to GEI [2, 21, 72]. Furthermore, environments with IPC scores close to or equal to zero have less contribution in the GE interaction and consequently play a crucial role in the stability of genotypes [2]. Based on the AMMI2 biplot analysis, genotypes 24, 22, 61, 42, 43, 16, 40, 25, 50, and 55 were identified as having high stability. However, among these, only genotypes 22 and 24, which demonstrated average yields exceeding the overall average, are recommended as suitable options for all environments. Meanwhile, genotypes 15, 11, 23, 46, 30, 58, 35, 7, 47, 38, 26, 59, and 60 showed specific adaptation (Fig. 1b). According to the biplot (Fig. 1b), genotype 46 showed high yield in saline environment on 2015, genotypes 35 and 58 in saline environment on 2016, genotypes 26, 47, 38, 59, and 60 in normal environment on 2016, and genotypes 11 and 15 in normal environment on 2015. AMMI analysis was also performed for each yield-related trait. The AMMI1 biplot for FSW indicated high stability for genotypes 4, 22, 24, and 25, but only genotype 4 exhibited an above-total average yield (Supplementary file 1). Genotype 25 was stable but low-yielding for the HD trait, whereas genotype 4 displayed stability and the highest average yield. Genotype 50, situated near the graph's center, exhibited high stability for the HD and grain yield traits (Supplementary file 2).

Genotypes 24, 25, and 55 displayed high stability for the PH trait. Genotypes 24 and 25 showed a high average yield, while genotype 55, despite high stability, had a below-average yield (Supplementary file 3). Based on the first two principal components of the AMMI2 biplot for FSW, genotypes 46 and 47, similar to their performance for grain yield, showed specific adaptation to the saline environment in 2015 and normal environments in 2016, respectively (Supplementary file 1). Regarding the HD trait, genotypes 46 and 11 showed high stability similar to what they showed for the grain yield, and genotype 50 was identified as the most stable genotype for this trait (Supplementary file 2). Regarding the PH trait, genotypes 55, 61, 43, and 4 showed high stability, while genotype 60 had specific adaptation to a normal environment in 2016 (Supplementary file 3). The stability analysis analysis based on the AMMI model for yield-related traits was similar to the results obtained for grain yield traits.

GGE Biplot

The GGE biplot is particularly useful for simultaneously visualizing the stability and performance of genotypes. The 'average vs. stability' view, commonly known as Average Environment Coordination (AEC), aids in simplifying the assessment of genotypes based on their mean performance and stability across diverse environments. The horizontal axis of the AEC, indicated by an arrow and a small circle, passes through the origin and approximates genotype performance. The vertical axis, with two arrows and perpendicular to the AEC axis, represents the genotype $\times$ environment interaction and determines the stability of genotypes. The longer the projection of a genotype on this axis, the less stable it is.

After removing environmental effects in GGE biplot analysis, the first two principal components explained 54.97% and 30.16% of the total variability, respectively. GGE biplot AEC views indicated that genotypes 4, 6, 46, and 59 had the highest yields, while genotypes 54, 56, 16, 43, 3, 45, and 33 had the lowest (Fig. 2a). Genotypes 55, 50, 31, 34, 41, 49, and 24 were stable due to their closeness to the AEC. Genotypes 45, 1, 33, and 54 exhibited the lowest yield and stability. Genotypes 15, 18, 23, and 30 had an average yield close to the overall average but were unstable. Genotypes 43, 56, 12, 42, 3, and 16 had the lowest yield but had high stability. Genotypes 26, 22, and 44 were characterized by high yield and moderate stability. Figure 2b shows a polygon view of the GGE biplot for the "which won where" pattern of 61 oilseed sunflower genotypes in four environments.

The polygon connects the genotypes furthest from the biplot's center, enclosing the remaining genotypes. Perpendicular lines are drawn from the biplot origin to the polygon sides partitioning it into distinct sectors. Segmenting aids to recommend specific genotypes for particular environments. Genotypes 4, 46, 15, 54, 16, 7, and 47 are placed at the polygon's vertices. For example, genotype 46, located at the vertex of the polygon and proximate to the normal and saline stress environments in 2015, indicates its favorable performance under those conditions. Similarly, the placement of genotypes 47 and 7 at the vertex of the polygon, proximate to the normal and saline stress environments in 2016, shows their desirable performance under those conditions. Genotypes closer to the origin of the biplot indicate general adaptability compared to those located at the vertex. Therefore, genotypes 50, 55, and 34 with average yield near the overall mean are considered genotypes with general adaptability (Fig. 2b). Genotypes at the vertex, without environmental influence, exhibit low yields in some or all conditions. Meanwhile, genotypes near the origin of the biplot are suitable for low-yield situations due to their stability. Based on GGE biplot analysis for the HD trait, genotype 46 was determined to possess the highest

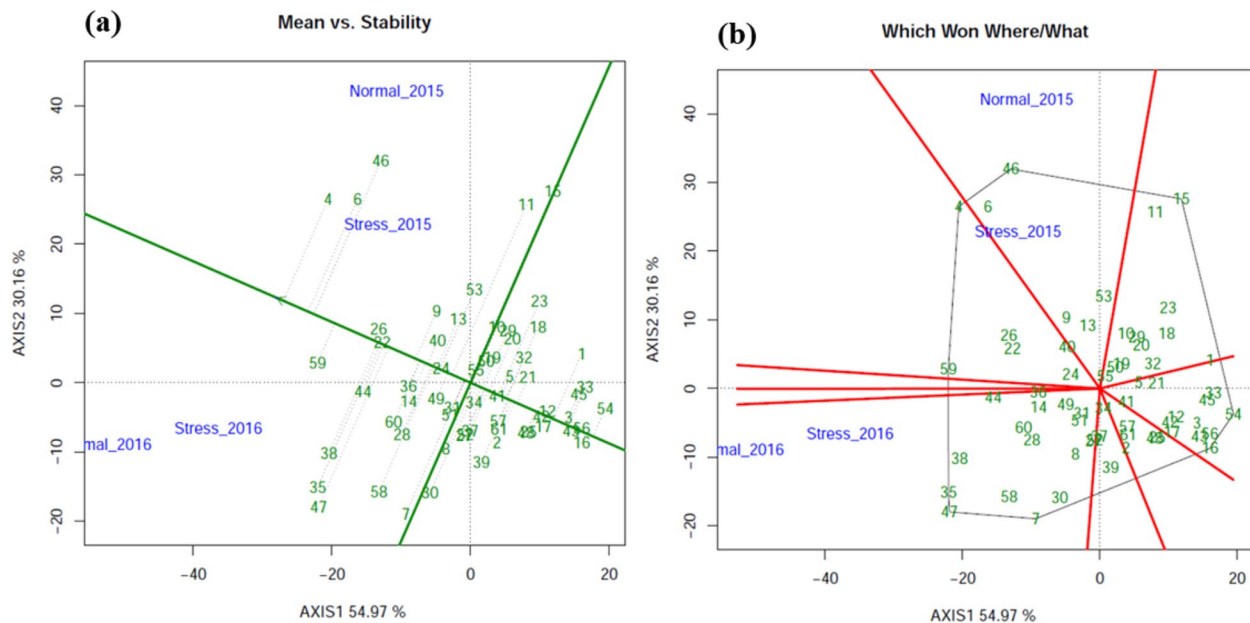


Fig. 2 **a** Average environment coordinate (AEC) views of GGE biplot for ranking of 61 oilseed sunflower genotypes (green dots) based on grain yield mean and stability in four environments (blue text). AXIS 1 and AXIS 1 are the first and second principal components, respectively. The codes of the genotypes are listed in Table 1. **b** Polygon views of the GGE biplot for the "which won where" pattern of 61 oilseed sunflower genotypes (green dots) in four environments (blue text), which helps to visualize the interaction patterns and to determine which genotypes performed better in which environment for grain yield

mean trait and stability, conversely, genotypes 1 and 33 showed the lowest mean trait and least stability. Concerning to the PH trait, genotypes 4 and 59 showed the highest trait mean, while genotypes 54 and 1 were unstable (Supplementary Fig. S4). Overall, genotype 1 showed the lowest mean and was unstable, considering most of the studied traits. Analysis of the 'which-won-where' plot revealed that for the HD trait, genotypes 46 and 6 were at the polygon vertices, and for the PH trait, genotypes 54, 46, and 47 were similarly positioned, aligning with their grain yield performance (Supplementary Fig. S5).

Yield vs. WAASB Biplot

The biplot of yield (Y) vs. WAASB (Fig. 3) provided superior genotype selection simultaneously based on yield grain and stability. In the $Y \times WAASB$ biplot, grain yield is plotted on the horizontal axis (X) and WAASB values on the vertical axis (Y). Drawing vertical lines from the overall average of two variables (yield (Y) and WAASB index) on the x and y axes divides the biplot into four quadrants. The first quadrant includes genotypes 15, 23, 18, 1, 54, and 30, characterized by high WAASB values (implied less stability due to their high contribution to GEI) and low yield. The second quadrant includes genotypes 11, 7, 58, 60, 28, 38, 35, 47, 59, 44, and 46, characterized for high WAASB values (implied less stability due to their high contribution to GEI) but high grain yield. The third quadrant includes genotypes 29, 27, 20, 39, 21, 32, 52, 41, 34, 55, 5, 25, 57, 61, 42, 17, 43, 16, 56, 12, 3, 48,

2, and 10 characterized for low WAASB values (implied high stability due to their low contribution to GEI) but low grain yield. Finally, the fourth quadrant includes genotypes 6, 4, 26, 22, 14, 9, 13, 36, 49, 51, 31, 19, 40, and 24, characterized by low WAASB values (implied high stability due to their low contribution to GEI) and high grain yield. Genotypes 8 and 10, on the border of the third and fourth quadrants, have yields equal to the overall mean and high stability (Fig. 3). In both years, the normal conditions, due to the highest WAASB index, had higher discriminatory power and played a more significant role in the interaction.

Genotype ranking based on stability and yield weights

WAASBY superiority index was calculated to select stable and high-yielding genotypes by 50:50 weighting between mean performance and stability (Fig. 4). Blue and red circles represent higher and lower scores of the WAASBY index, respectively. In calculating WAASBY, different weights were assigned to grain yield and the WAASB index. Genotypes 4, 6, 22, 24, 40, 9, 26, 55, 46, 13, 34, 14, 36, 31, 50, 51, 44, 57, 59, 19, 37, 41, 61, 27, 52, 5, 25, 10, 2, 49, 53, and 42 showed high WAASBY values. Genotypes 59, 44, and 46, while high-yielding, were unstable. In contrast, genotypes 27, 52, 41, 34, 55, 5, 25, 57, 61, 42, and 2 showed lower-than-average yields but were stable. Finally, the remaining genotypes showed high yield and stability. Depending on the weight of the

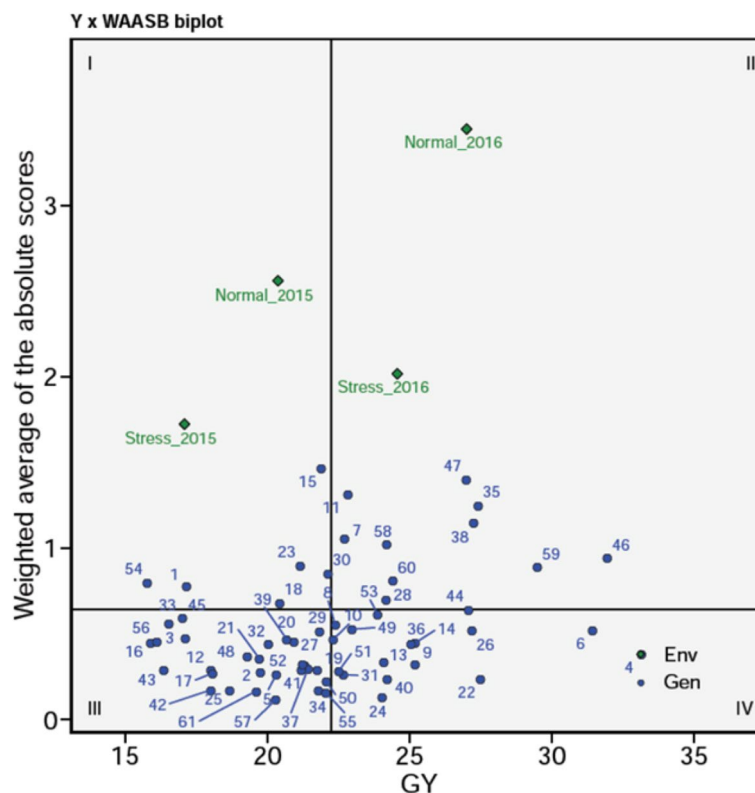


Fig. 3 Grain yield (GY) × WAASB biplot for selection of 61 oilseed sunflower genotypes for grain yield and stability

two variables, stability and mean yield, a comprehensive evaluation of genotype stability can be achieved (Fig. 4b).

Multi-Trait Stability Index (MTSI)

The ranking of sunflower genotypes based on the multi-trait stability index (MTSI) is presented in Fig. 5. Considering all studied traits and with a selection intensity of 10%, nine sunflower genotypes, including 4, 61, 25, 50, 55, 26, 32, 40, and 34 showed the lowest MTSI values; indicating high mean yield and stability among the studied sunflower genotypes.

Discussion

Sunflower is recognized as one of the most important oilseed crops, due to its high adaptability, suitability for agricultural mechanization, and low labor requirements [45]. As an environmental stress, salinity has multiple negative impacts on sunflower plants, notably reductions in yield and yield components. Various studies have reported decreases in seed yield, number of seeds per head, and oil content in saline conditions [17, 24, 26, 39, 40, 67]. Breeding high-yielding cultivars for saline conditions, like other quantitative traits, is affected by GEI. GEI can weaken the association between phenotypic and genotypic values [18]. Two strategies exist to mitigate the $G \times E$ interaction effects: (1) Partitioning diverse environments into smaller and more homogeneous environments

to develop genotypes with specific adaptability to those environments, and (2) Identifying genotypes with general adaptability to diverse environments [22]. In the present study, the yield and stability of 61 oilseed sunflower genotypes were evaluated across non-saline (normal) and saline environments. Analysis of variance revealed significant GEI effects. This means the ranking of sunflower genotypes given studied traits varies depending on environmental conditions. These findings are per the previous studies that reported substantial GEI for seed yield in sunflowers [7, 9, 48, 50, 74, 85]. This highlights the need for comprehensive investigations into the nature and extent of GEI, which cannot be fully addressed using traditional analysis of variance [35]. Indeed, the observed variation in yield data necessitates further analysis to improve the efficiency of genotype selection. In other words, the GEI effect highlights the need to consider genotype adaptability alongside yield in breeding programs [73]. Because of being the environment as the main and primary source of variation in sunflower hybrids [29, 42, 77], $G \times E$ provokes the most significant challenges in sunflower hybrid breeding programs. This interaction results in yield variation among genotypes across different environments, thereby complicating the selection of suitable genotypes. To investigate GEI interaction, various statistical methods have been developed and proposed. While univariate statistics are simple to

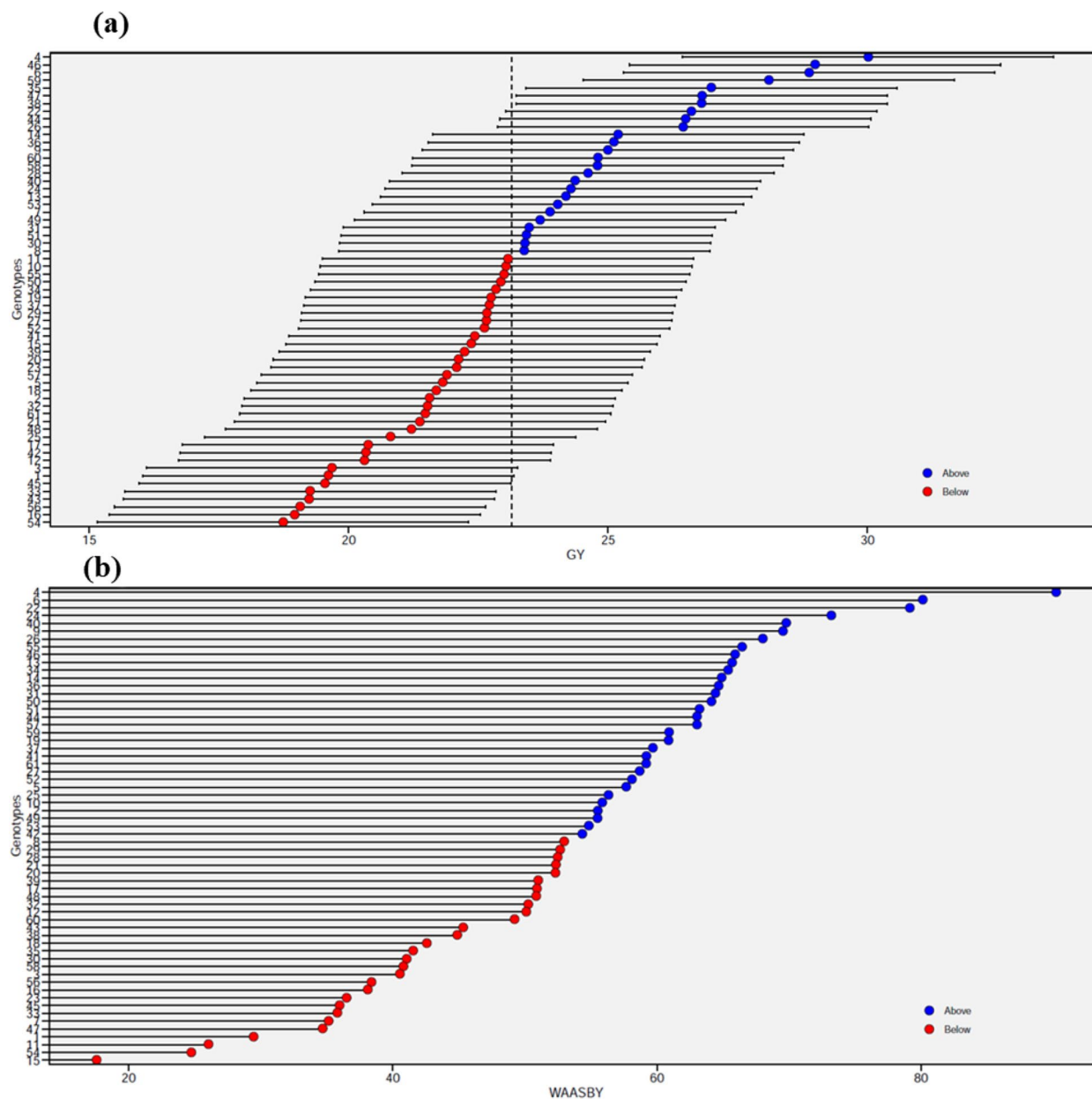


Fig. 4 **a** The best mean linear unbiased prediction values for grain yield in oilseed sunflower genotypes. **b** Estimated values of WAASBY index for (a) grain yield in oilseed sunflower genotypes, considering weights of 50 for yield and 50 for stability.

calculate and can straightforwardly interpret GEI interaction, they cannot reveal the complex patterns in GEI data [6]. Multivariate methods such as AMMI, GGE, and BLUP are introduced as powerful techniques for analyzing and detecting genotype-by-environment interaction patterns. These methods are suitable for the analysis of complex data from multi-environment trials and help researchers to identify stable genotypes. Previous studies have primarily focused on applying the AMMI and GGE methods to investigate GEI in sunflower, for example, the evaluation of sunflower genotypes in six locations because of oil content and fatty acid composition and the

introduction of stable genotypes [80], assessment the stability and adaptability of 12 sunflower genotypes across five regions in Iran in view of seed yield [7], use of AMMI biplot to interpret genotype-environment interaction in 24 sunflower hybrids evaluated in 12 environments [42], use of GGE biplot for introducing stable and high-yielding hybrids in the experiments realized for evaluating 11 new hybrids beside four commercial hybrids in four environments [29], use of WAASB for stability analysis beside the evaluation of disease resistance of active sunflower germplasm against *Plasmopara halstedii* race 100 in four semi-arid locations in India [20]. In this study,

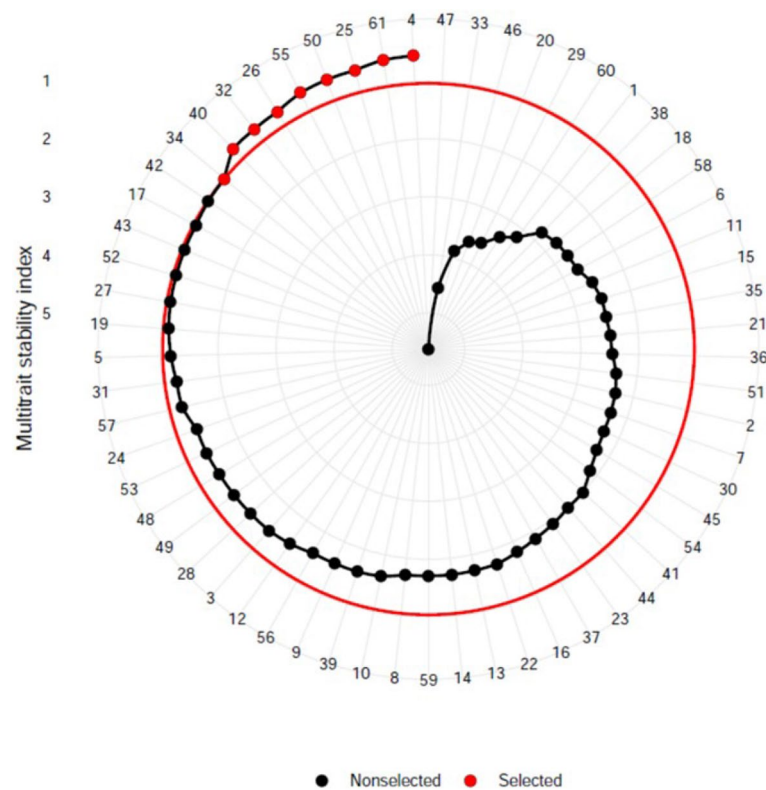


Fig. 5 Ranking 61 oilseed sunflower genotypes based on multi-trait stability index (MTSI) considering 10% selection intensity

we combined these methods with the BLUP method to achieve a more accurate assessment of genotype stability. The novelty of this study resides in the simultaneous application of several uni- and multi-variate methods for a thorough analysis of GEI data, aiming to optimize the selection of stable genotypes. As expected, the various statistical methods elucidated the different aspects of our dataset structure. These findings are consistent with previous reports on various crops [41], [79], [44], [36].

Genotypes 50, 59, 22, 24, 4, 6, 44, 31, 25, and 12 demonstrated high stability across various parametric and non-parametric methods. Genotypes 4, 6, 44, 22, and 59 exhibited high stability and yield. Genotype 4, demonstrating high yield and stability across all studied environments, is a promising candidate for salt-tolerant hybrid development. However, selecting high-yield and stable genotypes using parametric and non-parametric stability models remains challenging. Alternative approaches are required to identify genotypes capable of adaptation to diverse environments while maintaining high performance [79].

Considering the AMMI biplot, which illustrates the complex relationships between genotypes and environments, as well as the AMMI stability index that reflects the genotype's variability across various environments, the genotypes 24, 22, 50, 48, 40, 25, and 55 have been identified as a suitable source for genes associated with

adaptability to a wide range of environmental conditions. Therefore, these genotypes are suitable parents for breeding yield stability. Similarly, the IPCA scores indicate the stability of genotypes, where an IPCA value close to zero reflects high stability. Consequently, genotypes 22 and 24, exhibiting IPCA values near zero, are classified as highly stable. The AMMI2 model effectively identifies high-yielding, stable genotypes and the correlation among environments [47]. In AMMI2 biplots, genotypes distant from the plot's center and near an environment-specific axis are considered environment-specific, typically yielding well in that environment [84].

AMMI2 biplot analysis revealed that certain genotypes exhibited superior adaptability to specific environmental conditions. Therefore, the proximity of genotypes 11 and 15 to the 'normal environment 2015' axis indicates their high yield and superior adaptation to that environment compared to the other three environments. Or being close to the saline environment on the 2015 axis of genotype 46 about yield and plant height, being close to the saline environment on the 2016 axis of genotypes 35, 30, and 58 about yield, genotype 30 about head diameter, and genotype 58 about plant height and yield, or being close to the normal environment on 2016 axis of genotypes 28, 44, 47, 38, 59, and 60 about seed yield and genotypes 44, 47, 59 and 60 about five-grain weight and genotypes 60 and 47 about plant height shows their good performance

and adaptation to these environments. In other words, these findings highlight the superior adaptability of these genotypes to specific environmental conditions and their potential for use in breeding programs to develop cultivars with stable performance across diverse environments. Therefore, it can be concluded that the stability of grain yield can result from the stability of yield components. This finding is consistent with previous studies on other crops [5, 10], determined that environmental conditions predominantly influence seed yield variability in sunflowers, indicating restricted adaptability and a preference for genotypes to excel in particular environments. The significant differences in the relative performance of genotypes across diverse environments attest to the presence of GEI and environmental heterogeneity. Based on mean yield and IPCA1 scores, genotypes with moderate yield exhibited higher stability than those with a high mean yield. This aligns with the findings of [2]. Furthermore, our results suggest that a genotype exhibiting high stability and performance for one trait may not necessarily show the same characteristics for others. This complex phenomenon can be attributed to the control of traits by different genes, variations in gene expression across genotypes, and the influence of variable environmental factors such as temperature and humidity [23]. GGE biplot analysis visually represents the relationships between genotypes and environments, effectively revealing genotype performance and stability [35]. The GGE biplot analysis indicated that genotypes 4, 46, 15, 54, 16, 7, and 47, located at the polygon vertices, exhibited high adaptability to the experimental environments they can be recommended for specific adaptability. In contrast, genotypes 50 and 55, located close to the origin, showed a lesser response to environmental variations and are more suitable for use in breeding programs aimed at developing genotypes with broad adaptability. The ideal experimental environment is characterized by the highest PC1 score, which enhances the ability to detect the main effects of genotypes. The salinity stress environments, in both years, exhibited the highest PC1 scores, indicating a greater capacity to differentiate between genotypes, thus presenting them as ideal settings for evaluating germplasm and identifying salt-resistant genotypes. Genotypes 4, 6, and 46 are identified as promising candidates for normal and salinity stress conditions in 2015 due to their superior performance. Notably, genotype 6 demonstrated remarkable adaptability to the saline environment in 2015. Given these findings, genotypes 4 and 6 are suggested as suitable parents for the development of new hybrids with high yield and wide adaptability across different environmental conditions. Previous studies have also confirmed the high potential of these genotypes for tolerance to environmental stresses, including salinity and phosphorus deficiency. Ahmadpour et al.

[4] identified genotypes 4, 6, 46, and 59 with the highest single-plant yield under both normal and salinity stress conditions. In another study, Darvishzadeh et al. [14] identified genotype 4 as the most drought-tolerant. Furthermore, [68, 69] demonstrated that genotypes 4 and 59 exhibited higher yield and yield components under phosphorus deficiency stress conditions, with less reduction in quantitative and qualitative traits.

As highlighted by Yan [82], yield stability, while important, is not the sole criterion for genotype selection, it must be evaluated in conjunction with high mean yield to ensure optimal performance in breeding programs. The results of this study showed that genotypes 26, 22 and 44 possess a favorable combination of high yield and stability. However, for a more comprehensive selection of ideal genotypes, the Weighted Average of Absolute Scores from the Best Linear Unbiased Prediction (WAASB) model, which simultaneously considers both stability and yield, was used [28, 51, 65]. This method, proposed by Olivoto et al. [60], enables the concurrent selection for stability and yield by balancing the response variable (yield) with the WAASB stability score. Consequently, genotype 4 with a high WAASB index, had the highest grain yield and stability. Additionally, genotypes 6, 22, and 24 demonstrated high mean yields and acceptable stability levels. Genotypes 22 and 24 consistently demonstrated high stability and above-average yield across all statistical analyses, making them promising candidates for breeding programs focused on stable and broadly adaptable cultivars. Moreover, this study employed a MTSI to verify the simultaneous selection of stable genotypes based on GY and three key yield components: HD, FSW, and PH. These traits were selected based on previous findings by Khalifani et al. [46], which demonstrating their significant impact on sunflower grain yield. Based on the stability analysis of these traits, genotypes 4, 61, 25, 50, 55, 26, 32, 40, and 34, originating from various research centers, were identified as stable genotypes. Previous studies have shown that recommending cultivars based on both mean performance and the stability of multiple agronomic traits provides greater accuracy and reliability [61]. Consistent with this, our findings indicated that genotypes 4, 6, 22, and 24, due to combination of their desirable stability and high yield, are promising candidates for breeding programs aimed at developing high-performing, stable sunflower cultivars with broad adaptability. These findings underscore the necessity of multivariate approaches for selecting stable, high-yielding genotypes across various environments, which can enhance our understanding and interpretation of the complexities associated with genotype-by-environment interactions (GEI).

Conclusion

Combining numerical and graphical methods for assessing genotype stability allows for more accurate and reliable recommendations for selecting suitable genotypes for new cultivars. The results of this study indicated that although different stability methods vary in their computational details, they generally yield similar results. For instance, genotype 4 was consistently identified as a stable genotype with desirable performance across most methods and is thus recommended as a suitable parent in future breeding programs. Additionally, our findings indicate that Seed yield stability is closely linked to the stability of its component traits, reflecting the high genetic potential present within sunflower populations for enhancing multiple traits. Furthermore, genotypes selected based on the stability of multiple traits originated from different research institutions, highlighting the broad genetic diversity available for improving sunflower traits. The selected genotypes should be further evaluated under diverse environmental conditions, to validate these findings. Given the results of this study and previous research, genotype 4 is considered a promising candidate with high stability and desirable performance under various stress conditions (salinity, drought, and phosphorus deficiency). This genotype can be used as a parent in breeding programs to develop hybrid cultivars or as a foundation population to create genetic diversity. Overall, the results of this study demonstrate that combining multiple stability assessment methods and considering multiple agronomic traits is a powerful tool for selecting suitable genotypes for sunflower breeding programs. These genotypes can contribute to the development of cultivars with stable performance and broad adaptability. These genotypes can contribute to developing cultivars with stable performance and broad adaptability.

Abbreviations

AMMI	Additive Main Effects and Multiplicative Interaction
BLUP	Best Linear Unbiased Prediction
FSW	100-Seed Weight
GEI	Genotype × Environment Interaction
GGE	Genotype Main Effect plus Genotype × Environment Interaction
GGE	Genotype main effect (G) and genotype by environment interaction (GE)
GY	Grain Yield
HD	Head Diameter
MTSI	Multi-Trait Stability Index
PH	Plant Height
RCBD	Randomized Complete Block Design
IPCA	Principal Component of Interaction
AEC	Average Environment Coordination
WAASB	Weighted Average of Absolute Scores from BLUP
WAASBY	Weighted Average of Absolute Scores from BLUP and Yield

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Authors' contributions

Author contributions: S.K. conducted the experiments, analyzed the data, interpreted the results, and prepared the first draft of the manuscript. R.D. conceived and supervised the study, contributed to experimental design, data interpretation, and manuscript revision. N.A. contributed to data analysis, interpretation of results, and critical revision of the manuscript. R.S.R. contributed to statistical analyses, interpretation of results, and manuscript editing. All authors read and approved the final manuscript.

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Data availability

The data supporting the findings of this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable. This study did not involve human participants, human data, or animals.

Consent for publication

Not applicable.

Competing Interests

The authors declare no competing interests.

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