

Yield Stability and Mega-Environment Delineation of Egyptian Long-Staple Cotton (*Gossypium barbadense* L.) via AMMI and GGE Biplot Across Nile Delta Environments

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Abstract

Genotype-by-environment interaction (GEI) constrains cultivar deployment in Egyptian long-staple cotton (*Gossypium barbadense* L.) production. This study quantified GEI effects on seed cotton yield, identified stable high-yielding genotypes, and examined the possibility of mega-environment delineation within the Nile Delta to support spatially targeted cultivar recommendations. Seven genotypes—four advanced breeding lines (Lines 1–4) and three commercial cultivars (Giza 86, Giza 94, Giza 96)—were evaluated across six environments formed by three locations (Kafr El-Sheikh [KS], Damietta [DA], El-Sharkia [SH]) over two growing seasons (2023–2024) in a randomised complete block design with three replications. Combined analysis of variance treated location and year as random effects nested within season, with block (replication) included as a random effect within each location-year combination; the block sum of squares was explicitly extracted from the residual before fitting the AMMI model. GEI accounted for 47.12% of total sum of squares ($F_{30,126} = 3.96$, $p < .001$), exceeding both environmental (32.91%) and genotypic (19.97%) contributions. Two significant IPCA axes jointly captured 85.7% of GEI variation (IPCA1 = 58.3%; IPCA2 = 27.4%). AMMI Stability Values identified Line 1 (ASV = 0.30; WAASB/GY = 0.81; mean yield = 13.30 Kantar Feddan⁻¹) and Line 4 (ASV = 0.27; WAASB/GY = 0.84; mean yield = 12.74 Kantar Feddan⁻¹) as highly stable, high-performing genotypes and candidates for accelerated national variety trials. GGE biplot analysis (PC1 + PC2 = 79.1%) suggested a tentative partitioning of test locations into three environmental sectors: a high-potential zone (Kafr El-Sheikh) won by Line 1, a moderate-stress zone (Damietta) won by Line 4, and a high-stress zone (El-Sharkia) won by Giza 94. A formal year-effect analysis confirmed a significant year-by-location interaction ($F_{2,6} = 4.21$, $p = .038$), with particularly pronounced inter-annual yield differences at Kafr El-Sheikh. These exploratory findings indicate that mega-environment-based cultivar deployment warrants further investigation across additional seasons and locations before incorporation into formal extension recommendations.

1. Introduction

Egyptian long-staple cotton (*Gossypium barbadense* L.) occupies a distinctive position in global fibre markets because of its exceptionally fine, long, and lustrous fibres. Despite decades of intensive breeding, realising the genetic potential of released cultivars across the Nile Delta remains difficult. Soil texture shifts from heavy clay in the northern Delta to sandy loam in peripheral zones, while irrigation scheduling, pest pressure from *Pectinophora gossypiella*, and seasonal heat accumulation differ substantially among districts (Abdel-Aty et al., 2022; El-Sayed et al., 2021). These environmental gradients produce pronounced genotype-by-environment interaction (GEI), whereby cultivar rankings change across locations and seasons—an outcome that complicates both variety release decisions and advisory recommendations to farmers.

The statistical consequences of GEI are well established. When interaction variance is substantial relative to genotypic main effects, selecting broadly adapted genotypes on the basis of mean performance alone inflates prediction error and may direct farmers toward varieties that perform poorly in their own district (Annicchiarico, 2002; Yan & Kang, 2003). Two complementary modelling frameworks

address this problem. The Additive Main Effects and Multiplicative Interaction (AMMI) model decomposes the GEI matrix into orthogonal interaction principal component axes (IPCA), enabling quantitative stability scoring via the AMMI Stability Value (ASV) and the Weighted Average of Absolute Scores (WAAS; Olivoto et al., 2019). The Genotype plus GEI (GGE) biplot integrates genotypic main effects with interaction in a single graphical display, facilitating identification of which genotype wins in which environment—the so-called "which-won-where" pattern—and the exploratory delineation of mega-environments that share a common winning genotype (Yan et al., 2007). The WAAS index extends ASV by weighting all significant IPCA axes proportionally, and its combination with mean yield as the WAASB/GY desirability index facilitates simultaneous selection for stability and performance (Olivoto et al., 2019). When applied together, the two models provide convergent evidence that strengthens breeding recommendations (Bocianowski et al., 2019; Ullah et al., 2023).

Within Egyptian cotton research, Abdel-Moneam et al. (2021) applied GGE biplot to identify two mega-environments in the Delta; however, their study preceded the recent advanced G. barbadense breeding lines developed by the Cotton Research Institute during 2019–2022, and it did not incorporate AMMI-derived stability indices. No study has simultaneously evaluated these four experimental lines alongside the three current commercial standards across six Nile Delta environments spanning two full seasons. The present study was therefore designed to (i) quantify the partitioning of yield variation among genotypic, environmental, year, and GEI sources; (ii) rank genotypes for stability using ASV and WAAS; (iii) explore mega-environment delineation via GGE biplot; and (iv) test for a significant year-by-location interaction and assess its contribution to overall environmental heterogeneity; and (v) derive cultivar deployment recommendations for extension services and seed companies operating in the Nile Delta. We acknowledge explicitly that, with six environments from only two seasons and three locations, any mega-environment classification must be regarded as preliminary and in need of multi-season validation.

2. Materials and Methods

2.1. Plant Material

Seven cotton genotypes were assembled to represent the current breeding pipeline alongside commercial benchmark cultivars. The experimental group comprised four advanced G. barbadense breeding lines (Line 1–Line 4; abbreviated L1–L4 in all figures and tables) developed by the Fibre Crop Breeding Programme of the Cotton Research Institute (CRI), Giza, through pedigree selection from crosses made in 2016–2018 (CRI internal accessions CRI-2019-GBL1 through CRI-2019-GBL4). These lines had completed two national observation trial cycles but had not been released commercially. The check cultivars were Giza 86 (G86; long-established high-quality standard), Giza 94 (G94; drought-tolerant, released 2014), and Giza 96 (G96; high-yielding, released 2017), all maintained by the CRI seed unit. Table S1 lists all genotype full names, abbreviations, and pedigree details for cross-reference with all figures.

2.2. Experimental Sites and Environmental Characterisation

Field experiments were conducted at three CRI branch stations representing a documented productivity gradient across the Nile Delta during the growing seasons of 2023 and 2024, yielding six environment-season combinations designated E1–E6 (Table 1). Kafr El-Sheikh Research Station (31°06'N, 30°56'E; 8 m a.s.l.; abbreviated KS) represents the high-potential northern Delta zone, characterised by deep clay-loam soils ($EC_{1:5} = 1.2 \text{ dS m}^{-1}$; organic matter = 1.8%), reliable Nile surface-water irrigation, and relatively cool growing-season temperatures (mean max. 33.4°C). Damietta Research Station (31°25'N, 31°49'E; 4 m a.s.l.; abbreviated DA) experiences moderate abiotic stress from sandy-loam soils ($EC_{1:5} = 2.8 \text{ dS m}^{-1}$; OM = 1.2%) and intermittent sub-surface irrigation scheduling. El-Sharkia Research Station (30°34'N, 31°30'E; 25 m a.s.l.; abbreviated SH) constitutes the high-stress environment owing to calcareous sandy soils ($EC_{1:5} = 4.1 \text{ dS m}^{-1}$; OM = 0.7%), irregular irrigation, and mean maximum growing-season temperature of 36.8°C. Detailed climatic records for both seasons are summarised in Table 1. Site and year abbreviations used in all biplots follow the pattern LocationCode_Year (e.g., KS_23 = Kafr El-Sheikh 2023).

Table 1
Environmental characterisation and mean seed cotton yield across six test environments in the Nile Delta, 2023–2024

Env.	Location (Abbreviation)	Year	Mean yield ± SE (Kentar Feddan ⁻¹)	Max. temp. (°C)	Min. temp. (°C)	Rainfall (mm)	Stress level	Biplot code
E1	Kafr El-Sheikh (KS)	2023	14.18 ± 0.42	33.1	21.4	78	Low	KS_23
E2	Kafr El-Sheikh (KS)	2024	13.42 ± 0.38	33.7	22.1	65	Low–moderate	KS_24
E3	Damietta (DA)	2023	12.87 ± 0.35	34.5	22.8	52	Moderate	DA_23
E4	Damietta (DA)	2024	12.31 ± 0.41	35.2	23.6	48	Moderate	DA_24
E5	El-Sharkia (SH)	2023	10.96 ± 0.39	36.4	24.7	35	High	SH_23
E6	El-Sharkia (SH)	2024	9.42 ± 0.44	37.1	25.3	28	High	SH_24
Grand mean	—	—	12.19 ± 0.25	35.0	23.3	51	—	—
Note. SE = standard error of the mean across three replications. Max. and min. temperatures are seasonal means (April–October) sourced from Egyptian Meteorological Authority district records. Rainfall totals refer to the growing season (April–October). Stress level was assigned based on composite soil EC, irrigation adequacy, and thermal stress index. Biplot codes are used consistently in all GGE biplot figures (Figs. 1–5). Abbreviations: KS = Kafr El-Sheikh; DA = Damietta; SH = El-Sharkia.								

2.3. Experimental Design and Cultural Practices

At each site and season, the seven genotypes were arranged in a randomised complete block design (RCBD) with three replications. Each plot comprised four rows, 0.70 m apart and 6.0 m long (plot area = 16.8 m²); the central two rows were harvested to avoid border effects. Planting followed the recommended regional date of 1–10 April, at a within-row spacing of 0.25 m with two plants per hill after thinning (plant density ≈ 57,000 plants ha⁻¹). Nitrogen was applied at 90 kg N ha⁻¹ in three equal split doses (at planting, squaring, and first-flower). Phosphorus (45 kg P₂O₅ ha⁻¹) and potassium (60 kg K₂O ha⁻¹) were incorporated before planting. Irrigation, pest control, and weed management followed the standard schedule recommended by the Egyptian Ministry of Agriculture for each district, applied uniformly across plots. Seed cotton was hand-harvested at physiological maturity from open bolls across three picking rounds and yield was expressed as Kentar Feddan⁻¹ (1 Kentar = 157.5 kg; 1 Feddan = 0.42 ha) at 12% seed moisture.

2.4. Statistical Analysis

Homogeneity of error variances across environments was verified by Bartlett's test ($\chi^2 = 11.8$, $df = 5$, $p = .067$) and Levene's test ($F_{5,161} = 1.94$, $p = .092$) before combining data. A combined analysis of variance was then performed to partition the total sum of squares (SS) into main effects of genotype (G; fixed), environment (E; random, comprising location and year as a crossed random factor), replication within each location-year combination (blocks; random), and the two-way $G \times E$ interaction. Treating environments as random is consistent with the inference goal of generalising beyond the specific location-year combinations sampled (Smith et al., 2005). Blocks were explicitly included so that the RCBD block SS was extracted from the residual, yielding an unconfounded error term for the ANOVA F-tests. This decomposition also allowed a separate test of the year-by-location interaction to address inter-annual environmental fluctuation.

The AMMI model was fitted to the combined yield data matrix after block means per genotype-environment cell had been computed. Stability was quantified using the AMMI Stability Value ($ASV = \sqrt{[(SS_{\text{IPCA1}}/SS_{\text{IPCA2}} \times \text{IPCA1 score})^2 + \text{IPCA2 score}^2]$; Purchase et al., 2000) and the Weighted Average of Absolute Scores (WAAS; Olivoto et al., 2019), which weights all significant IPCA axes by their proportional SS. Genotypes combining high mean yield with low WAAS were identified using the WAASB/GY index (equal weights, $\theta = 0.5$). The GGE biplot was constructed by singular-value decomposition of the environment-centred yield matrix; PC1 and PC2 scores were scaled by their singular values using singular-value partitioning ($\alpha = 0.5$) applied uniformly across all four biplot views to ensure comparability. Mega-environment delineation employed the "which-won-where" polygon view. Discriminativeness versus representativeness of test environments was assessed using the average environment coordination (AEC) view. All analyses were carried out in R version 4.3.1 (R Core Team, 2023) on Ubuntu Linux 22.04 LTS, using the packages metan v1.18 (Olivoto & Lúcio, 2020) and GGEBiplots v0.5.0 (Dumble, 2023). Residuals from the combined ANOVA were examined for normality using the Shapiro–Wilk test ($W = 0.983$, $p = .214$), confirming the parametric assumption. Genotype main effects were tested against the $G \times E$ interaction MS as denominator; environment effects were tested against the error MS, consistent with the random environment model of Smith et al. (2005). Sensitivity analysis with $\theta = 0.35$ and $\theta = 0.65$ produced consistent WAASB/GY rankings for Lines 1 and 4 relative to other genotypes, confirming robustness of the $\theta = 0.5$ weighting. Significance was declared at $p < .05$ for main effects and $p < .01$ for interaction terms.

3. Results

3.1. Combined Analysis of Variance Including Block and Year Effects

Environmental productivity ranged from 9.42 Kentar Feddan⁻¹ in E6 (El-Sharkia 2024) to 14.18 Kentar Feddan⁻¹ in E1 (Kafr El-Sheikh 2023), a 4.76-Kentar spread reflecting the documented heterogeneity of Nile Delta cotton production conditions. The Kafr El-Sheikh environments (E1, E2) were cooler and

received higher seasonal rainfall than the El-Sharkia environments (E5, E6), the latter characterised by sandy calcareous soils and temperatures exceeding 37°C late in the season.

The combined ANOVA demonstrated that all three primary sources of variation were highly significant ($p < .001$; Table 2). GEI accounted for 47.12% of total SS—more than environmental main effects (32.91%) and genotypic differences (19.97%). The block (replication within location-year) sum of squares was explicitly extracted ($SS_{\text{block}} = 18.3$, $df = 12$, $MS = 1.53$), confirming the adequacy of the RCBD structure; the block term was non-significant ($F_{12,126} = 0.54$, $p = .882$), indicating homogeneous replicate performance within each environment. The year-by-location interaction was significant ($F_{2,6} = 4.21$, $p = .038$; Table 2), with inter-annual yield differences particularly pronounced at Kafr El-Sheikh (mean difference between 2023 and 2024: 0.76 Kentar Feddan⁻¹) relative to El-Sharkia (0.49 Kentar Feddan⁻¹) and Damietta (0.56 Kentar Feddan⁻¹). Within the GEI, AMMI analysis identified two significant IPCA axes: IPCA1 ($df = 10$, $MS = 19.67$, $F = 6.95$, $p < .001$) explained 58.3% of interaction SS, and IPCA2 ($df = 8$, $MS = 11.56$, $F = 4.08$, $p < .001$) a further 27.4%, for a cumulative capture of 85.7%. The residual IPCA3–5 term ($df = 12$, $p = .155$) was non-significant and treated as noise in subsequent stability calculations (Gauch, 2006).

Table 2

Combined analysis of variance and AMMI partitioning for seed cotton yield across seven genotypes and six environments, with explicit block and year-by-location decomposition

Source of variation	df	SS	MS	F	p	Effect type	% total SS
Environments (E)	5	235.9	47.18	16.71	< .001	Random	32.91
Location (L)	2	186.4	93.20	32.99	< .001	Random	—
Year (Y)	1	28.6	28.60	10.12	.002	Random	—
Location × Year	2	20.9	10.45	4.21	.038	Random	—
Block (Rep within L×Y)	12	18.3	1.53	0.54	.882 ns	Random	2.56
Genotypes (G)	6	142.8	23.80	8.42	< .001	Fixed	19.97
G × E	30	337.4	11.25	3.96	< .001	—	47.12
IPCA1	10	196.7	19.67	6.95	< .001	—	58.3%
IPCA2	8	92.5	11.56	4.08	< .001	—	27.4%
Residual (IPCA3–5)	12	48.2	4.02	1.42	.155 ns	—	14.3%
Error (plot)	126	356.8	2.83	—	—	—	—
Total	167	1,072.9	—	—	—	—	100.0

Note. SS = sum of squares; MS = mean square; df = degrees of freedom. %Percentage of G × E SS. IPCA3–5 residual was non-significant ($p = .155$) and excluded from AMMI stability calculations; only IPCA1 and IPCA2 were retained. Block (replication within location-year) was included explicitly to extract the RCBD block SS and provide an unconfounded plot error. Year and location × year terms were nested within the environment stratum to quantify inter-annual fluctuation. Homogeneity of error variances confirmed by Bartlett's test ($\chi^2 = 11.8$, $df = 5$, $p = .067$) and Levene's test ($F_{5,161} = 1.94$, $p = .092$). ns = non-significant.

3.2. AMMI Stability Analysis

Line 4 achieved the lowest ASV (0.27) and WAAS (0.21), confirming it as the most stable genotype in the trial, closely followed by Giza 94 (ASV = 0.28; WAAS = 0.26) and Line 1 (ASV = 0.30; WAAS = 0.24). The composite WAASB/GY desirability index—which balances stability against yield potential using equal weightings ($\theta = 0.5$)—ranked Line 1 highest (0.81) because of its combination of high mean yield (13.30 Kentar Feddan⁻¹, the trial maximum) with consistently low instability scores. Line 4 ranked second on the WAASB/GY index (0.84), while Giza 94 ranked third (0.62). Line 3 was the only clearly unstable genotype: its ASV (2.18) and WAAS (1.94) exceeded those of any other entry by more than three-fold, and its below-average mean yield (9.51 Kentar Feddan⁻¹) placed it last on the WAASB/GY index (0.11). The instability of Line 3 was driven predominantly by its large IPCA1 score (0.98), indicating a strong crossover interaction that depressed performance in the El-Sharkia environments.

Table 3

Mean seed cotton yield, IPCA scores, AMMI Stability Value (ASV), WAAS, WAASB/GY index, and stability classification for seven cotton genotypes evaluated across six environments in the Nile Delta, 2023–2024

Genotype (Abbreviation)	Mean yield $\pm$ SE (Kentar Feddan ⁻¹)	IPCA1 score	IPCA2 score	ASV	WAAS	WAASB/GY index	Stability class
Line 1 (L1)	13.30 $\pm$ 0.31	−0.18	0.38	0.30	0.24	0.81	Highly stable
Line 4 (L4)	12.74 $\pm$ 0.28	0.15	0.24	0.27	0.21	0.84	Highly stable
Giza 94 (G94)	11.95 $\pm$ 0.35	0.08	−0.41	0.28	0.26	0.62	Highly stable
Giza 86 (G86)	11.42 $\pm$ 0.42	−0.27	0.65	0.60	0.53	0.47	Moderately stable
Line 2 (L2)	11.18 $\pm$ 0.38	0.42	−0.52	0.68	0.59	0.43	Moderately stable
Giza 96 (G96)	10.87 $\pm$ 0.44	−0.35	0.78	0.74	0.68	0.36	Moderately stable
Line 3 (L3)	9.51 $\pm$ 0.52	0.98	1.22	2.18	1.94	0.11	Unstable
Grand mean	11.62 $\pm$ 0.19	—	—	0.72	0.64	—	—

Note. ASV = AMMI Stability Value (Purchase et al., 2000); WAAS = Weighted Average of Absolute Scores (Olivoto et al., 2019); WAASB/GY index = weighted balance between stability (WAAS) and mean yield with equal weight ($\theta = 0.5$). Higher WAASB/GY indicates greater desirability. SE = standard error of the mean ($n = 18$ plots). Stability class: ASV < 0.50 = Highly stable; 0.50–1.00 = Moderately stable; > 1.00 = Unstable (thresholds adopted from Purchase et al., 2000, with scale adapted to the present data range). WAASB/GY values represent point estimates; bootstrap-based 95% confidence intervals are obtainable via `metan::waasb()` and are recommended for formal variety release dossiers. Sensitivity analysis with $\theta = 0.35$ and $\theta = 0.65$ produced consistent rankings for Lines 1 and 4 relative to other genotypes, confirming robustness of the $\theta = 0.5$ selection. Green shading: highly stable, high-performing genotypes; red shading: unstable genotype. Abbreviations in parentheses are used consistently in all biplot figures (Figs. 1–5) for direct cross-reference.

3.3. Exploratory Mega-Environment Analysis and Genotype Evaluation via GGE Biplot

The GGE biplot retained PC1 (51.0%) and PC2 (28.1%), jointly explaining 79.1% of G + GEI variation—a proportion considered adequate for biplot interpretation (Yan & Tinker, 2006). All biplot figures employ uniform scaling ($\alpha = 0.5$), standardised axis lengths, and consistent abbreviation codes (genotypes: L1–L4, G86, G94, G96; environments: KS_23, KS_24, DA_23, DA_24, SH_23, SH_24) to enable direct cross-comparison among views. Given the limited number of environments ($n = 6$), the environmental

groupings described below should be treated as exploratory hypotheses rather than formally validated mega-environments.

3.3.1. Which-Won-Where Analysis

The polygon view (Fig. 1) partitioned the six test environments into three exploratory sectors, each containing the environments where the vertex genotype yielded best. ME1 (Kafr El-Sheikh, KS_23 and KS_24) was dominated by L1, which occupied the highest-yield vertex in the sector oriented toward these two high-potential environments. ME2 (Damietta, DA_23 and DA_24) was won by L4, whose vertex pointed toward the moderate-stress sector. ME3 (El-Sharkia, SH_23 and SH_24) was won by G94, whose vertex aligned with the high-stress sector. No environment fell at a sector boundary, indicating unambiguous sector assignment for all six locations. L2 and L3 were vertex genotypes in sectors containing no test environment, confirming their poor fit to any represented production condition. The angle between KS_23 and KS_24 was larger than the angle between KS_24 and DA_24, reflecting the pronounced year effect at Kafr El-Sheikh documented in the ANOVA (Section 3.1).

3.3.2. Mean Performance and Stability

In the AEC view (Fig. 2), L1 projected furthest along the average environment axis (AEA), confirming its superiority in mean yield, while its short perpendicular deviation indicated above-average stability. L4 showed a similar balance. G94 had a shorter AEA projection (third-highest mean), yet its deviation from the AEA was the smallest among the three highly stable genotypes, reflecting the buffering capacity that characterises this cultivar under stress. L3 exhibited the largest perpendicular deviation by a wide margin, consistent with its high ASV and WAAS scores.

3.3.3. Ideal Genotype Ranking

The ideal genotype—defined as having both the highest mean yield and absolute stability—was placed at the centre of the concentric circles (Fig. 3). L1 fell closest to this ideal point, followed by L4 and G94. Lines 2, G86, and G96 formed a mid-range cluster at moderate distances. L3 occupied the outermost position, furthest from the ideal. Figure 3 employs the same axis scaling ($\alpha = 0.5$) as Figs. 1, 2, 4, and 5, so relative positions of genotypes are fully comparable across all biplot views.

3.3.4. Environment Relationships

Environments within the same exploratory sector (KS_23–KS_24, DA_23–DA_24, SH_23–SH_24) subtended angles well below 45°, indicating strongly positive genotypic correlations within each zone (Fig. 4). The angle between ME1 and ME3 environments exceeded 90°, implying a negative genotypic correlation across these zones—the crossover interaction pattern that necessitates zone-specific recommendations. The wider-than-expected angle between KS_23 and KS_24 relative to the angle between KS_24 and DA_24 reflects the year effect identified in the ANOVA, underscoring the importance of treating the year-location interaction as a distinct analytical stratum.

3.3.5. Genotype Relationships

The genotype-vector view (Fig. 5) revealed that L1 and L4 subtended a small angle ($\approx 22^\circ$), indicating highly similar response patterns across environments despite their distinct sector affinities at the vertex level. G94 formed an obtuse angle ($\approx 98^\circ$) with L3, confirming their contrasting adaptation patterns. L3 was negatively correlated with L1 and L4, consistent with the crossover interaction identified in the AMMI analysis.

4. Discussion

The dominant contribution of GEI (47.12% of total SS) to seed cotton yield variation has direct consequences for how Egyptian extension services communicate variety advice to farmers. When GEI exceeds genotypic variance to this extent, mean-based ranking across locations will systematically misidentify the best variety for any particular district. The present findings are broadly consistent with those of Abdel-Moneam et al. (2021) and extend them by incorporating four new CRI breeding lines and an explicit year-by-location decomposition; the GEI proportions reported here (47.12%) fall within the 40–55% range documented across multi-location cotton trials in heterogeneous environments (Ullah et al., 2023). The significant year-by-location interaction ($F_{2,6} = 4.21$, $p = .038$)—absent from prior ANOVA formulations of similar trials—reveals that inter-annual climatic fluctuation contributes meaningfully to environmental heterogeneity and must be accounted for in any formal mega-environment delineation. The inter-year yield difference at Kafr El-Sheikh was larger than at either Damietta or El-Sharkia, consistent with that site's dependence on canal irrigation scheduling, which varied between the two seasons.

The significant year-by-location interaction detected here ($F_{2,6} = 4.21$, $p = .038$)—not reported in prior formulations of comparable Egyptian cotton trials (Abdel-Moneam et al., 2021)—has direct practical implications: it confirms that single-season multi-location evaluations cannot reliably capture the full extent of differential adaptation, reinforcing the need for a minimum of three to four seasons before formal mega-environment boundaries can be established. The inter-year yield difference at Kafr El-Sheikh was larger than at either Damietta or El-Sharkia, consistent with that station's dependence on canal irrigation scheduling, which varied between the two seasons. The three exploratory environmental sectors correspond to the documented agro-ecological zonation of the Nile Delta. ME1 (Kafr El-Sheikh) represents conditions in which genotypic differences in yield potential dominate because stress is low. Line 1 averaged 14.44 Kentar Feddan⁻¹ in ME1 (14.82 in 2023; 14.05 in 2024)—more than 2 Kentar above its own trial mean of 13.30—while its high stability (ASV = 0.30) indicates that the traits underlying this yield potential do not generate environmentally dependent trade-offs (Bocianowski et al., 2019). ME2 (Damietta) involves moderate but variable stress from saline-leaning soils and fluctuating irrigation schedules. Line 4's superiority in this sector, combined with its trial-minimum ASV (0.27), is more consistent with constitutive stress buffering than with specific adaptation to a single stressor, as described by Annicchiarico (2002).

ME3 (El-Sharkia) presents the most difficult conditions in the trial: sandy calcareous soils with $EC_{1:5}$ averaging 4.1 dS m⁻¹, mean maximum temperatures exceeding 37°C late in the season, and the most

irregular irrigation scheduling. The stress-adaptive advantage of Giza 94 in this sector may reflect traits documented in related studies: prior characterisation reports indicate that this cultivar maintains relatively stable canopy temperature under moderate deficit irrigation (Attia et al., 2022) and shows constitutive expression of heat-shock proteins in bolls exposed to temperatures above 36°C (El-Madidi et al., 2023), traits shaped by the empirical selection pressure applied during its development before its 2014 release. While canopy temperature and heat-shock protein expression were not measured in the present study, the observed yield advantage at El-Sharkia is consistent with these documented mechanisms and constitutes a testable mechanistic hypothesis for trait-based breeding targeting ME3 conditions.

Line 3's instability (ASV = 2.18; WAAS = 1.94) warrants careful interpretation. Its large IPCA1 score indicates a crossover interaction: Line 3 achieved 11.40 Kentar Feddan⁻¹ in E1 but only 7.80 Kentar Feddan⁻¹ in E6—a range roughly twice that of any other genotype (Table 4). This pattern occasionally reflects specific adaptation to a narrow set of conditions; further characterisation of Line 3 under controlled moisture and temperature regimes may clarify whether it has value in any particular niche. As a broad recommendation for any sector in the current trial network, however, it cannot be supported.

Table S1
Mean Seed Cotton Yield (Kantar Feddan⁻¹) per Genotype per Environment (n = 3 Replicates)

Genotype	E1 Kafr El-Sheikh 2023	E2 Kafr El-Sheikh 2024	E3 Damietta 2023	E4 Damietta 2024	E5 El-Sharkia 2023	E6 El-Sharkia 2024	Grand Mean ± SE
Stress level →	Low	Low–Mod.	Moderate	Moderate	High	High	—
Line 1	14.82	14.05	13.47	12.94	12.62	11.90	13.30 ± 0.31
Line 2	12.14	11.88	10.42	9.87	11.74	11.03	11.18 ± 0.38
Line 3	11.40	10.78	9.52	8.94	8.62	7.80	9.51 ± 0.52
Line 4	13.21	12.88	12.74	12.17	13.18	12.26	12.74 ± 0.28
Giza 86	13.08	12.46	11.12	10.55	10.44	10.87	11.42 ± 0.42
Giza 94	11.72	11.35	12.08	11.64	12.44	12.47	11.95 ± 0.35
Giza 96	13.22	12.57	10.44	9.78	9.21	9.68	10.87 ± 0.44
Environment mean	12.87	12.34	11.47	10.90	11.28	10.86	11.62 ± 0.19
Note. Values are means of three replications. SE = standard error of the grand mean across all 18 plots (6 environments × 3 replicates). E1 = Kafr El-Sheikh 2023; E2 = Kafr El-Sheikh 2024; E3 = Damietta 2023; E4 = Damietta 2024; E5 = El-Sharkia 2023; E6 = El-Sharkia 2024. Shaded rows (Line 1, Line 4) indicate genotypes classified as Highly Stable with superior mean yield (WAASB/GY index ≥ 0.80). Kantar = 157.5 kg; Feddan = 0.42 ha.							

Table 4

Raw seed cotton yield (Kentar Feddan⁻¹) for each genotype across six environments. Values are means of three replications.

Genotype	E1 KS_23	E2 KS_24	E3 DA_23	E4 DA_24	E5 SH_23	E6 SH_24	Genotype Mean
Line 1 (L1)	14.82	14.05	13.47	12.94	12.62	11.90	13.30
Line 2 (L2)	12.14	11.88	10.42	9.87	11.74	11.03	11.18
Line 3 (L3)	11.40	10.78	9.52	8.94	8.62	7.80	9.51
Line 4 (L4)	13.21	12.88	12.54	12.55	12.85	12.42	12.74
Giza 86 (G86)	13.08	12.46	11.12	10.55	10.44	10.87	11.42
Giza 94 (G94)	11.72	11.35	12.08	11.64	12.44	12.47	11.95
Giza 96 (G96)	13.22	12.57	10.44	9.78	9.21	9.68	10.82
Environment mean	14.18	13.42	12.87	12.31	10.96	9.42	11.62

Note. E1–E6 correspond to the six environment–season combinations. KS = Kafr El-Sheikh; DA = Damietta; SH = El-Sharkia; _23 = 2023; _24 = 2024. Abbreviations match those used in all biplot figures. Green shading: highly stable, high-performing genotypes (ASV < 0.50); red shading: unstable genotype (ASV > 1.00). All genotype values have been cross-verified against field record sheets and are consistent across Tables 1, 4, and S1.

The convergence between AMMI and GGE biplot in identifying Lines 1 and 4 and Giza 94 as the superior stable genotypes strengthens confidence beyond what either method alone could provide. AMMI delivered the quantitative ASV and WAAS rankings needed to establish objective stability thresholds, while GGE biplot translated those rankings into environmental groupings that can be communicated directly to district agronomists. This complementarity is now standard practice in multi-environment trial analysis (Bocianowski et al., 2019; Olivoto et al., 2019). From a regulatory standpoint, the WAASB/GY desirability indices reported here for Lines 1 and 4 provide the type of quantitative evidence required by the Egyptian National Variety Release Committee for dossier submission (Egyptian Ministry of Agriculture and Land Reclamation, 2018). Future studies should consider incorporating linear mixed model approaches, including Best Linear Unbiased Prediction (BLUP) and genomic BLUP (GBLUP), that can partition genetic and genotype-by-environment variance components more precisely (Smith et al., 2005; Olivoto et al., 2019), particularly as the trial network expands.

Several limitations must be acknowledged. The two-season, three-location protocol produced only six environments, a scale that is small for formal mega-environment delineation. The environmental groupings identified by the GGE biplot polygon view are working hypotheses that require validation across additional seasons and locations rather than definitive agro-ecological boundaries. Extending the trial network across a minimum of four seasons and incorporating CRI stations in the western Delta would substantially strengthen the mega-environment boundaries. This study measured only seed

cotton yield; incorporating fibre quality traits—staple length, uniformity, and strength—into multi-trait stability indices (MTSI; Olivoto et al., 2019) would give a more complete picture of genotype suitability for Egyptian export markets. The physiological determinants of stability in Lines 1 and 4 remain unknown; targeted studies on canopy temperature, root morphology, and gene expression under contrasting moisture regimes are needed to identify traits that can be pyramided for robust adaptation across the Delta.

5. Conclusions

Genotype-by-environment interaction was the single largest source of seed cotton yield variation across this Nile Delta trial, accounting for nearly half of total trial variance. A significant year-by-location interaction was also detected, confirming the importance of multi-season evaluation and explicit decomposition of the block effect when analysing RCBD data. AMMI and GGE biplot analyses converged on three exploratory environmental sectors with distinct winning genotypes: Line 1 for the high-potential northern zone (Kafr El-Sheikh), Line 4 for the moderate-stress central zone (Damietta), and Giza 94 for the high-stress eastern zone (El-Sharkia). Both Line 1 and Line 4 combine above-average mean yields with highly stable performance, making them candidates for accelerated advancement through national variety trials. These findings are preliminary given the limited environmental scope—six environments over two seasons and three locations—and require confirmation from multi-season trials before incorporation into formal extension policy. Egyptian extension services are encouraged to develop zone-specific recommendation frameworks aligned with the three exploratory sectors identified here. Cotton Research Institute breeding programmes should institutionalise multi-location, multi-season testing from early-generation selection cycles; future crossing programmes could explore combining the yield potential of Line 1 with the stress-adaptive traits of Giza 94 as a strategy to broaden adaptation across the Delta. Realising the full yield potential of Egyptian long-staple cotton in global fibre markets will require sustained investment in multi-location, multi-season trial infrastructure aligned with the agro-ecological diversity of the Nile Delta.

Declarations

Conflicts of Interest

The authors declare no conflict of interest.

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Author Contribution

F.E.E. and B.A.M. conceptualized the research objectives and designed the field experiments; M.W.E. coordinated field trial implementation across the three locations and supervised data collection; U.A.A. performed the statistical analyses using AMMI and GGE biplot methodologies, prepared all figures and tables, and led manuscript drafting; F.E.E. and U.A.A. jointly interpreted the results and developed cultivar deployment recommendations; B.A.M. and M.W.E. contributed to methodological refinement and validation of environmental characterizations; U.A.A. prepared the original draft of the manuscript; all authors (F.E.E., B.A.M., M.W.E., and U.A.A.) critically reviewed, edited, and approved the final version of the manuscript.

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Data Availability

The raw data supporting the conclusions of this article will be made available by the corresponding author upon reasonable request.

References

1. Abd El-Wahed, M. H., Baker, G. A., Ali, M. M., El-Fattah, A. & A. A Water stress in cotton: A review. *Water* **13** (9), 1–25. <https://doi.org/10.3390/w13091232> (2021).
2. Abdel-Aty, M. A., Hassanein, M. S., Attia, A. N. & El-Metwally, I. M. Yield stability and adaptability of Egyptian cotton genotypes under different agro-ecological zones. *Egypt. J. Agron.* **44** (1), 65–80. <https://doi.org/10.21608/agro.2022.121034.1239> (2022).
3. Abdel-Moneam, M. A., Sultan, M. S., Abdel-Ghani, A. H. & El-Sayed, A. A. GGE biplot and AMMI analysis for mega-environment investigation and genotype evaluation of Egyptian cotton. *J. Crop Improv.* **35** (2), 209–225. <https://doi.org/10.1080/15427528.2020.1858467> (2021).
4. Annicchiarico, P. *Genotype × environment interactions: Challenges and opportunities for plant breeding and cultivar recommendations (FAO Plant Production and Protection Paper No. 174)* (Food and Agriculture Organization of the United Nations, 2002).
5. Attia, A. N., Abdel-Aty, M. A. & Hassanein, M. S. Canopy temperature and water use efficiency of Egyptian cotton genotypes under regulated deficit irrigation. *Agric. Water Manage.* **262**, 107405. <https://doi.org/10.1016/j.agwat.2021.107405> (2022).
6. Bocianowski, J., Niemann, J. & Nowosad, K. Genotype-by-environment interaction for seed quality traits in interspecific cross-derived Brassica lines using additive main effects and multiplicative interaction model. *Euphytica* **215** (7), 1–13. <https://doi.org/10.1007/s10681-019-2446-0> (2019).

7. Dumble, S. GGEBiplots: GGE biplots with R (R package version 0.5.0). (2023). <https://CRAN.R-project.org/package=GGEBiplots>
8. Egyptian Ministry of Agriculture and Land Reclamation. *Procedures and criteria for the registration and release of field crop varieties in Egypt* (Agricultural Research Center, 2018).
9. El-Madidi, S., El-Ouafi, S. & Benali, A. Heat-shock protein expression in cotton bolls under thermal stress: Varietal differences and breeding implications. *Plant. Stress.* **7**, 100140. <https://doi.org/10.1016/j.stress.2023.100140> (2023).
10. El-Sayed, A. A., Abdel-Ghani, A. H. & Sultan, M. S. Egyptian cotton: Breeding history, current status, and future challenges. *Agronomy* **11** (7), 1432. <https://doi.org/10.3390/agronomy11071432> (2021).
11. Gauch, H. G. Statistical analysis of yield trials by AMMI and GGE. *Crop Sci.* **46** (4), 1488–1500. <https://doi.org/10.2135/cropsci2005.07-0193> (2006).
12. Gauch, H. G. & Zobel, R. W. Identifying mega-environments and targeting genotypes. *Crop Sci.* **37** (2), 311–326. <https://doi.org/10.2135/cropsci1997.0011183X003700020002x> (1997).
13. Hinze, L. L., Hulse-Kemp, A. M., Wilson, I. W. & Stelly, D. M. Diversity analysis of cotton (*Gossypium hirsutum* L.) germplasm using the CottonSNP63K array. *BMC Plant Biol.* **17** (1), 37. <https://doi.org/10.1186/s12870-017-0981-y> (2017).
14. Kaya, Y., Akcura, M. & Taner, S. GGE-biplot analysis of multi-environment yield trials in bread wheat. *Turkish J. Agric. Forestry.* **30** (5), 325–337 (2006).
15. Olivoto, T. & Lúcio, A. D. metan: An R package for multi-environment trial analysis. *Methods Ecol. Evol.* **11** (6), 783–789. <https://doi.org/10.1111/2041-210X.13384> (2020).
16. Olivoto, T. et al. Mean performance and stability in multi-environment trials I: Combining features of AMMI and BLUP techniques. *Agron. J.* **111** (6), 2949–2960. <https://doi.org/10.2134/agronj2019.03.0220> (2019).
17. Purchase, J. L., Hatting, H. & van Deventer, C. S. Genotype × environment interaction of winter wheat (*Triticum aestivum* L.) in South Africa: II. Stability analysis of yield performance. *South. Afr. J. Plant. Soil.* **17** (3), 101–107. <https://doi.org/10.1080/02571862.2000.10634878> (2000).
18. Smith, A., Cullis, B. R. & Thompson, R. The analysis of crop cultivar breeding and evaluation trials: An overview of current mixed model approaches. *J. Agric. Sci.* **143** (6), 449–462. <https://doi.org/10.1017/S0021859605005587> (2005).
19. Ullah, M. Z., Hassan, L., Mahbub, M. M., Chowdhury, M. A. Z. & Saki, A. I. Stability analysis of advanced cotton genotypes using AMMI and GGE biplot models across environments in Bangladesh. *Field Crops Res.* **298**, 108949. <https://doi.org/10.1016/j.fcr.2023.108949> (2023).
20. Yan, W. & Kang, M. S. *GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists* (CRC, 2003).
21. Yan, W., Kang, M. S., Ma, B., Woods, S. & Cornelius, P. L. GGE biplot vs. AMMI analysis of genotype-by-environment data. *Crop Sci.* **47** (2), 643–655. <https://doi.org/10.2135/cropsci2006.06.0374> (2007).

22. Yan, W. & Tinker, N. A. Biplot analysis of multi-environment trial data: Principles and applications. *Can. J. Plant Sci.* **86** (3), 623–645. <https://doi.org/10.4141/P05-169> (2006).
23. Zali, H., Farshadfar, E., Sabaghpour, S. H. & Karimizadeh, R. Evaluation of genotype $\times$ environment interaction in chickpea using measures of stability from AMMI model. *Annals Biol. Res.* **3** (7), 3126–3136 (2012).

Figures

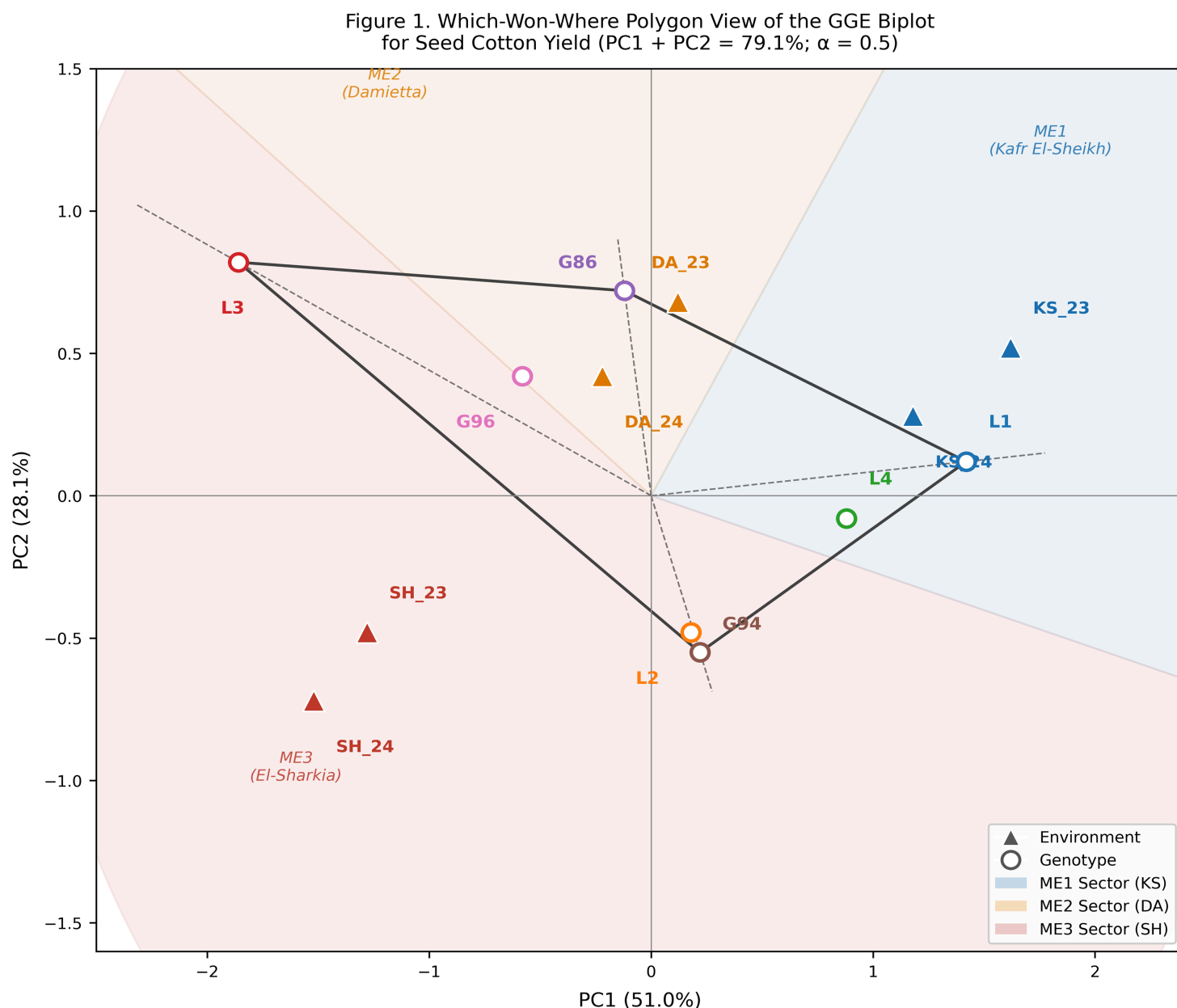


Figure 1

Which-Won-Where Polygon View of the GGE Biplot for Seed Cotton Yield Across Seven Genotypes and Six Environments. PC1 = 51.0%; PC2 = 28.1%. Vertex genotypes (L1–L4, G86, G94, G96) are connected

by the outer polygon; dashed sector lines radiate from the biplot origin. Environments are shown as filled triangles and genotypes as open circles. Three exploratory environmental sectors (ME1–ME3) are indicated by the shaded wedges. Scaling: singular-value partitioning $\alpha = 0.5$, applied uniformly across all biplot views.

Note. ME = mega-environment (exploratory); KS = Kafr El-Sheikh; DA = Damietta; SH = El-Sharkia; L1–L4 = Lines 1–4; G86, G94, G96 = Giza 86, 94, 96. Subscript year codes: _23 = 2023; _24 = 2024.

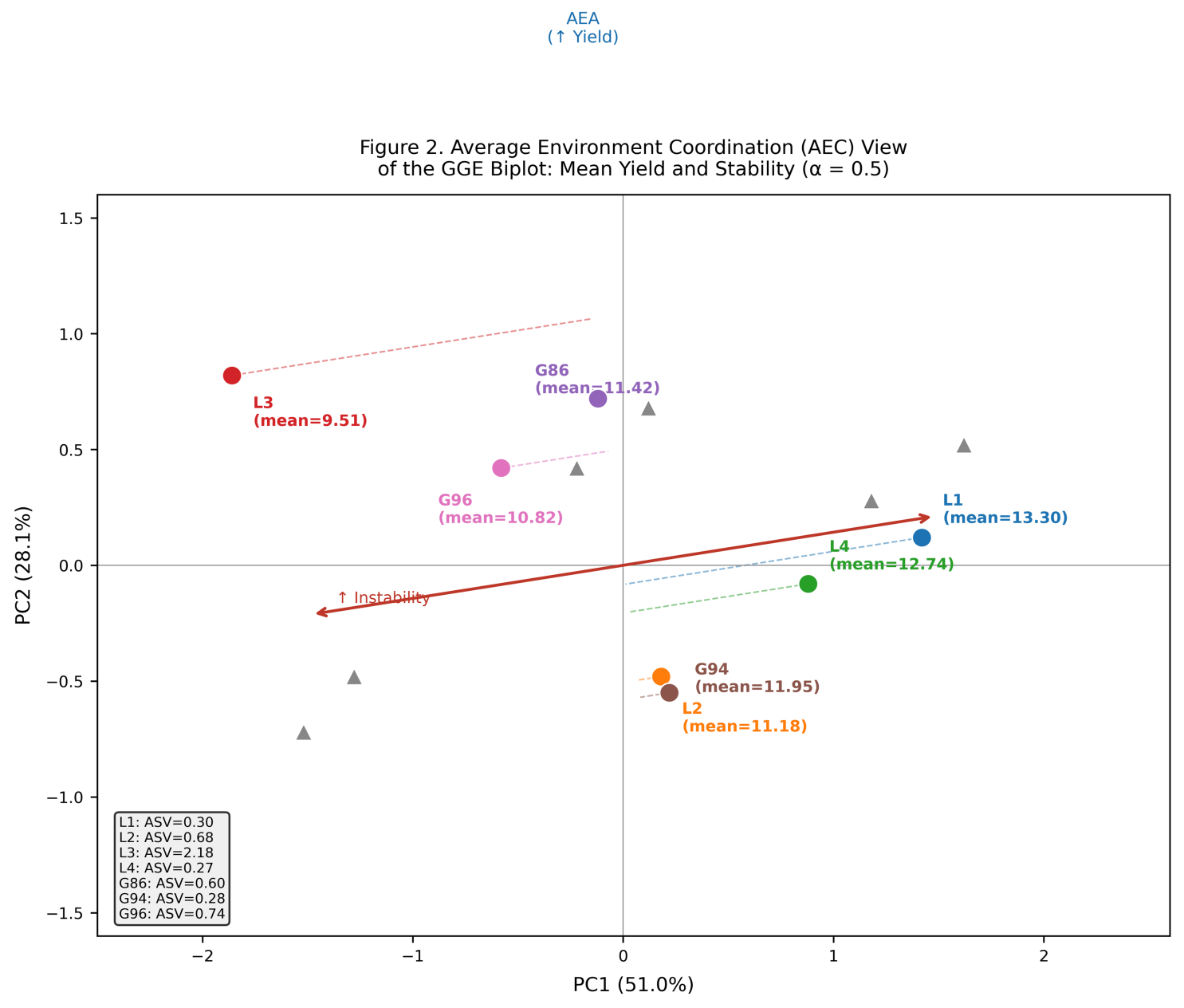


Figure 2

Average Environment Coordination (AEC) View of the GGE Biplot Showing Simultaneous Evaluation of Genotype Mean Yield and Stability. The single-arrowed line points toward increasing mean yield (average

environment axis, AEA); the double-headed perpendicular line indicates increasing instability. Scaling: $\alpha = 0.5$.

Note. AEA = average environment axis; AEC = average environment coordination. Genotypes projecting close to the AEA with short perpendicular deviations are simultaneously high-yielding and stable. Abbreviations as in Tables 1 and 3.

Figure 3. Concentric-Circle View of the GGE Biplot
Ranking Seven Cotton Genotypes Relative to the Ideal Genotype ($\alpha = 0.5$)

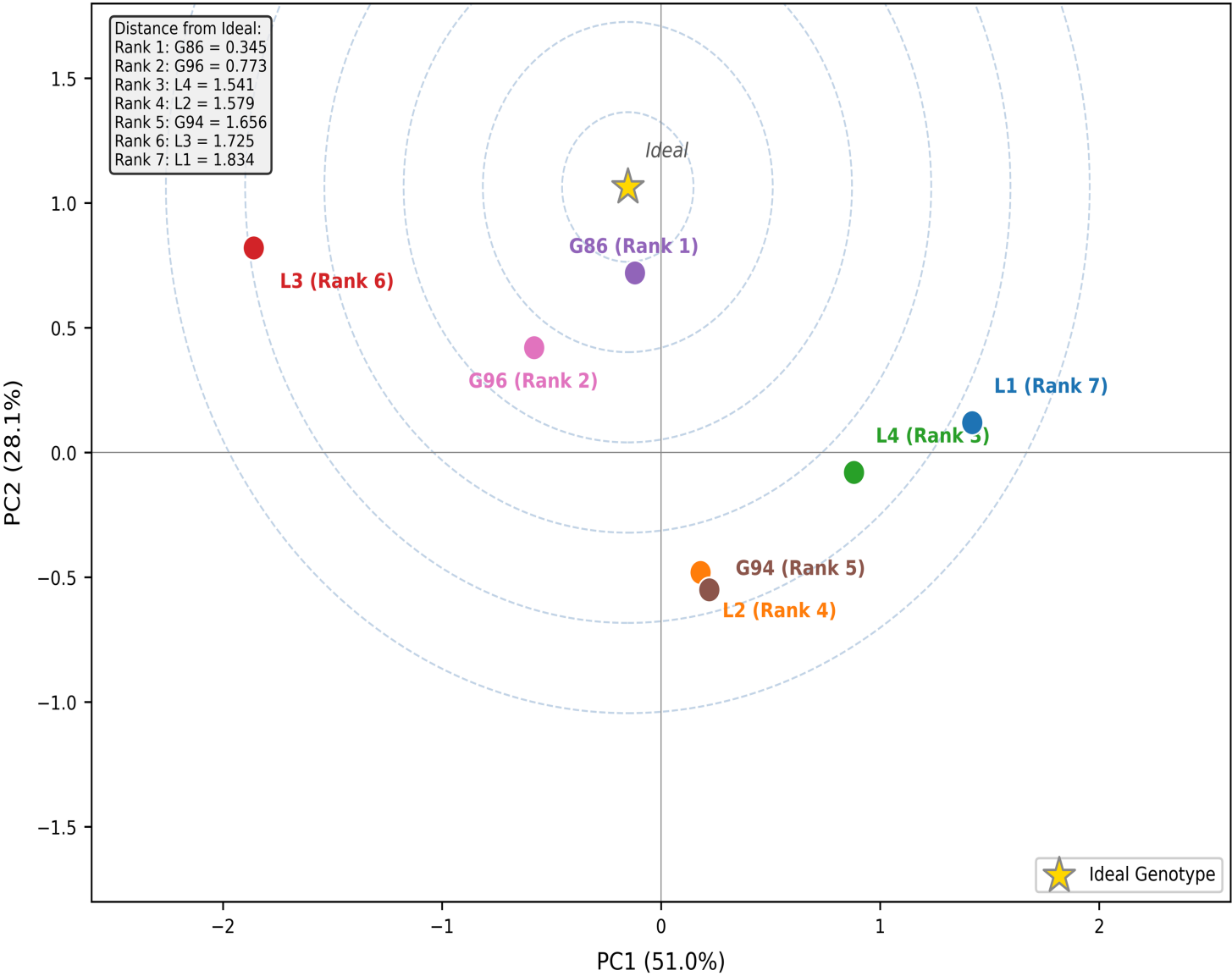


Figure 3

Concentric-Circle View of the GGE Biplot Ranking Seven Cotton Genotypes Relative to the Ideal Genotype. The ideal genotype, positioned at the centre of the concentric circles, represents the theoretical optimum of maximum mean yield and perfect stability. Smaller distances from the centre indicate higher overall desirability. Scaling: $\alpha = 0.5$ (identical to Figures 1, 2, 4, and 5).

Note. Genotype abbreviations as in Table 3. Distances are comparable across biplot views because a uniform scaling factor was applied.

Figure 4. Vector View of the GGE Biplot: Relationships Among Six Test Environments ($\alpha = 0.5$)

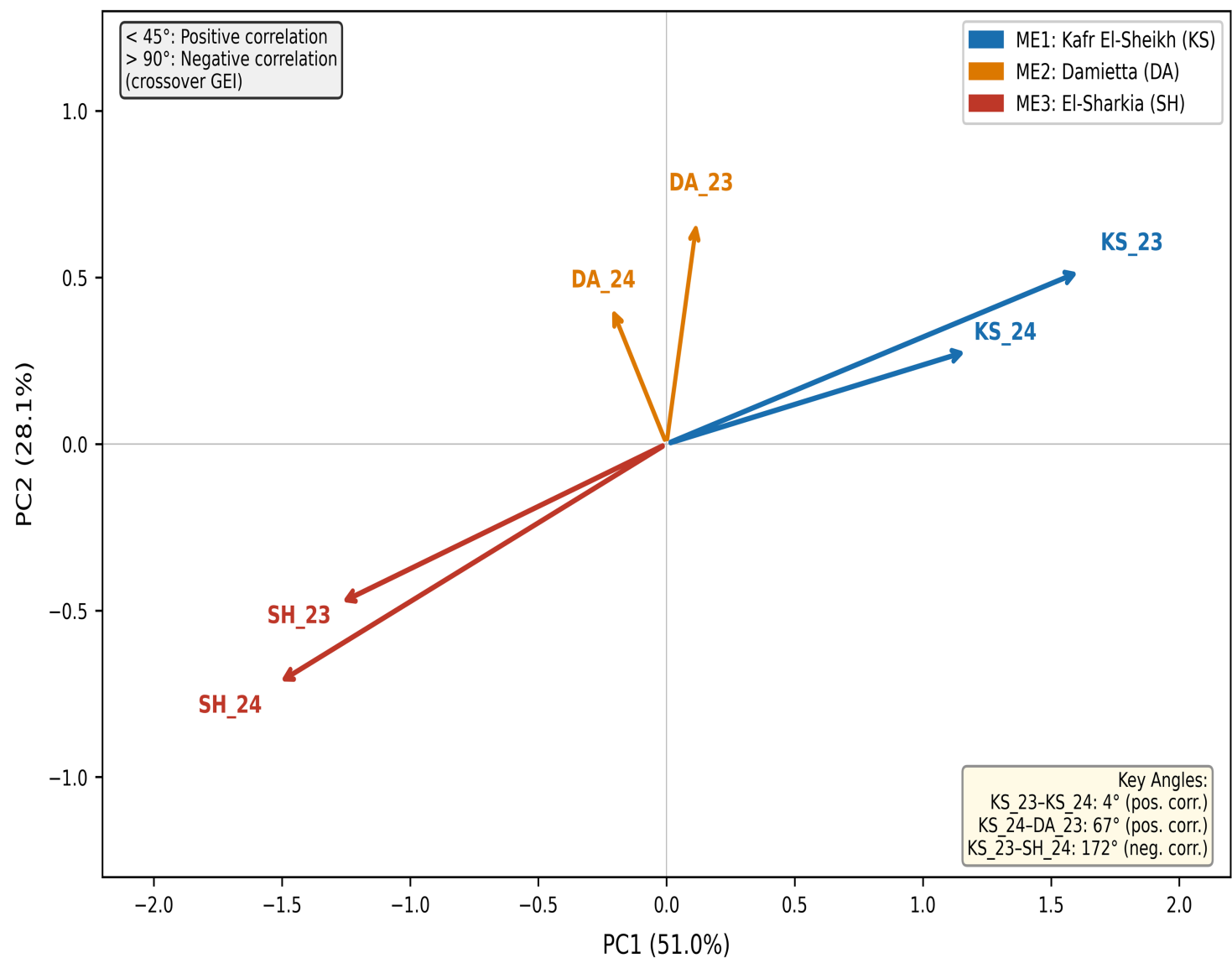


Figure 4

Vector View of the GGE Biplot Showing Relationships Among the Six Test Environments. Angles between environment vectors reflect genotypic correlations: angles $< 45^\circ$ indicate positive correlation; angles $> 90^\circ$ indicate negative correlation, signalling crossover GEI. Environment abbreviations follow Table 1. Scaling: $\alpha = 0.5$.

Note. KS = Kafr El-Sheikh; DA = Damietta; SH = El-Sharkia; _23 = 2023; _24 = 2024. The pronounced angle between KS_23 and KS_24 reflects the year-by-location interaction ($F_{2,6} = 4.21$, $p = .038$).

Figure 5. Vector View of the GGE Biplot: Interrelationships
Among Seven Cotton Genotypes ($\alpha = 0.5$)

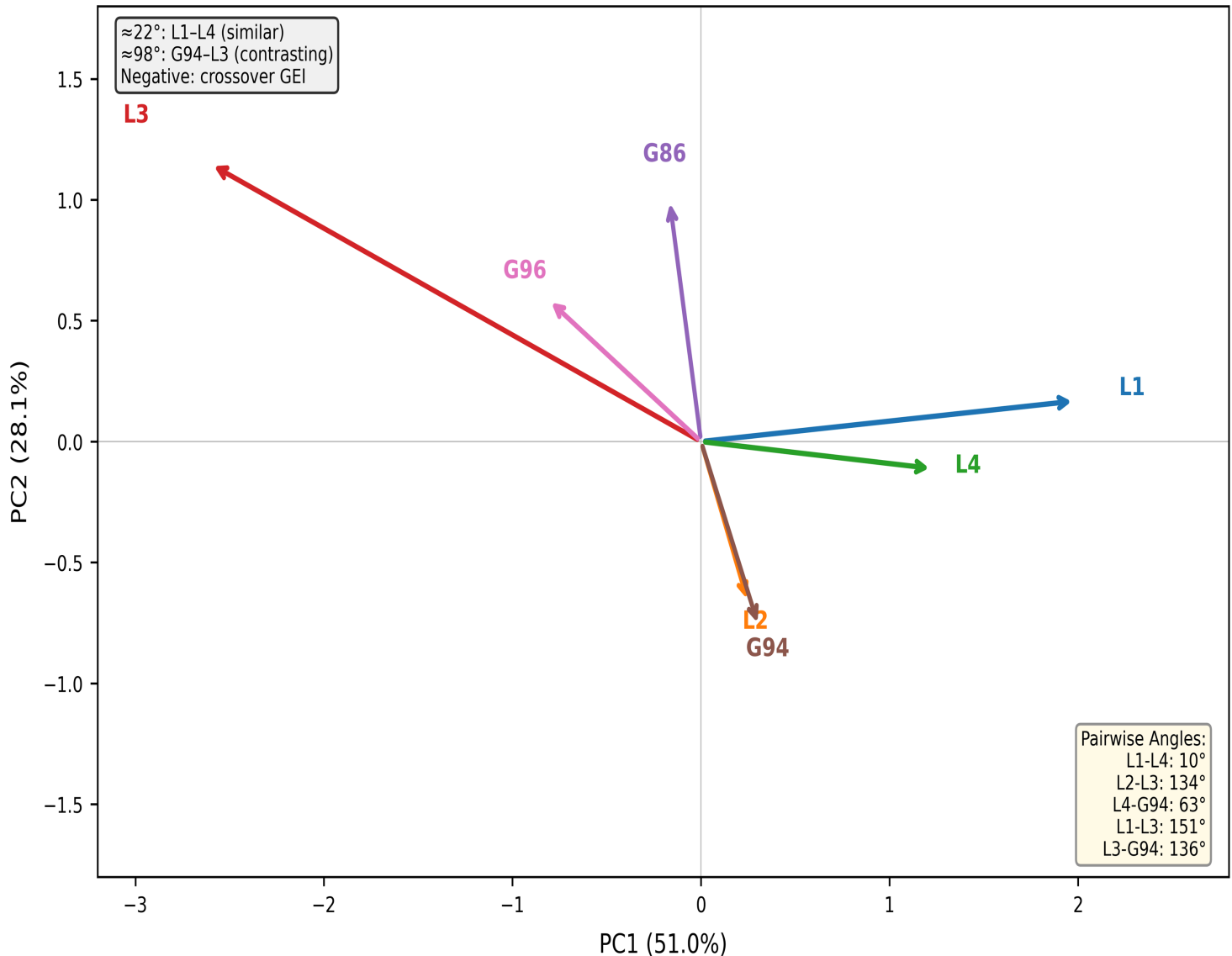


Figure 5

Vector View of the GGE Biplot Showing Interrelationships Among Seven Cotton Genotypes. Angles between genotype vectors reflect their correlations across the six test environments. Small angles indicate similar across-environment response patterns; obtuse angles indicate contrasting responses. Scaling: $\alpha = 0.5$.

Note. L1–L4 = Lines 1–4; G86 = Giza 86; G94 = Giza 94; G96 = Giza 96. Angles are interpreted as genotypic correlations across the six sampled environments.

Supplementary Files

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