

Integrated models approach for multi-environmental trial: uncovering Genotype-by-environment interaction and stability investigation of durum wheat (*Triticum durum* Desf.) varieties

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Abstract

Exploring crop performance and response across diverse conditions has become increasingly strenuous due to the combined effects of climate change and genotype-by-environment interaction. In this regard, evidence concerning the extent of genotype $\times$ environment pattern on the grain yield of the released Tunisian durum wheat varieties amid the changing climate is still scarce. This investigation sought to assess the grain yield stability of nine durum wheat varieties using multi-environment trials (13 environments), combining two locations namely Kef and Beja across six and seven cropping seasons, respectively. To this end, a holistic approach integrating the Additive Main Effect and Multiplicative Interaction (AMMI), Weighed Average Absolute Scores of BLUPs (WAASB) and the Genotype + Genotype $\times$ Environment interaction (GGE) was adopted. The combined analysis revealed significant effects of genotypes ($P < 0.01$), environments ($P < 0.001$), and their interaction ($P < 0.001$) on grain yield. Among all sources of variance, environment accounted for the largest proportion of variation (92.08%), followed by the genotype $\times$ environment interaction (6.43%), and genotype (1.48%). Through the investigation of specific interactions, four distinct mega-environments with four winning varieties Dhahbi, INRAT100, Om Rabiaa and Maali were uncovered. The results further identified Salim and Razzak as exhibiting the highest stability. By giving insight into the performance and stability of the released Tunisian varieties, this study lay the groundwork for the development of guidelines and varietal recommendations under varying climate scenarios.

Background

Durum wheat (*Triticum turgidum* L. var. *durum* Desf.), one of the most ancient winter wheat cultivars, is proven to be a staple crop throughout the world [1]. It is grown principally in North America and the Mediterranean Basins where countries such as Italy, Tunisia, Algeria and Turkey are the primary consumers. Various byproducts are commonly derived from durum wheat, including couscous, freekeh, pasta, bulgur and a range of bakery products, as it serves as a principal source of vital dietary components [2]. In Tunisia, Durum wheat, which is predominantly rainfed, exhibited over the years significant yields fluctuations, closely related to the ongoing changes in the weather patterns [3]. Forewarnings mentioned severe yields losses as the acute rise in annuals temperatures, drastic downturn in annuals precipitations and the exhaustion of water supplies have adversely affected durum wheat productivity [4]. In the light of these challenges, breeders have extended extensive efforts to regularly generate high yielding varieties that meet the specific socio-economical requirements [1, 3]. However, although the yield trait features solid genetic foundations, its phenotypic expression is strongly dependent on the combined effect of the environment (E), genotype (G), and the genotype $\times$ environment interaction (GEI), particularly under changing climate conditions [5, 6]. The occurrence of this GEI may weaken the association between the genotypic basis and the phenotypic outcome, hindering the ability of the wheat varieties to sustain high yield performance over several seasons and across different locations [6, 7]. In this regard, accurate quantification and understanding of the GEI is crucial [8]. Hence, the relevance and significance of multi-environments trials, for crop adaptability and stability evaluation [9]. As such, a range of univariate and multivariate statistical tools and models have been put forward for

the multi-environments analysis. Amongst them, the Additive Main Effect and Multiplicative Interaction (AMMI) and Genotype + Genotype $\times$ Environment interaction model (GGE) are two reliable and well-established approaches in GEI assessment [7, 8]. Both these tools are derived from the principal component analysis (PCA) technique. However, the principal distinction between these two models is that while the AMMI allocates the interaction components into separate PCAs, the GGE model entails the joint assessment of genotypes and GEI [7, 10]. These two approaches offer distinct advantages and are often employed in conjunction in multi-environments trials [11]. The main strength of the AMMI model is that it brings together the merit of the principal component analysis (PCA) and ANOVA, through singular value decomposition (SVD), providing a comprehensive approach for the estimation of the main effects and GEI while explaining a substantial proportion of the interaction's sum of squares [11]. Albeit, GGE method stands out due to its additional specifications including the ranking of genotypes in relation to their yield and stability with the "mean vs. stability" illustration, the assessment of the test environments based on their discriminative ability and representativeness with the "discriminating power and representativeness" biplot and the identification of specific adaptation through the "which-won-where" graphic visualization [8, 13, 14]. Uncovering specific adaptation within the tested genotypes and environments is the foremost attribute of the GGE model and is at the outset of the selection process of outstanding genotypes and cultivars. It categorizes the test environments into macro-environments that are homogenous (mega-environments) and subsequently recommends specific genotypes for each of the identified specific mega-environments [7, 13, 14]. Mega-environments are often described as a subset of or a cluster of environments with relatively consistent biotic and abiotic stresses and homogeneous cropping systems, where the GEI patterns are reproducible and consistent [15]. Consequently, these environments foster similar genotype responses and favor the same most suitable cultivars. This feature offers benefits to agronomists and breeders through diminishing the GEI effect and therefore improving selection efficiency, rearranging the environments or ecological regions by the specificity of the genotypes or cultivars, eliminating the non-performing environments and streamlining the handling of time and resources [13, 15]. Complementing the above-discussed approaches, the Best Linear Unbiased Prediction (BLUP) is another MET's alternative analytical method, delivering efficient estimates of genotype mean yields through mixed models [11]. To capitalize on the complementary strengths of AMMI and BLUP, the WAASB index (Weighed Average Absolute Scores of BLUPs) was proposed as a hybrid approach, and the biplot WAASB $\times$ GY offering a comprehensive, two-dimensional representation of both stability and productivity through the inclusion of all IPCAs of the model was established. The WAASB can be considered as a mixed-effects extension of the AMMI-derived stability indices. Nevertheless, its main assets lie in its greater robustness and reduced sensitivity to outliers and its enhanced accuracy in quantifying the stability under complex GEI scenarios [16]. Multi-environments trials analytic methods are constantly evolving to enhance the accuracy of the GEI deciphering. In this context, studies addressing this field often tend to integrate several modeling approaches instead of relying on one single approach [17]. To date, several studies documented the use of these methods to unveil stables or specifically adapted genotypes, uncover ideal environments or/and displays mega-environmental formation patterns in various crops including Barley [8, 13], bread wheat [6, 18], maize [15,

19], rice [9, 20] and Durum Wheat [6, 18, 21, 22]. Within this framework, the objective of this study is to assess the adaptation potential of existing varieties under farmers' conditions by exploring genotype × environment interactions in MET's, particularly under the ongoing climate change. A further aim is to develop adaptation guidelines that support region-specific varietal recommendations and optimize the efficient use of commercial varieties.

Materials and methods

Genetic material and experimental sites

In this experiment, nine durum wheat improved varieties (e.g., 'Karim', 'Razzek', 'Om Rabiaa', 'Khiair', 'Nasr', 'Maali', 'Dhahbi', 'INRAT100' and 'Salim') tracing their origins to the crossbreeding national program, initiated during the 1950s Green Revolution, served as a material for the field (Table 1). Reflecting major strides in agricultural improvement, these varieties were designed to raise productivity for Tunisian farmers, particularly in rainfed environments, by enhancing genetic grain yield potential and fostering widespread use of improved cultivars. Their registration and development history are outlined in Table 1. The tested varieties were assessed for their grain yield across 13 environments under rainfed conditions. Each of the tested environments was defined by the combination of location and planting season. The trial included two locations Kef-Boulifa (semi-arid region) and Beja (sub-humid region) reflective of the main rainfed production areas of durum wheat in Tunisia, with seven cropping seasons (2016–2017, 2017–2018, 2018–2019, 2019–2020, 2021–2022, 2022–2023, and 2023–2024) for the location Beja and six cropping seasons (2016–2017, 2017–2018, 2018–2019, 2020–2021, 2021–2022, and 2023–2024) for Kef. The soil of the experimental field of the region Beja is identified as vertic with 87.53 ppm available N, 80.00 ppm available P, 621.00 ppm available K, 2.10% of organic matter, 2.30% CaCO_3 , and pH = 7.00. While the soil of the trial location Kef / Boulifa is recognized as clay-loamy with 98.00 ppm available N, 16.53 ppm available P, 510.00 ppm available K, 1.41% of organic matter, 25.67% CaCO_3 , and pH = 7.91. The climatic conditions (monthly minimum, maximum, and mean temperature, relative humidity and total rainfall) for the two trial locations throughout the seven cropping seasons were presented in Table S1.

Table 1
List and origin of the selected durum wheat varieties [23–25].

Genotypes	Name	Origin and registration date
G1	Dhahbi	Local cross (INRAT) released in 2018
G2	INRAT100	Cross (INRAT / CIMMYT) released in 2017
G3	Salim	Local cross (INRAT) released in 2009
G4	Maali	Local cross (INRAT) released in 2007
G5	Nasr	Cross (INRAT/ ICARDA) released in 2004
G6	Om Rabiaa	Cross (INRAT/ICARDA) released in 1996
G7	Khlar	Cross (INRAT/CIMMYT) released in 1992
G8	Razzak	Cross (INRAT/ICARDA) released in 1987
G9	Karim	Cross (INRAT/CIMMYT) released in 1980

Field experiment and trial management

The experimental layout followed a randomized complete block design (RCBD) comprising three replications ($n = 3$). Three blocks were partitioned into 27 plots each, totaling 81 plots. Each plot covered an area of 6 m², comprising six rows of 6 m long. A row spacing of 0.2 m and an inter-plot spacing of 0.5 m were adopted. Sowing was carried out in December by the mean of mechanical seeder at a consistent rate of 350 grains m⁻². Fertilization included a basal application of 100 kg ha⁻¹ Di-Ammonium Phosphate at sowing and three equal splits of ammonium nitrate (33.5% N, 100 kg ha⁻¹ each) at early tillering (Z13), stem elongation (Z16), and second node visible (Z32). Weeds were managed through an integrated strategy, combining chemical and mechanical methods.

Table 2. List and characterization of the thirteen test environments

E1				2016-2017
E2	Kef	36°11'10"N ; 84200"E	532	2017-2018
E3				2018-2019
E4				2020-2021
E5				2021-2022
E6				2023-2024
E7				2016-2017
E8	Beja	36°43'N; 912E	161	2017-2018
E9				2018-2019
E10				2019-2020
E11				2021-2022
E12				2022-2023
E13				2023-2024

EnV, test environments.

Statistical Analysis

In this study, all the statistical analyses were executed using R software version 4.5.0 [26]. Grain yield data of the studied durum wheat genotypes across thirteen environments was processed by a combined variance analysis (ANOVA) and the mean performance was visualized using boxplots and heatmap by means of 'ggplot2', 'agricolae', 'dplyr' and metan packages. The AMMI was carried out to estimate the grain yield data for the i th genotype in the k th environment (y_{ik}) using the following model [27]:

$$y_{ik} = \mu + \alpha_i + \tau_k + \sum_{n=1}^p \lambda_n a_{in} t_{kn} + \rho_{ik} + \epsilon_{ik}$$

where μ represents the grand mean, α_i is the additive effects of genotype i , τ_k refers to the mean of the environment k , λ_n is the singular value associated with the n -th interaction principal component axis (IPCA), a_{in} and t_{kn} are the i -th element and the k th element of the n -th eigenvector and ρ_{ik} the residual remaining when not all IPCAs are included in the model. The corresponding AMMI1, AMMI2 and AMMI1 chart to reveal "which-won where" biplots were generated, and thirteen stability parameters derived from the AMMI model were calculated: Sum across environments of GEI modelled by AMMI (AMGE) [28], AMMI Stability Index (ASI) [29], AMMI Stability Value (ASV) [30], AMMI-based stability parameter (ASTAB) [31], Sum across environments of absolute value of GEI modelled by AMMI (AVAMGE) [32], Annicchiarico's D parameter values (DA) [33], Zhang's D Parameter (DZ) [34], Sums of the Averages of the Squared Eigenvector Values (EV) [35], Stability Measure Based on Fitted AMMI Model (FA) [36], Modified AMMI Stability Index (MASI) [37], Modified AMMI Stability Value (MASV) [38], Sums of the

Absolute Value of the IPC Scores (SIPC) [28], and Zobel and Annicchiarico's stability index (Za) [32]. A slopegraph of the genotypic ranking based on these parameters was subsequently illustrated. This model and its derivatives were computed through the 'metan' package. For a more in-depth analysis the grain yield data was furthermore assessed using the Weighted Average of Absolute Scores Stability (WAASB) method, which integrates elements of the AMMI and BLUP models. The WAASB value as introduced by Olivoto et al., [16] was computed using the following equation:

$$WAASB = \frac{\sum_{i=1}^P |IPCA_{in} \times EP_i|}{\sum_{i=1}^P EP_i}$$

Where $IPCA_{in}$ represents the score of the genotype or environment n in the i th interaction principal compound while EP_i symbolizes the amount of the variance explained by the i th IPCA. The WAASBY index, a superiority metric that integrates the weights of both the genotypic grain yield performance and WAASB stability was determined as follows:

$$WAASBY_n = \frac{(rG_n \times \theta_G) + (rW_n \times \theta_W)}{(\theta_G + \theta_W)}$$

Where rG_n and rW_n measure the rescaled grain yield (G_n) and WAASB (W_n) values for the n th genotype, respectively and θ_G and θ_W represents the weights for the grain yield variable and stability. These metrics were determined according to the method described by Olivoto et al., [16], using the 'metan' package and the related biplot (Y x WAASB) and heatmap of ranking of genotype according to the WAASB/GY ratio were constructed accordingly. Similarly, the Genotype plus Genotype x Environment interaction (GGE) method was utilized to delineate mega-environments (MGEs) and identify winning genotypes through the "which-won-where" biplot, to determine genotypes with high average grain yield and stability across environments by the "Mean vs Stability" biplot and to reveal ideals environments combining discriminating power and representativity by the means of "Discriminativeness vs representativeness" and "Ranking Environments" biplots. These biplots were generated using 'metan' package following the model introduced by Yan and Kang., [39] and described by Olivoto and Lúcio.,[40]. Moreover, to capture the genotype responses (GY) across environments with explanatory variables (maximum temperature (°C), minimum temperature (°C), average temperature (°C), relative humidity (%) and precipitation (mm)), the partial least square (PLS) regression model was employed and the PLS biplot offering a comprehensive overview of environments, and climatic factors was generated [41].

Results

Variance analysis and mean grain yield performance

The Genotype (G), Environment (E), and Genotype-by-Environment Interaction (GEI) effects on grain yield (GY) were investigated through the combined analysis of variance. The results revealed highly significant effects of environment ($P < 0.001$), genotype ($P < 0.01$), and the GEI ($P < 0.001$) on grain yield (Table 3).

The environment was the highest contributor to the total variation and accounted for 92.08%. This observed variation is further supported by the disparity of the average grain yield values of the tested environments, which spanned from 339.57 g plot⁻¹ for E4 to 3443.05 g polt⁻¹ for E11. Overall, the environments E11, E10, E9, E7, E8 and E3 showed higher average grain yield values (Fig. 1a, b). The genotypic variance and the GEI effects contributed with 1.48% and 6.43% of the total recorded variance, respectively. The observed notable genotypic heterogeneity is subsequently verified with the difference in the assessed durum wheat varieties average GY performance across the 13 evaluated environments. Among them, G2 (2011.27 g polt⁻¹), G6 (2040.88 g polt⁻¹) and G1 (1982.98 g polt⁻¹), followed by G3 (1911.86 g polt⁻¹) recorded the highest average GY values. Whereas the genotypes G7 (1708.57 g polt⁻¹) and G8 (1676.22 g polt⁻¹) displayed the lowest average GY values.

Table 3
Combined analysis of variance for GY of nine durum wheat varieties across tested environments

Source	df	SS	F value	%(G + E + GEI)
E	12	498100504	302.6157***	92.08
G	8	8035656	2.771324**	1.48
G x E	96	34794882	3.987992 ***	6.43
** significant at ($P < 0.01$); *** significant at ($P < 0.001$); df, degree of freedom; SS, sum of square; F, F value; % respect (E + G + G E) from Sum Sq				

Additionally, the existence of a marked GEI effect suggests a variation in the GY mean values of the genotypes and their ranking across the test environments. In fact, G3, the highest yielding genotype in E6, showed the lowest performance in E12, and G8 the best yielding genotype in E4 recorded the lowest mean GY value in E5 and E8. Overall, the highest average GY (4033.75 g polt⁻¹) was observed for the genotype G6 in environment E11, and the lowest average GY (186.49 g polt⁻¹), was registered by G7 in E4 (Fig. 1b).

Additive Main Effect and Multiplication Interaction (AMMI) model.

The AMMI model depicted the deconstruction of the GEI into significant interaction principal components uncovering the underlying structure of its interaction-related multiplicative variance. Results indicated that the main effects E (92.08%) and G (1.48%) and the GEI (6.43%) were highly significant ($P < 0.001$). Consequently, the GEI effect was subsequently partitioned into five statistically significant interaction principal component axes (IPCA). The five IPCAs collectively explained (95.4%) of the total GEI variability and the IPCA1, IPCA2, IPCA3, IPCA4 and IPCA5 contributed individually with 42.2%, 22.9%, 16.6%, 7.7%, and 6%, respectively. IPCA1 and IPCA2 contributed together 65.1% of the total GEI effect (Table 4).

Table 4

Additive Main effects and Multiplicative Interaction (AMMI) analysis of variance for grain yield of nine durum wheat genotype evaluated across thirteen environments

Source	df	SS	MS	F value	Proportion (%)	Accumulated (%)
E	12	498100503.81	41508375	302.6157***		
G	8	8035656	1004457	11.05202***		
G x E	96	34794882	362446.7	3.987992***		
IPCA1	19	14687461	773024.3	8.51***	42.2	42.2
IPCA2	17	7961805	468341.4	5.15***	22.9	65.1
IPCA3	15	5769014	384600.9	4.23***	16.6	81.7
IPCA4	13	2682415	206339.6	2.27**	7.7	89.4
IPCA5	11	2090376	190034.2	2.09*	6	95.4
Residuals	312	28355969	90884.52			
Total	563	609431338.81	1082471			

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; df, degree of freedom; SS, sum of squares; MS, mean squares; IPCA, Interaction Principal Component.

To illustrate the contribution of the GEI effect, the AMMI1 biplot was constructed by plotting the mean GY of the main additive effects E and G against the interactive principal component score IPCA1 score [42]. The abscissa corresponds to the average GY of E and G, and the ordinate represents the interaction effects [43] (Fig. 2a). Therefore, based on the distribution patterns of the studies environments and genotypes, the environments were clustered into two groups. The first subset comprising E11, E10, E7, E3, E8, E9 appeared on the right side of the grand mean, indicative of higher productivity, with E11 outperforming all others. The second subset included the environments E4, E6, E1, E2, E12, E5 and E13 positioned successively from the left side of the overall mean indicated poorer GY performance. Environment E4 emerged as the lowest yielding. Similarly, the genotypes G6, G2, G1 and G3 mapped in the right direction recorded GY values greater than the overall mean, and genotypes G8, G4, G9, G7 and G5 plotted in the left side of mean axis were underperforming. Additionally, the closer the IPCA1 scores are from zero, the lower the contribution of the GEI and the more stable the genotype across the tested environment [13]. Consequently, in the range of IPCA1 scores, G8 and G3 were considered the most stable genotypes over all the sampled environments.

The AMMI2 biplot was generated by plotting the genotypes and environments against the two first IPCAs (IPCA1 and IPCA2) to simultaneously showcase the effects of environments and genotypes and apprehend the genotypic response across different test environments (Fig. 2b). In this biplot, the length of an environment's vector from its origin indicates the extent of the GEI contributed by that environment

and therefore, reflects its discrimination capacity. A similar inference is applied to the assessment of genotypes, as genotypes with limited IPCAs scores and therefore positioned in the vicinity of the origin of the coordinates are regarded as genotypes with the lowest GEI and the widest adaptability [13, 44]. According to these considerations, E8, E9, E13 and E7, located further away from origin, were considered the most interactive environments and displayed the strongest capacity to discriminate among genotypes. In addition, the genotypes G3, G8 and G9 were identified as the most stable among the tested genotypes.

For a more in-depth analysis, an AMMI “which-won-where” chart of estimates of genotypes nominal GY was generated (Fig. 2c). This plot allows the delineation of mega-environments (MGEs), the identification of genotypes with superior performance in specific environments and the determination of broadly stable genotypes across all environments. The genotypes are represented as linear regressions plotted against the AMMI’s first IPCA scores of the environment. The intercepts correspond to the G, while the slopes represent the scope of the GEI [45]. Consequently, the genotype G6 emerged as the superior performer within the mega-environment MGE1 (E1, E11, E10, E2, E12, E4, E5, E6, E13, E8), while G1 emerged as the winner in MGE2 (E9, E7, E3), as delineated by the IPCA1 scores. Additionally, G3 and G8 attained the highest stability across the tested environments.

AMMI’s stability indexes

Genotype ranking depending on the average GY performance and AMMI-derived stability indexes is illustrated in Fig. 3. The genotypes G6, G2 and G1 held the leading position with respect to the mean GY ranking, while G3 achieved the top rank under ASTAB, ASI, ASV, AVAMGE, DA, FA, MASI, MASV and ZA indices, and the third rank based on DZ, EV, and SIPC. Similarly, G8 was the highest ranking according to EV and DZ and it ranked second under ASTAB, ASI, ASV, AVAMGE, DA, FA, MASI, MASV, SIPC and ZA. Accordingly, these two genotypes were deemed the most stable among the studied set.

The weighted average of absolute scores (WAASB)

For a thorough investigation of the genotypes ranking patterns, the WAASB index was calculated and a biplot plotting the GY values with the WAASB results was constructed (Fig. 4c). The biplot “YxWASSB” grouped the samples into four quadrants [13]. The first quadrant (I) included the genotypes G4, G5 and G9 with GY below the overall average and a high contribution to the total GEI. The second quadrant encompassed the genotypes G2, G1 and G6 which are deemed unstable but highly productive, and the environments E9, E11, E8, E7 and E10 exhibiting good discrimination capacity and outstanding productivity. The third quadrant (III) grouped genotypes G8 and G7 displaying low productivity but broad adaptability and environments E4, E6, E2, E1, E12, E5 and E13 demonstrating low productivity and low discriminating ability. The fourth quadrant (IV) comprising genotype G3 and environment E3, reflects high genotypic performance paired with wide adaptation and high environmental productivity associated with a limited ability to distinguish between genotypes. Overall, genotypes G1, G2, G6 and G3 emerged as the top yielding genotypes, and G7, G8 and G3 as the most consistent across environments. It is

important to highlight that G3 showed a balanced performance in terms of GY and stability. Furthermore, E11 and E10 stood out as the most productive environments, whereas E9 rose to prominence for its superior discriminative capacity.

These results are further corroborated by the outcome of Fig. 4b, which depicts a WAASB/GY ratio heat map grouping and ranking the durum wheat genotypes using the weighted combinations of WAASB index and GY. The right side of the map places greater emphasis on GY performance, while the left side reflects the stability of the genotype. Accordingly, the first cluster (marked in green), which displays conditions conducive to high yield and instability, comprised the genotypes G1 and G2. The second cluster (depicted in red) consisted of genotypes G3 and G6, which exhibited favorable yields performance and high stability for G3, and in contrast moderate stability and high grain yield performance for G6. Genotypes G4, G5 and G9 (blue colored) were characterized by low productivity and limited stability, with G4 showing the greatest instability across environments. The fourth group (G7 and G8), highlighted in black, showed good stability but poor productivity. The genotypic stability rankings, based on the number of IPCAs considered in the WAASB analysis, are displayed in Fig. 4a. It was revealed that the genotype ranking was influenced by the number of IPCAs included in the WAASB calculation. This became apparent from the inclusion of the second IPCA as the stability ranking of G9, G6 and G5 was altered when IPCA2 was accounted for. Interestingly, G4 remained the most unstable genotype across all IPCA calculated, and G7 occupied the third rank irrespective of the number of IPCA's considered. G8 and G3 ranked first and second in the range of IPCA1, but their positions were reversed upon inclusion of the second principal component and persisted across the seven IPCA's

GGE biplot's analysis

The outcome of the GGE biplot's analysis provides an exhaustive overview of genotypic behavior, environmental efficiency and their interaction. In this analysis, PC1 and PC2 explained respectively 41.37% and 29.41% of the total variation and accounted cumulatively for 70.78%. A comprehensive evaluation of the environmental variation through the GGE biplots "discriminativeness vs representativeness" and "Ranking Environments" views was conducted (Fig. 5a, b). The biplot "discriminativeness vs representativeness" define and distinguish environments by their proficiency in reflecting the average target environment (longer vectors) and in their ability to emphasize distinct genotypic responses (acute angle the Average Environment Coordination abscissa (AEC)) [42]. While the "Ranking Environments" biplot rate and classify the test environments with respect to a conceptual ideal environment (situated at the midpoint of the concentric circles) that combine these two features [44]. In this regard, the results showed that E9, E8, E13 and E7 displayed longer vectors and were therefore considered as the most effective in discriminating among genotypes, whereas E6, E5, E4, E1 and E12 with the shortest vector exhibited the least discriminating power. Likewise, it was observed that E6, E5, E7, E13, E8 and E12 presented smaller angles with AEC, revealing a stronger representativeness capacity. Conversely, the environments E9, E3, E4 and E1 were the least representative of the target

environment (Fig. 5a). Additionally, the analysis revealed that the environments E8, E13 and E7 which encompassed both attributes were positioned the closest to the ideal environment (Fig. 5b).

An additional interesting feature of the “discriminativeness vs representativeness” biplot is its concise illustration of the environmental correlations. The association between two environments can be inferred by the estimation of the cosine of the angle between the two vectors that reflects two definite environments [44]. Accordingly, the environments E9-E3, E7-E5, E13-E8-E12, E12-E2, E2-E10-E11, E4-E1 showed positives correlations, while environment E6 located at the origine of the biplot displayed a positive relationship with each test environment (Fig. 5a). The genotypic examination was undertaken using the biplots “Mean vs Stability” which characterize genotypes regarding their GY performance and their stability across the test environments (Fig. 6). Yield performance is portrayed by the positioning of the genotypes on the AEC abscissa, crossing the origin of the axes and marked by an arrow pointing to the positive direction. Genotypes mapped on the positive end of the axis demonstrate a high mean GY and the opposite holds true. Moreover, stability is rendered by the length of the vectors of projection as shorter vectors indicate stable genotypes and longer vectors showcase higher instability [46]. Consequently, it was revealed that G7, G8, G9, G5 and G4 were identified as low yielding genotypes, and among this group G8 was the most stable genotype, succeeded by G7. Conversely G2, G6, G1 followed by G3 were defined as the best yielding genotypes, and among them G3 was recognized as the most stable genotype. Overall G8 and G3 exhibited the greatest stability, while G4 and G1 displayed high inconsistency. Furthermore, to uncover specific adaptive responses, the GGE biplot “which-won-where” was generated (Fig. 7). Results showed that all environments were divided into four main sectors, which embodies four distinct MGEs delineated as follows: MGE1 encompassed E9 and E3, MGE2, comprising E5 and E7, MGE3, including E8, E13, E11, E10, E2 and E12 and MGE4, grouping E1 and E4.

The genotypes G1, G2, G6 and G4 located at the vertices of the four sectors were more responsive to their respective sectors MGE1, MGE2, MGE3 and MGE4. On the other hand, G3 and G9 positioned closer to the origin of the biplot, were deemed less adapted to their respective MGE. The genotypes G8, G7 and G5, grouped in a sector devoid of any of the test environments, were considered low yielding genotypes across all environments.

Associations between environmental performance and climatic factors

The correlation analysis between mean GY and climatic variables and the factorial regression model were used to determine the most significant explanatory climatic variables that are impacting GY performance among the thirteen test environments.

The correlation analysis between mean GY and climatic variables, maximum temperature (Tmax), minimum temperature (Tmin), average temperatures (Tavg), relative humidity (RH) and rainfall (Precip) of the thirteen test environments is portrayed in Fig. 8. Results revealed that GY was positively associated with precipitation ($r = 0.75$, $p < 0.01$) and relative humidity ($r = 0.75$, $p < 0.01$). Maximum, minimum and

average temperature showed weak and non-significant correlations with GY, suggesting a marginal contribution to GY variation in this study. Partial Least Squares regression with kernel PLS fitting and cross-validation identified two components as optimal for explaining the variation in GY. These components minimized prediction error (RMSEP = 770.4) while explaining 63.83% of GY variability and 98.45% of climatic predictor variability. Among the predictors, precipitation was the most influential variable (VIP = 2.07), followed by relative humidity (VIP = 0.79), indicating that water availability and atmospheric moisture were key determinants of GY variation. While Tmax, Tmin, and Tavg with VIP scores < 0.3, contributed only marginally to this model (Table S3).

The PLS biplot illustrating the association between HY and climatic variables, is shown in Fig. 9. The explanatory variables relative humidity (RH) and rainfall (Precip) exhibited the longest vectors from the origine of the biplot indicating that these variables are the main explanatory factors of the variation in GY. Whereas Tmax, Tmin and Tavg with shorter vectors positioned near the center of the plot, were clustered closely together and pointing to the opposite direction of RH and precipitation, suggesting a tradeoff pattern between temperature and moisture related, and showcasing the minor role of temperature-related factor in explaining the variability among environments compared to water-related factors.

Results further showed that E7, E8 and E9 were positioned in the direction of the precipitation and relative humidity vectors in the biplot, indicating that these environments benefited from higher water availability and atmospheric moisture. Among them E9 benefited the most from both factors. The environment E10 was located closely aligned with RH vector, indicating that this environment experienced the highest relatively high humidity. While E3 and E11 were situated closer the Precip vector suggesting good precipitation conditions. The environments E13 and E12 were oriented to some extent in the direction of Precip vector and aligned with Tmax and Tavg vectors indicating that although characterized by adequate rainfall conditions they underwent high temperatures. Conversely, the environments E4, E2, E1, E5 and E6 were arranged in the opposite direction of the main explanatory variables (Precip and RH), reflecting drier conditions. Their position, distant from the origin, suggests that their conditions deviated substantially from the mean climatic pattern, but in a direction opposite to the factors that contributed positively to yield performance. To conclude, the two-component PLS model effectively distinguished environments based on a moisture–temperature gradient, with precipitation and RH explaining most of the response variability, and highlighted substantial climatic contrasts among environments, particularly between hot–dry and cool–humid sites.

Discussion

The ongoing climate change represents a critical challenge for wheat production globally, necessitating the adoption of resilient varieties and adaptive management practices to ensure sustainable yield under increasingly unpredictable environmental conditions. Durum wheat grown in Tunisian rainfed conditions often faces Mediterranean type climate with erratic and insufficient precipitation, high temperatures and annual climate variability [5]. Against the backdrop of limited scientific evidence addressing the specific

and broad adaptability of the entire set of Tunisian durum wheat commercial varieties released by the national program, multi-environment trials are valuable for evaluating the varieties performance and stability through the quantification and elucidation of the complexity of GEI [20]. In the present study, significant effects of environment, genotype and GEI on GY variation were uncovered through the ANOVA and AMMI analysis. Similar results were reported [6, 47, 48], for the wheat crop. Overall, the environment emerged as the main source of variation, and this result can be explained by the presence of a substantial variability among the studied environments. In fact, the present trial covered two locations representatives of two different bioclimatic zones (semi-arid and sub-humid) featuring distinct climatic and edaphic conditions and extended over six to seven cropping seasons in each location. In relation to this, Lee et al., [20] suggested that environmental variability can be achieved by strengthening the location effect, such as geographic separation and contrasts between climate zones. While Reckling et al., [49] outlined that strengthening the temporal effect driven by year-to-year variation in weather and management conditions should be considered. Despite being less pronounced than the environmental effects, the significant genotypic variability highlights the presence of inherent genetic differences among the investigated durum wheat varieties. The genotype effect observed may be attributed to the genetic background of the nine investigated varieties. And within this study, some of the tested varieties exhibited closely related genetic makeup and other genetically distinct lineages. These results align with those of Lee et al., [20], which mentioned that genetic variability can be generally achieved through crosses between inter-subspecific cultivars or accessions with diverse genetic backgrounds. Moreover, the relative contribution of the GEI (6.43%) to the GY variability, suggests the presence of a disparate genotypic performance across the test environments [50], the existence of various mega-environments hosting different leading varieties, and further justify the need for a stability and adaptability analysis for GEI interpretation [51]. Consequently, in the current study, many statistical models and tools such as AMMI, WAASB and GGE were used to assess and quantify the magnitude of GEI effect. In the AMMI model, the GEI effect was further divided into five significant IPCAs, shedding more light on the complexity and magnitude of the GEI in our study [13]. The first two IPCAs (IPCA1 and IPCA2) explained 42.2% and 22.9% of the GEI effect, respectively, and captured together about 65.1% of the GEI effect. This outcome represents more than half of the variation, reflecting their effectiveness in cross validating the GY variation explained by the GEI in the given trial [44]. Indeed, Wodebo et al., [52] supported the notion that with an IPCA1 accounting for a greater sum of squares than the genotypic main effect, the model is considered to have adequately explained the entire GEI component. Similarly, Gauch et al., [53] showed that when the first two interaction IPCAs contribute to a large proportion of the variation in GY, they can effectively be used to assess genotypic performance. Within this work, the AMMI1 and AMMI2 biplot were applied to study the performance of the genotypes, environments and their interactions. The AMMI1 associates the variability of genotypes with environmental effect, enabling the identification of high yielding and stable genotypes and favorable environments. While the AMMI2 captures the GEI effect by investigating the contribution of IPCA1 and IPCA2 components to the explained variation, thus providing a general insight into the response of genotypes within each test environment. Overall, in the AMMI's biplots, the main effects with near-zero IPCA scores reflect limited interactions and greater stability [42]. Hence, among the tested genotypes, G6, G2, G1 and G3 delivered superior yield

performances, while genotypes G8, G4, G9, G7 and G5 showed limited productivity. Interestingly, whereas the AMMI1 model identified G8 and G3 as the most stable genotypes, over all the sampled environments, the AMMI2 biplot, included G9 as a genotype with consistent performance. The observed difference in the outcome between the two biplots could be attributed to the number of IPCAs considered, as the AMMI1 relies on the first IPCA only for stability analysis while the AMMI2 includes the first two IPCAs. As reported by Sharifi et al., [54], considering the number of IPCAs factored into the analysis, the ranking of some genotypes may or may not vary. In view of the fact that the AMMI model does not inherently provide a quantitative stability metric, needed for the assessment of genotypes ranking [7], twelve stability indices derived from the model were computed and investigated alongside the average grain yield of each genotype across all environments. Similarly, G6, G2 and G1 held the leading position with respect to the mean GY ranking, while G3 and G8 stood out for their stability. To provide stronger evidence for the assessment of genotypic stability and productivity, the WASSB index was incorporated in the course of our study. The main advantage of the WAASB lies in its ability to incorporate all IPCA components which allows for the inclusion of GEI patterns overlooked in IPCA1 when ranking genotypes [55]. The “YxWAASB” biplot and “WASSB/GY ratio” heatmap categorized genotypes according to their WAASB values and average performances. The corresponding results were in line with those of the AMMI model with the exception for G7 and G9. The genotype G9, considered stable under the AMMI model, was not classified as stable by the WAASB model. While, G7 showed the opposite pattern, being stable in the WAASB model and not in the AMMI’s. A subsequent decomposition of the IPCA’s revealed that the genotype ranking was influenced by the number of IPCAs included in the calculation of the model, further strengthening our earlier inference. To reach a realistic conclusion in the selecting of high-yielding and stable genotypes, an additional breakdown of the GEI through the GGE biplot was carried out. The key strength of the GGE model resides in its capacity to efficiently filter the environmental effect noise and highlight the genotype and GEI effects, thus allowing a more robust and consistent assessment of genotype stability and productivity [56]. In the present study, the GGE model through PC1 and PC2 captured 5.7% greater GY variability compared to AMMI model, further demonstrating its reliability in the evaluation of the test genotypes yield potential and consistency [13]. The GGE analysis corroborated our earlier findings, providing congruent results with the AMMI model. Similarly, G2 (INRAT100), G6 (Om Rabiaa), G1 (Dhahbi) followed by G3 (Salim) were defined as the best yielding genotypes. These results are in line with previous reports, on the Tunisian durum wheat varieties, demonstrating the superiority of Dhahbi, INRAT100, Salim and Om Rabiaa in term of GY potential [3, 57]. Otherwise, our study emphasized the greater stability of G8 (Razzak) and G3 (Salim), as opposed to the other genotypes. Indeed, as documented by Ayed et al., [58] Salim is acknowledged as drought-tolerant variety, owing to its dense foliage and sustained stay-green feature. Similarly, a study conducted by Slama et al., [59] highlighted the superiority of Razzak variety performance under various water conditions. These results suggests that the adoption of these two varieties alongside the recommended management package could offer a promising strategy for climate change adaptation in Tunisian farms [60]. Overall, our findings underscore G3 (Salim) as a promising variety that offers both high yield potential and resilience to climate change.

In general, the occurrence of a significant GEI in multi-environment trials highlights the importance of an accurate characterization of the test environments. Accordingly, rigorous assessment of the test environments is warranted to ascertain their performance, discriminative power, representativeness, mutual associations, and redundancy [46]. Within this framework, the present study centered on a detailed examination of the test environments. According to the “AMMI1” and “YxWAASB” biplot’s, the environments E11, E10, E7, E3, E8 and E9 demonstrated the highest productivity, while E4, E6, E1, E2, E12, E5, and E13 emerged as low yielding. The correlation matrix relating climatic factors to GY in all test environments, along with the Partial Least Squares biplot, revealed that the relative humidity and rainfall were the key determinants of the GY variation. In line with these results, the environments E11, E10, E7, E3, and E9 benefited from adequate rainfall ranging between 377.8 mm and 532.4 mm. In contrast, environments E4, E6, E1, E2 and E5 experienced water with rainfall amounts (198.4–290 mm) markedly lower than the average durum wheat water requirements in Tunisia (381–443 mm) [61]. However, it is worth noting that E11, the highest-yielding environment, did not record the highest water and relative humidity levels. In the same way, E4, which recorded the lowest yield, did not experience the least amount of rainfall and relative humidity. This may suggest the presence of additional factors contributing to GY variability. Interestingly, with E3 as the exception, due to its high-water input (532.4 mm), all high-yielding environments were situated in Beja, while the low-yielding environments E4, E6, E1, E2 and E5 were in Kef. This may highlight the importance of soil conditions and management practices on GY. According to Adham et al., [62], GY variability is shaped in large part by the soil type and its characteristics. Otherwise, the discriminative ability of an environment reflects its effectiveness in selecting superior genotypes, thus allowing the identification of environments where performance rankings are more reliable and where potential trade-offs may arise [63]. In this respect, the environments E8, E9, E13 and E7 consistently emerged as the most discriminatory environments across the AMMI, WAASB and GGE models. Nonetheless, Nesa et al., [46] contend that environments must achieve a balance of discrimination and representativeness to enable relevant evaluation of genotypes. From this perspective, the GGE biplot “discriminativeness vs representativeness” and “Ranking Environments” views, offers a twofold measure, illustrating both features [63]. Accordingly, E8, followed by E13 and E7 which combined both attributes and featured favorable moisture conditions (rainfall and relative humidity), were in our case deemed ideal, and therefore appropriate targets for testing the GY of varieties [13]. Similar results were obtained by Adham et al., [62], which highlighted the role of favorable climatic conditions in defining the ideals environments. A noteworthy benefit of the “discriminativeness vs representativeness” biplot is the evaluation of the correlations among the test environments as within associated environments, the similarity in the climatic patterns, limit their capacity to discriminate among genotypes [52]. Consequently, this analysis sheds light on the reliability of the tests environments and provides information for efficient planning of future sites, in terms of both time and cost [11]. In our study, environmental clustering can be attributed to shared characteristics, comparable rainfall amounts (E9-E3), common location (E13-E8-E12), or a mix of contributing factors (E2, E10, E11).

Additionally, environmental clustering has revealed distinct patterns indicative of MEGs formation for AMMI’s “which-won-where” and GGE “which-won-where” biplot. This discrepancy in the results between

the two graphical approaches may stem from their reliance on different metrics for mega-environments delineation. As referenced earlier, the AMMI chart uses IPCA1 scores reflecting GEI, whereas the GGE biplot relies on PC1 and PC2 scores, capturing the G + GEI effects [45]. It became apparent from these results that irrespective of the approach taken, the genotype G1 (Dhahbi) showed an enhanced response in favorable conditions. Otherwise, the variety G4 (Maali) proved to have specific adaptation to marginal environments. Convergent findings were reported by Ayed et al., [5].

Conclusion

The current investigation depicted the relevance of AMMI and GGE biplot approaches to study stability and adaptability through exploring GEI patterns. From the perspective of genotypes stability and productivity, the results obtained from the three methods were consistent. However, the apparent differences emerged between the implemented approaches during the examination of specific interactions and the stratification of the environment. This variation in the output can be partly attributed to differences in their underlying assumptions and model accuracy. As the GGE biplot was deemed more reliable for capturing specific interactions, its results were adopted in the current research. These two statistical methods complement each other by addressing the limitations of individual analysis models. Consequently, their combined integration can improve the precision and dependability of the results, establishing a robust theoretical framework for evaluating and selecting durum varieties with superior and stable grain yield outcome.

Abbreviations

GY: Grain Yield

GGE: Genotype + Genotype $\times$ Environment interaction

AMMI: Additive Main Effects and Multiplicative Interaction

WASSB: Weighed Average Absolute Scores of BLUPs

METs : Multi-environments trials

GEI : Genotype $\times$ Environment Interaction,

PCA: Principal Component Analysis

BLUP: Best Linear Unbiased Prediction

IPCA: Interaction Principal Compounds

AMGE: Sum across environments of GEI modelled by AMMI

ASI: AMMI Stability Index

ASV: AMMI Stability Value

ASTAB: AMMI-based stability parameter

AVAMGE: Sum across environments of absolute value of GEI modelled by AMMI

DA: Annicchiarico's D parameter values

DZ: Zhang's D Parameter

EV: Squared Eigenvector Values

FA: Fitted AMMI Model

MASI: Modified AMMI Stability Index

MASV: Modified AMMI Stability Value

SIPC: Sums of the Absolute Value of the IPC Scores

ZA: Zobel and Annicchiarico's stability index

MGEs : Mega-environments

AEC : Average Environment Coordination Abscissa

SS: Sum of Square

PLS: Partial Least Squares regression

RMSEP: Root Mean Square Error of Prediction

VIP: Variable Importance in Projection

Declarations

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Author Contributions

Conceptualization, S.A., C.A., and M.A; methodology, S.A. and C.A.; software, C.A.; validation, S.A. and C.A.; formal analysis, C.A.; investigation, S.A. and C.A.; resources, S.A.; data curation, S.A. and C.A; writing original draft preparation, C.A., K.B., and M.A; writing, review and editing, S.A., C.A. and K.B., H.M;

visualization, S.A., C.A. and K.B.; supervision, S.A. and H.M; project administration, S.A. and C.A.; funding acquisition, S.A. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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Figures

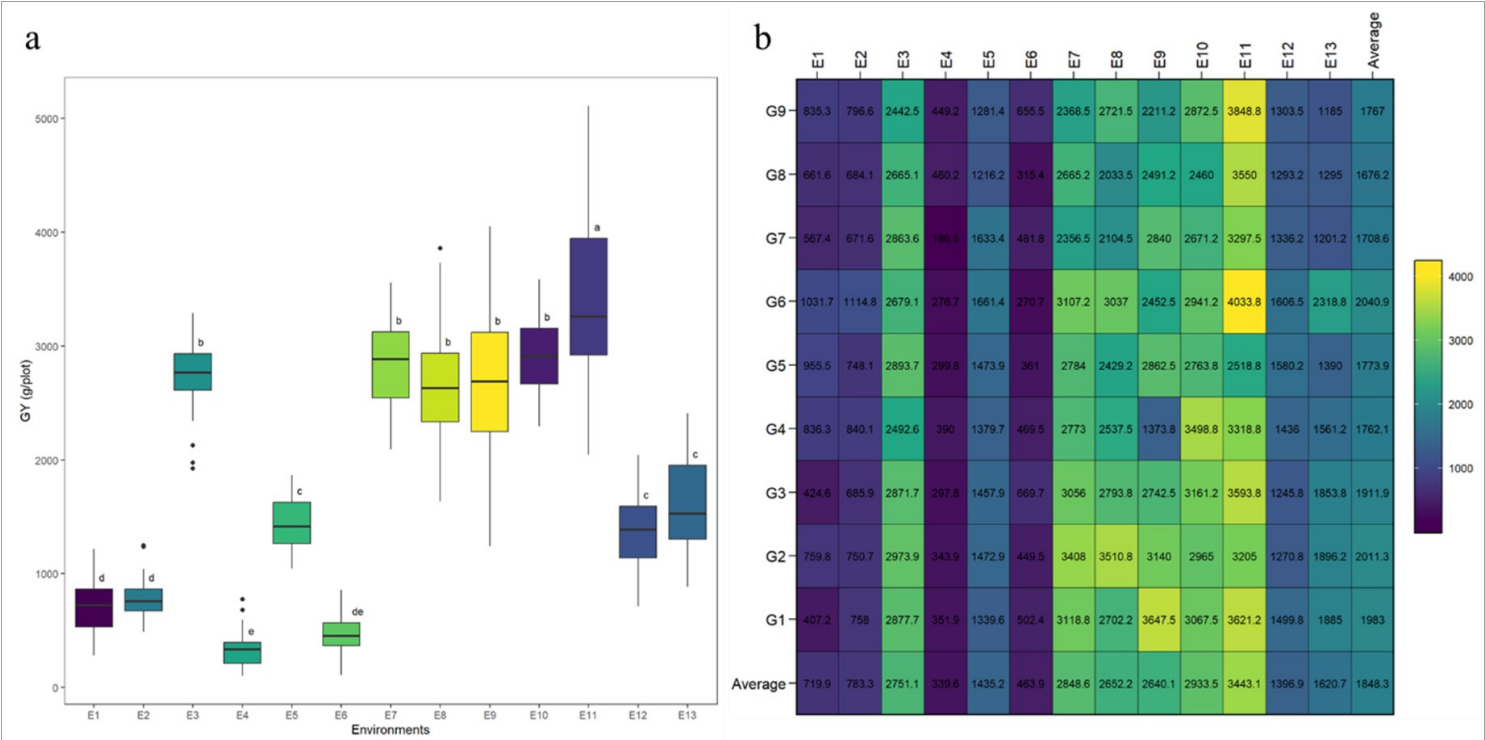


Figure 1

Performance of nine durum wheat genotypes under multi-environment trial. (a) Box plots showing the variation in GY among thirteen environments; Different lowercase letters convey significant variation based on the Tukey HSD test for post hoc comparison at $p \leq 0.05$. (b) Heatmap revealing the performance of 9 investigated genotypes of durum wheat across the tested environments based on the mean values of GY data.

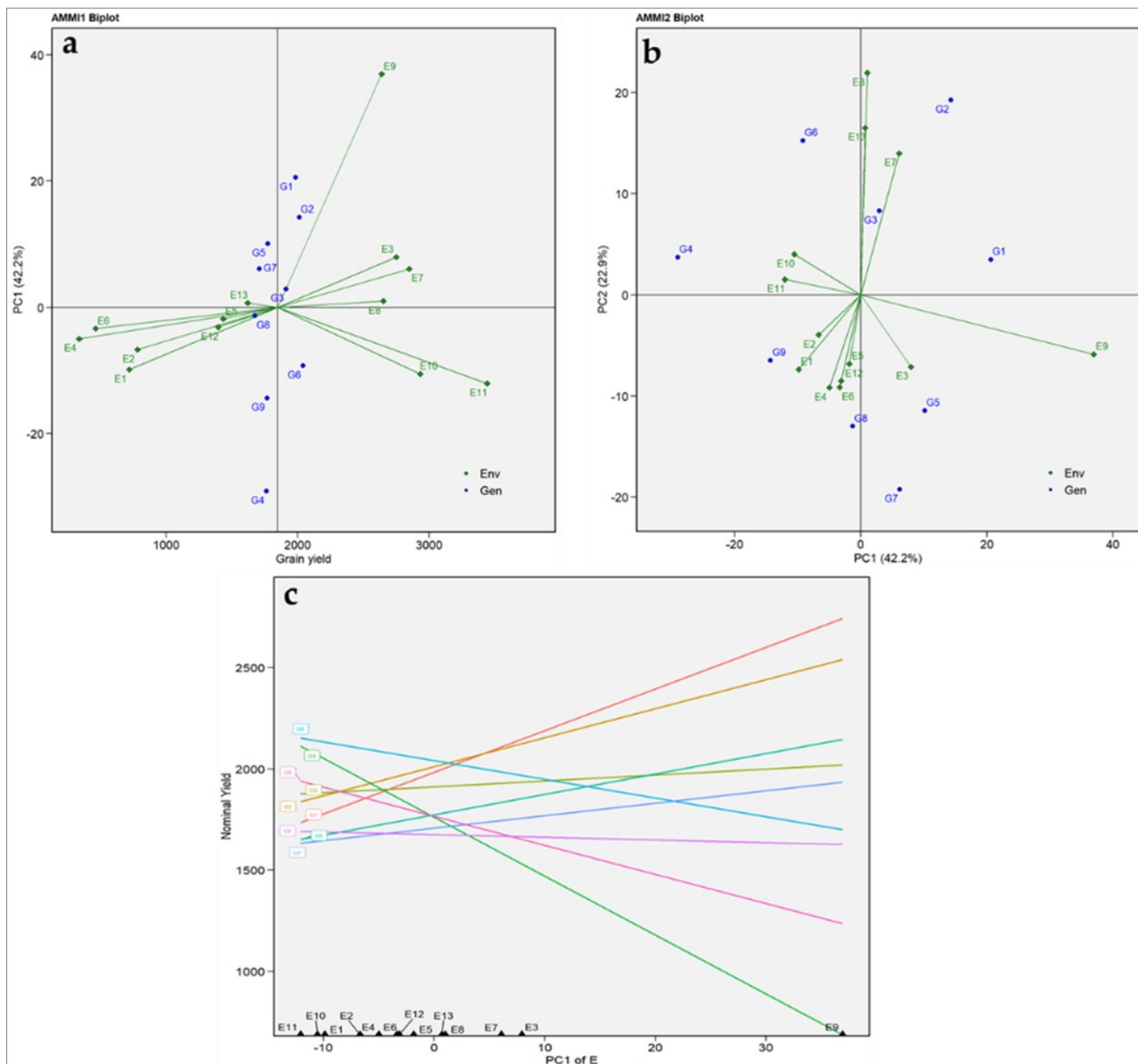


Figure 2

The AMMI1 (a) and AMMI2 (b) biplots displaying the GEI for the nine durum wheat genotypes across thirteen test environments. Nominal GY (c) of nine durum wheat genotypes plotted against the PC1 scores of tested environments

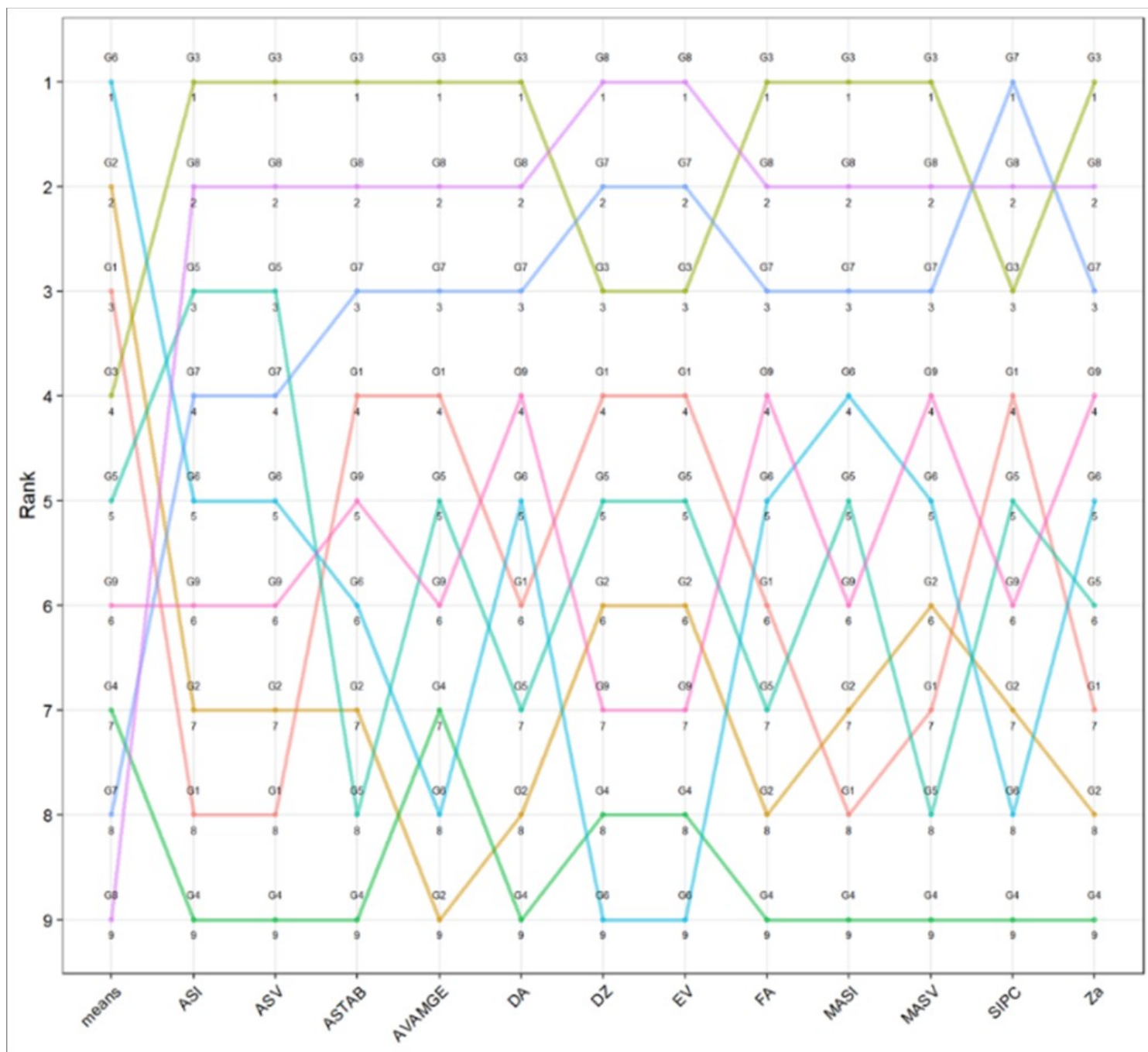


Figure 3

Ranking of nine durum wheat genotypes according to mean GY performance and AMMI stability indexes (ASI, ASV, ASTAB, AVAMGE, DA, DZ, EV, FA, MASI, MASV, SIPC and Za)

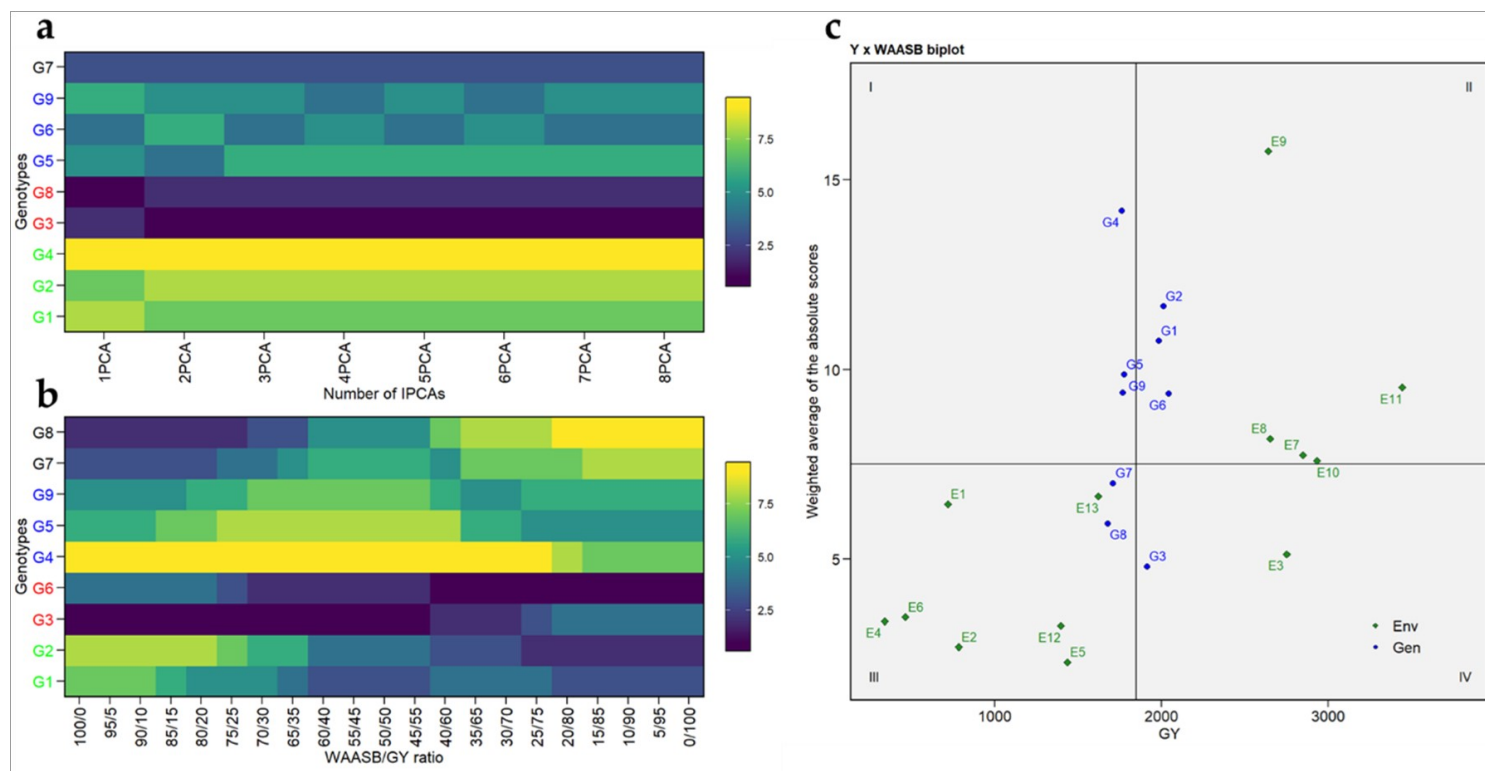


Figure 4

(a) Heatmap showing the ranking of nine durum wheat genotypes based on the number of IPCAs incorporated in the WAASB estimation, (b) Heatmap illustrating the rank of the wheat genotypes according to the different weights for the GY and stability (WAASB/GY ratio), (c) A biplot "YxWAASB" plotting the GY of nine wheat genotypes against WAASB index.

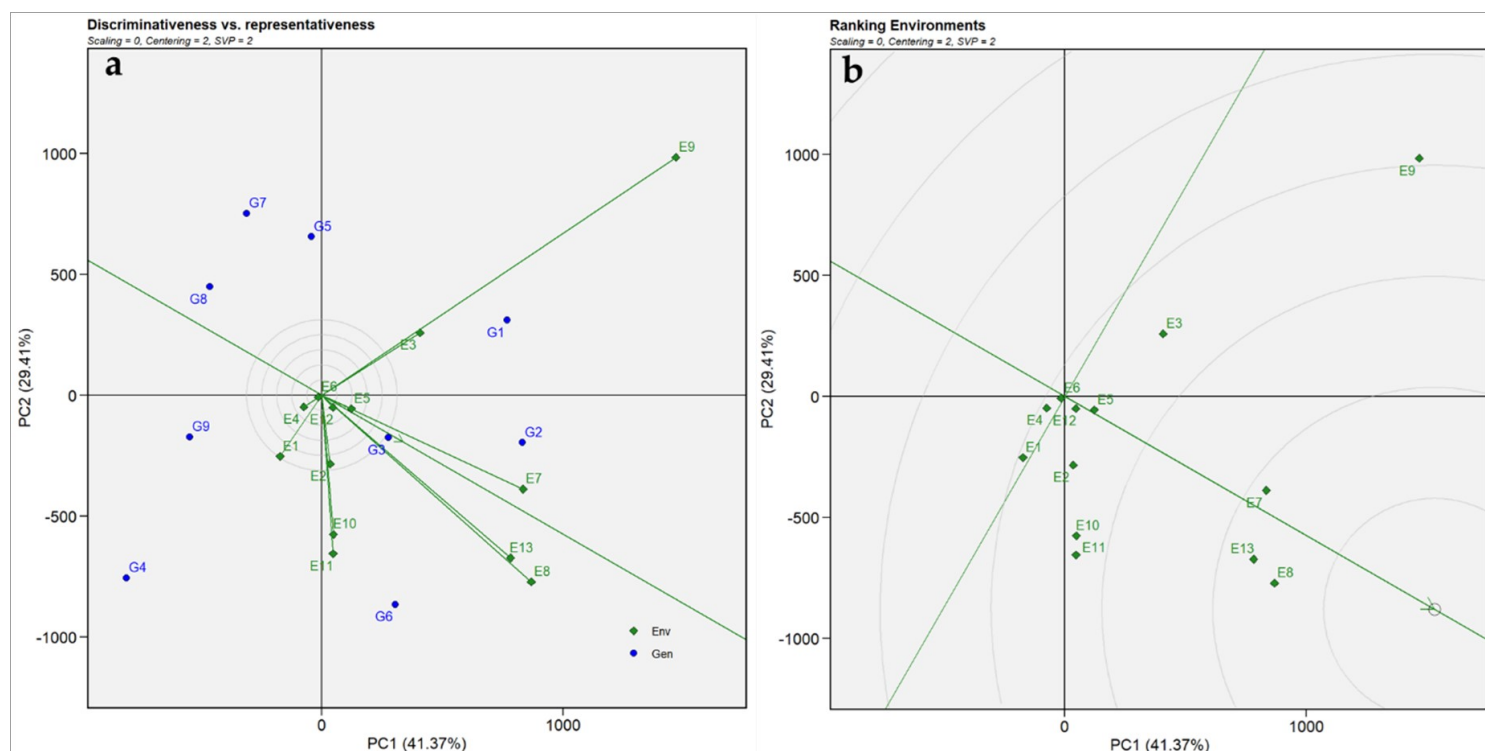


Figure 5

A GGE biplot analysis of GY of thirteen test environments. (a) A view of the representativeness and discriminating ability; (b) a view of the environments ranking in respect to an ideal environment

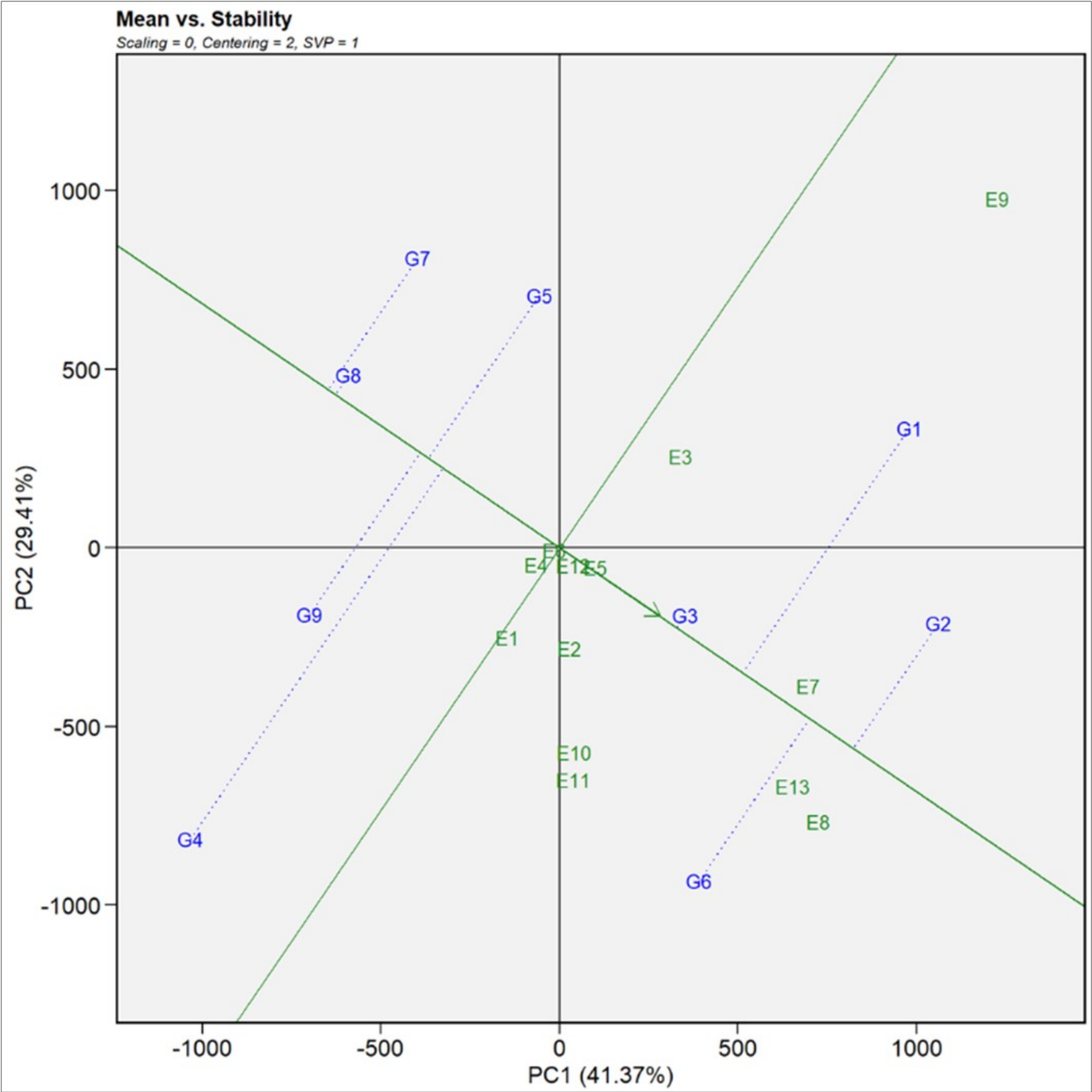


Figure 6

GGE biplot analysis of mean GY performance and stability of nine genotypes across thirteen environments

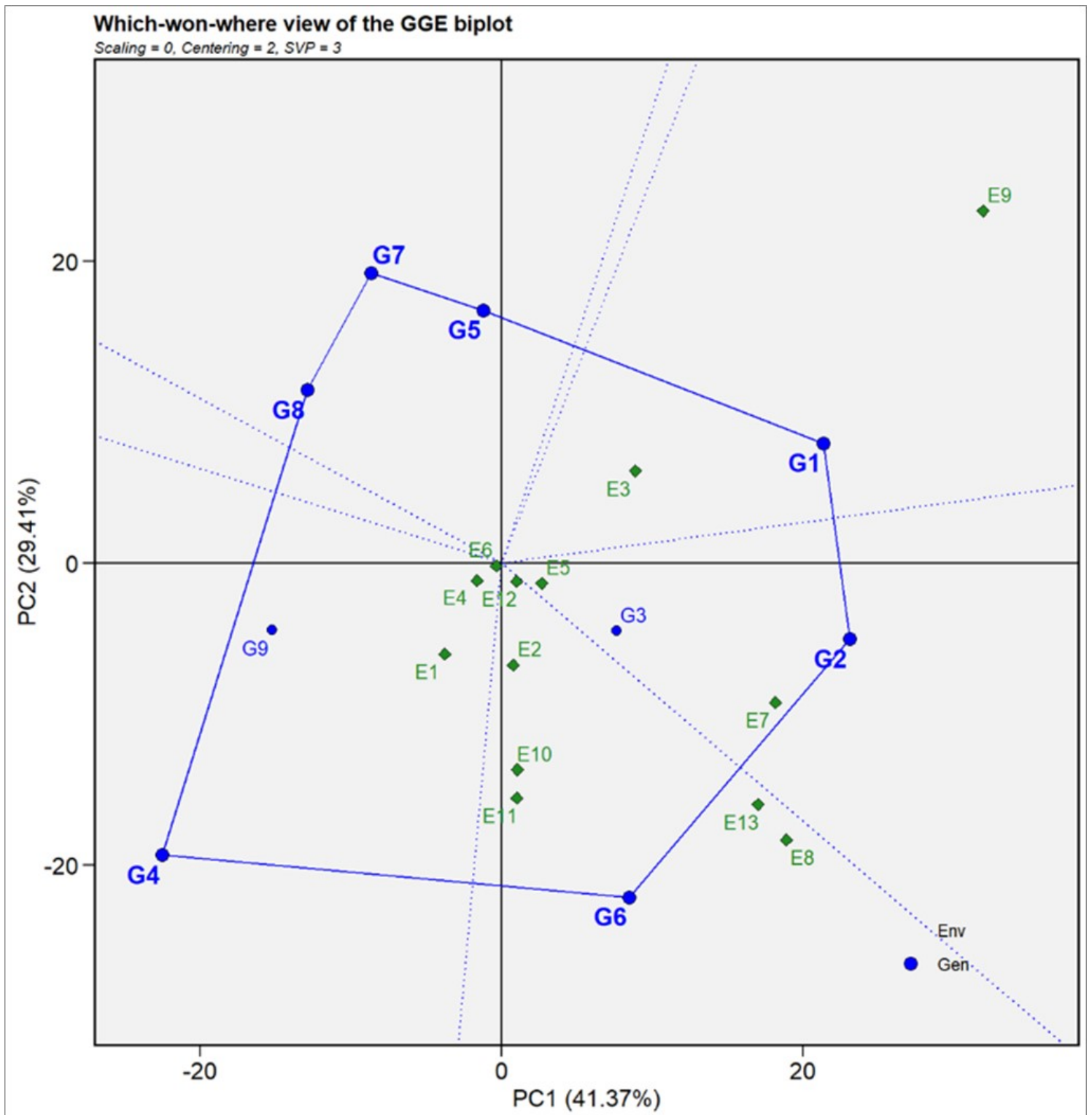


Figure 7

GGE biplot “which won and where” pattern analysis for GY

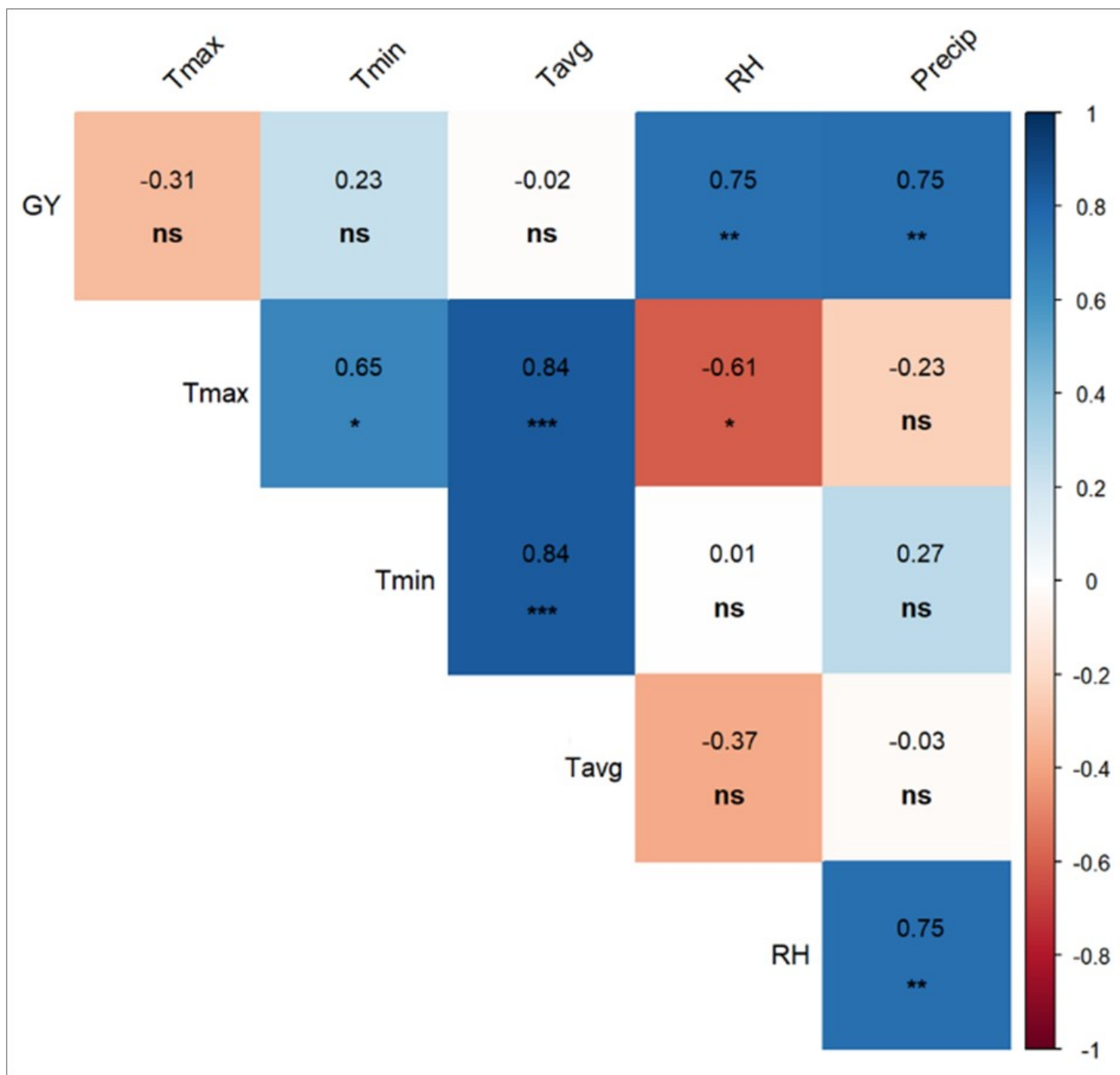


Figure 8

Heat map depicting the correlation coefficients between different climatic factors and mean GY of the test environments * significant at $P < 0.05$ and ** significant at $P < 0.01$

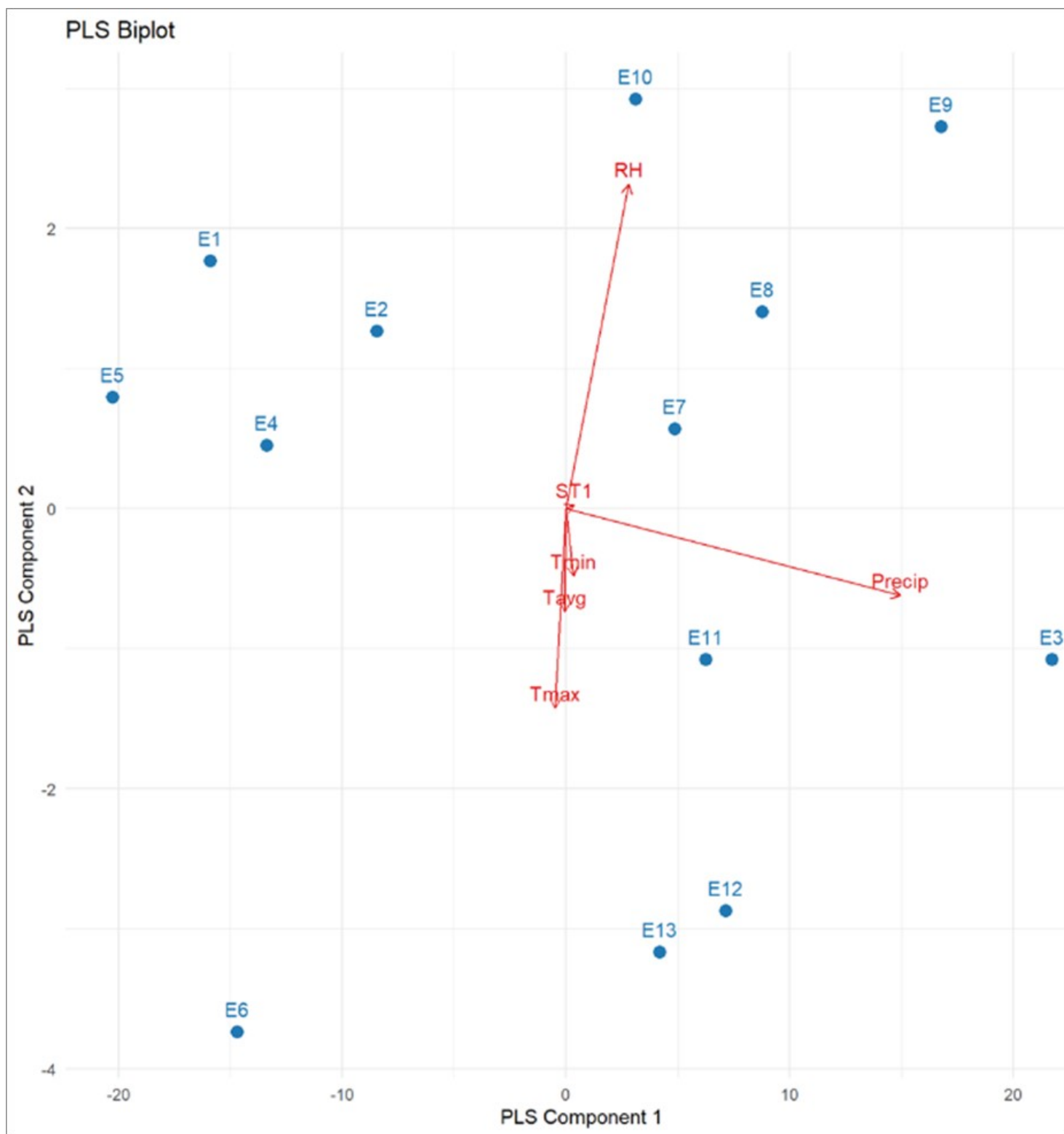


Figure 9

Partial least square (PLS) biplot representing the X-scores of nine durum wheat genotypes and the Y-loadings of thirteen test environments.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterials1.docx](#)