Evaluation of Sunflower Genotypes for Terminal Heat Stress Tolerance Using Yield-Based Indices and Multivariate Analysis

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Abstract

Heat stress (HS) is a major problem for sunflower production at the global level due to the effects of climate change. In this study, ten sunflower genotypes were compared to determine elite heat-tolerant ideotypes and gain insight into the association between traits under terminal heat stress. The experiment was carried out at the Karbala Research Station/Extension Farm, Iraq, in 25th July of 2025. The evaluated genotypes, comprising elite hybrids, commercial varieties, and breeding lines, included AKSF-9 (T1), COSFV-5 (T2), CMS-234A (T3), KBSH-44 (T4), PAC-36 (T5), EC-68414 (T6), DRSH-1 (T7), ARM-248B (T8), RHA-274 (T9), and CMS-108B (T10). The two contrasting sowing environments were used to subject the plants to terminal heat stress at the reproductive stage in a Randomized Complete Block Design. The ANOVA, Pearson correlation, PCA, and hierarchical clustering were used for the analysis of morpho-physiological, yield, and stress-tolerance indices. There was considerable genotypic variation between the environments tested. Under stress, the vegetative vigor, the earliest phenological development (heat escape), and the oil yield (60.5 g/plant) were significantly greater between the two genotypes, T7 (DRSH-1) and T4 (KBSH-44), than the other genotype under the three stress levels. Under stress, vegetative vigor, earliest phenological development (heat escape), and highest seed (127.0 g/plant) and oil yield (60.5 g/plant) were significantly higher in the two genotypes T7 (DRSH-1) and T4 (KBSH-44) than in the other genotype at all stress levels. They had the lowest SSI (0.407 and 0.527) and the highest Heat Tolerance Index. On the other hand, T6, T10, and T3 were very sensitive. Good positive correlations were observed between yield and tolerance traits, with negative correlations with stress penalty metrics, using Pearson correlations. The PCA and hierarchical clustering showed similar results and well clustered the genotypes into tolerant, moderate, and susceptible groups, with T7 having the highest heat-tolerant score based on composite response. Based on this, two genetic materials (T7 and T4) were identified as potential and resilient germplasm for direct cultivation in heat-sensitive agroecosystems and as parental lines in breeding programs, and thus require further testing in multi-environment trials.

Keywords: Helianthus annuus, Terminal heat stress, Stress tolerance indices, Multivariate analysis, Yield stability, Climate-resilient breeding

1. Introduction

Sunflower (Helianthus annuus L.) is a world-scale oilseed crop of agricultural and economic importance with high nutritional value and quality edible oil, which is rich in fatty acid (FA) linoleic acid, with a wide range of applications in animal feed and industrial applications (Adeleke & Babalola 2020). Sunflower is one of the world's biggest suppliers of vegetable oil and is an important contributor to the edible oil security of the world. It is regarded as a hardy crop,

versatile for use in a wide range of agro-climatic conditions, but slow climatic changes with unpredictable weather events are posing a threat to its productivity. The terminal heat stress frequently occurs at sensitive reproductive stages (flowering and seed filling) caused by sowing delays or increasing temperatures, and is a serious and increasing problem in sunflower production among the abiotic constraints (Sehgal et al., 2018).

High temperature during reproductive seasons has a dramatic impact on physiological and biochemical processes, such as reduced photosynthetic rate, loss of pollen fertility, premature maturity, and reduced seed-set (Roychowdhury et al., 2025). Therefore, heat stress has a profound effect on seed size, oil content, and the total yield per plant, which in turn impacts the profitability of the crop and the quality of the oil. The most economic and sustainable approach to mitigate these negative impacts is the development and deployment of heat-tolerant genotypes, which is a complex polygenetic quantitative trait that depends on several morpho-physiological and phenological mechanisms (Thingujam et al., 2025).

However, when considering the genotypes in relation to one criterion or simple yield parameters in stress, the adaptive response of the plant is not always reflected (Basu et al., 2016). There is a need for a holistic screening approach to understand the multifaceted aspects of vegetative vigor, reproductive output, and yield stability. Thus, it is necessary to combine the stress tolerance index with the use of advanced multivariate statistical analysis techniques. Multivariate statistical methods such as PCA, hierarchical clustering, and correlation analysis are used to reduce the dimensionality of the data, measure the genetic distance, and identify the most discriminating traits for indirect selection. These tools provide a useful way of understanding the interactions between traits and for grouping of genotypes based on their stress adaptation strategies (Redhu et al., 2026).

The aim of the present study was to assess the performance and heat stress tolerance of 10 sunflower genotypes (elite hybrids, commercial varieties, and breeding lines) under different sowing times (normal and late sowing). Specifically, the effects of heat stress at the end of the season on important phenological, morphological and yield-attributing traits were evaluated; heat tolerance was quantified using the Stress Susceptibility Index (SSI), Heat Tolerance Index (HTI), and a composite index of heat tolerance; and multivariate analyses (PCA, hierarchical clustering, and correlation matrices) were used to understand trait associations and classify the genotypes by heat tolerance. The overall objective of the study is to screen for promising lines that are tolerant to high temperatures for future breeding to create high-yielding varieties for climate-smart sunflowers.

2. Materials and Methods

2.1 Experimental Location, Sowing Dates, and Heat Stress Confirmation

The experiment was carried out at the Karbala Research Station / Extension Farm, Iraq, (Latitude 32.34° N, Longitude 44.03° E and Altitude 29 m above sea level) on the July 25th, 2025. To develop two sowing environments, the trial was sown under normal sowing conditions and under heat stress conditions.

Daily meteorological data from an automatically operated weather station, 50 m from the experimental field, were used to determine and quantify terminal heat stress. The heat stress intensity was measured by the mean daily maximum temperature (Tmax), and the cumulative Growing Degree Days (GDD) were calculated during the critical periods of flowering and seed filling. The environment at the late sowing had a very high terminal heat stress, with a mean Tmax of 41.5 °C and during anthesis and grain filling, respectively, compared to 31.2 °C under normal sowing. The large thermal difference successfully imposed the terminal heat stress during the reproductive stage, as per the regular heat stress screening for summer crops (Hussain et al., 2025).

2.2 Plant Material, Plot Dimensions, and Agricultural Practices

Elite hybrids, commercial varieties, breeding lines, and restorer/maintainer lines of ten sunflower (Helianthus annuus L.) genotypes were tested. Genotypes are specified in the exact genetic background, and the classification of the genotypes is detailed in Table 1 (e.g., T7: DRSH-1, T4: KBSH-44, T5: PAC-36).

The experiment was designed in a Randomized Complete Block Design (RCBD) three times over the three sowing environments. The experimental plot was 4 rows long, with a dimension of 4.0 m in length and 2.4 m wide. Row to row and plant to plant spacing was strictly maintained as 60cm and 25cm, respectively. All plots were prepared and fertilized (NPK fertilizer; 80:40:40kg/ha) in the same way based on local agronomic recommendations. No chemical pest or disease control measures were used during the growing season to avoid influencing the field evaluation of genotypes in an unnatural way by heat stress.

2.3 Data Collection and Oil Content Estimation

Data from five plants were collected from each replication, which were healthy, competitive, and randomly selected from the area under the net. Phenological traits (days to 50% flowering and days to physiological maturity) and morphological traits (plant height, stem diameter, leaf area, and flower head/disc diameter) were measured. The yield and yield contributing parameters recorded were number of seeds/head, 1000 seed weight (g), plant yield (g), and oil yield (g).

The determination of oil content (%) was done on harvested and dried seeds according to the standard method described by AOAC (2005) with the Soxhlet extraction method and petroleum ether (boiling point 40-60 °C). The oil yield/plant (g) was then obtained by multiplying the seed yield/plant by the percentage of oil content. Heat stress percentage yield reduction was determined by taking into account the differences between the yield of the heat-stressed seed and the yield of the seed grown under normal growing conditions.

2.4 Statistical Analysis and Genotype × Environment Interaction

Analysis of Variance (ANOVA) was conducted to examine the main effect of each of Genotype (G), Environment (E - sowing date), and Genotype × Environment (G×E) interaction. For analysis, the linear statistical model was:

$$Y_{ijk} = \mu + G_i + E_j + (G\times E)_{ij} + B_{k(j)} + \varepsilon_{ijk}$$

Y_{ijk} is the observed value, μ is the overall mean, G_i is the effect of the i-th genotype, E_j is the effect of the j-th environment, $(G\times E)_{ij}$ is the interaction effect between genotype and environment, $B_{k(j)}$ is the effect of the k-th block nested

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within the j-th environment, and ε_ijk is the random error. Means were compared by using the Least Significant Difference (LSD) test at 5% probability level ($p \leq 0.05$).

To explore interrelationships among the traits and genotype clustering, multivariate statistical methods were performed, including Principal Component Analysis (PCA), hierarchical Cluster Analysis (using Euclidean distance), and Pearson's correlation analysis. A standardized Z-value heat map was also created. The statistical analyses were performed with R software, version 4.2.1, and GenStat 18th edition.

2.5 Calculation of Heat Tolerance Indices and Composite Score

The sunflower genotypes were compared in terms of their relative heat tolerance by calculating stress tolerance indices based on the yield data obtained from normal (Y) and heat-stressed (Y□) environments.

The Stress Susceptibility Index (SSI) was computed according to the formula suggested by Fischer and Maurer (1978):

$$SSI = [1 - (Y□/Y□)] / D$$

where $D = 1 - (\bar{Y}/\bar{Y}□)$. In this case, Y and Ys are the yield of a particular genotype under normal and stress conditions; on the other hand, $\bar{Y}p$ and $\bar{Y}s$ are the mean yields of all genotypes under normal and stress conditions, respectively.

The Heat Tolerance Index (HTI) was determined by the formula used by Fernandez (1992):

$$HTI = (Y□ \times Y) / (\bar{Y}□)^2$$

whereby higher values of HTI equate to better adaptation and resistance to a stressful environment.

To give an integrated assessment, the parameters of yield and tolerance (i.e., SSI and HTI) were standardized by Z-scores to form a CHTS. The CHTS was calculated by adding the Z scores of positive traits together and subtracting the Z scores of negative traits. This composite metric allowed for the robust classification of the genotypes into tolerance groups.

3. Results and Discussion

3.1 Heat Stress Effects on Growth and Yield Traits

In the plant height, significant differences were found among sunflower genotypes (T6, 155.33 cm; T7, 188.00 cm) under heat stress, as illustrated in Figure 1A. The results of the statistical analysis (LSD = 2.61 cm, $p \leq 0.05$) showed 8 different performance groups, with T7 being the superior group ('a ') followed by T4 (182.00 cm, 'b') and T2 (175.67 cm, 'c'). The superior height of T7 and T4 shows vigorous vegetative growth and increased canopy expansion, which means that under thermal stress, they are able to intercept more light and generate more biomass (Table 2).

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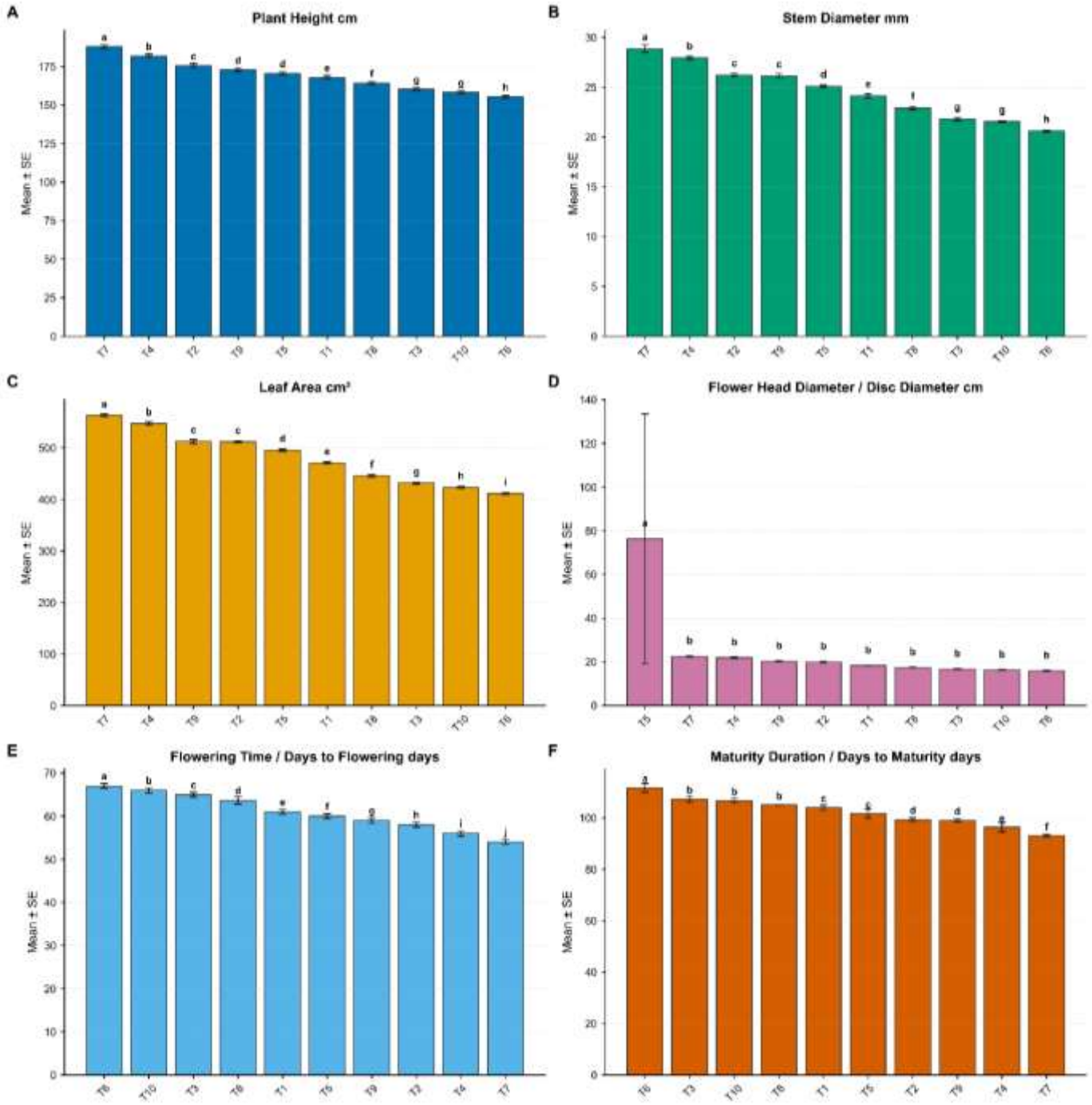


Figure 1. Mean performance of sunflower treatments for vegetative, yield, and heat tolerance traits under heat stress conditions.

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Table 1. Genetic Background and Classification of Experimental Sunflower Genotypes Evaluated Under Contrasting Sowing Environments

Category	Likely Real-World Sunflower Genotype	Treatment
Germplasm Breeding Line	AKSF-9	T1
Climate-Smart Variety	COSFV-5	T2
Cytoplasmic Male Sterile (CMS) Female Line	CMS-234A	T3
Elite Hybrid	KBSH-44	T4
Commercial Hybrid	PAC-36 / Proagro-6111	T5
Exotic Germplasm Line	EC-68414 (Representative Exotic Collection Line)	T6
National Check Hybrid	DRSH-1 (Directorate of Research on Sunflower Hybrid-1)	T7
Maintainer (B) Line	ARM-248B	T8
Restorer (R) Line	RHA-274	T9
Maintainer (B) Line	CMS-108B	T10

Table 2. Treatment-wise mean values of morphological, reproductive, yield, oil content, and heat stress tolerance indices in sunflower (*Helianthus annuus* L.).

Yield Reduction Due to Heat (%)	Mean Yield under Stress (g)	Stress Susceptibility Index (SSI)	Heat Tolerance Index (HTI)	Oil Yield per Plant (g)	Oil Content (%)	Seed Yield per Plant (g)	1000-Seed Weight (g)	Number of Seeds per Head	Days to Maturity	Days to Flowering	Flower Head Diameter / Disc Diameter (cm)	Leaf Area (cm²)	Stem Diameter (m)	Plant Height (cm)	Treatment

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18.66667	72.66667	0.836667	1.126667	35.8	40.06667	91.66667	66.66667	911.6667	104	61	18.23333	471	24.13333	168	T1
31.66667	55	1.18	0.823333	25.7	35.63333	70	55	766.6667	106.6667	66	16.3	423.3333	21.53333	158.3333	T10
13.66667	91.66667	0.65	1.35	47.63333	45.46667	108.6667	70	1076.667	99.33333	58	20.03333	511.6667	26.23333	175.6667	T2
29.66667	56.66667	1.123333	0.896667	25.4	37.33333	71.66667	56.66667	785	107.3333	65	16.6	431.6667	21.8	160.3333	T3
10	102	0.526667	1.483333	55.5	51.93333	120	72	1125	96.33333	56	21.9	547.3333	27.93333	182	T4
15.33333	81.66667	0.763333	1.216667	39.83333	43.96667	98.33333	65	976.6667	101.6667	60	76.26667	495.6667	25.1	170.6667	T5
35	50	1.286667	0.76	25.7	35.4	66.66667	53.66667	545	111.6667	67	15.86667	411.3333	20.6	155.3333	T6
6.666667	111.6667	0.406667	1.616667	60.5	50.43333	127	78.33333	1181.667	93	54	22.53333	563	28.86667	188	T7
25	65	1.03	0.95	30.63333	40.5	80	60	863.3333	105	63.66667	17.4	445.6667	22.9	164.3333	T8
15	87	0.706667	1.303333	45.5	45.6	106.6667	70	1013.333	99	59	20.26667	512.6667	26.13333	173	T9

Table 3. Principal component loadings of growth, phenological, yield, oil, and heat stress tolerance traits in sunflower (*Helianthus annuus L.*).

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PC10	PC9	PC8	PC7	PC6	PC5	PC4	PC3	PC2	PC1	Trait
0.368213	-0.20633	0.028304	0.305453	0.725703	-0.2635	0.039006	-0.06335	-0.05392	0.267664	Plant Height (cm)
-0.09918	-0.25034	0.025867	0.043823	-0.20911	0.010693	0.03839	-0.04383	-0.01719	0.269211	Stem Diameter (mm)
0.31708	-0.15556	0.478523	-0.05344	-0.28905	-0.01887	0.032134	-0.1573	0.006043	0.26872	Leaf Area (cm²)
-0.01589	0.045285	-0.01654	0.03356	0.008967	-0.09492	-0.01521	-0.04953	0.987164	0.057935	Flower Head Diameter / Disc Diameter (cm)
0.383261	0.097979	-0.15662	0.117021	-0.27332	-0.05043	0.163281	-0.00357	0.012057	-0.26893	Days to Flowering
0.144035	0.118373	-0.00781	-0.35717	0.357092	0.570872	-0.32344	-0.23853	0.043022	-0.26552	Days to Maturity
0.1239	0.201942	-0.12185	-0.32526	0.090378	0.114854	0.386053	0.67932	0.011646	0.260589	Number of Seeds per Head
-0.07273	0.426196	-0.08193	0.495756	-0.14641	0.036488	-0.54626	0.143486	-0.07844	0.26495	1000-Seed Weight (g)
0.302016	-0.0481	-0.18029	-0.24271	-0.28787	0.065779	-0.10707	-0.15104	-0.02011	0.268629	Seed Yield per Plant (g)
-0.15112	0.198012	0.009629	0.327538	0.017078	0.518315	0.558022	-0.39267	-0.0051	0.262669	Oil Content (%)
0.008368	-0.1064	-0.45029	-0.30796	-0.04441	-0.24569	-0.04205	-0.40517	-0.07408	0.265664	Oil Yield per Plant (g)
0.14994	0.37647	0.551974	-0.26912	0.047874	-0.04774	-0.13262	-0.0485	-0.01245	0.268872	Heat Tolerance Index (HTI)

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9	7									
0.53999	0.393947	-0.13892	0.151704	-0.07967	-0.14367	0.159254	-0.12869	-0.01634	-0.26869	Stress Susceptibility Index (SSI)
-0.19091	0.446426	-0.28344	-0.18846	0.137053	-0.19357	0.016743	-0.13181	-0.01771	0.268776	Mean Yield under Stress (g)
-0.31761	0.270926	0.288879	-0.12638	0.04024	-0.42941	0.221774	-0.22458	-0.08561	-0.26631	Yield Reduction Due to Heat (%)

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The stem diameter variation of different genotypes is depicted in Figure 1B, ranging from 20.60 mm(T6) to 28.87 mm (T7). Mean separation (LSD = 0.55 mm, $p \leq 0.05$) revealed eight statistical groups, with the stems of the thickest group being T7 (' a'), followed by T4 (27.93 mm, 'b'). High mechanical strength, lodging resistance, and good vascular capacity of water and nutrient translocation during the reproductive phase in heat stress, as proven by the good stem development of both T7 and T4.

It can be seen that the leaf area changes from the different varieties and ranges from 411.33 cm^2 (T6) to 563.00 cm^2 (T7), as shown in Figure 1C. The leaf area was found to be statistically different between the nine associated genotypes (LSD = 3.79 cm^2, $p \leq 0.05$). It was observed that the largest leaf area was for T7 (563.00 cm^2) and T4 (547.33 cm^2). The increased leaf area of these genotypes is due to the photosynthetic surface area available and their light-trapping potential, which could result in increased carbon assimilation during grain filling under thermal stress.

As can be seen in Figure 1D, there is an asymmetric distribution of flower head diameter, ranging from 15.87 cm (T6) to 76.27 cm (T5). The genotype T5 was found to be statistically different from the rest of the genotypes in the name group ' a ' (very large head diameter), while all other genotypes grouped together under the name group ‘b' (15.87–22.53 cm; LSD = 53.67 cm at $p \leq 0.05$). The other genotypes showed stable disc development, but there was high variability in T5 (SE = 57.12), which showed environmental sensitivity. T5 has an outstandingly large head size, indicative of a high reproductive sink; however, it should be multi-environment validated.

The flowering time ranged from 54.0 days (T7) to 67.0 days (T6) with statistically distinguishable flowering times between the different genotypes (LSD = 0.63 days, $p \leq 0.05$) as indicated in Figure 1E. The genotypes T7, T4, and T2 had the earliest flowering (54.0-58.0 days, 'h'-'j'); they are considered to have a heat-escape strategy, enabling pollination earlier than the heat stress. The T6, T10, and T3 cultivars were later in flowering (65.0-67.0 days), which might result in higher heat stress on the blooming floral parts during the flowering stage.

Maturity duration varied from 93.00 (T7) to 111.67 days (T6) and was separated into six statistical groups (LSD = 2.43 days, $p \leq 0.05$) as shown in Fig. 1F. The maturity of the plants did not differ significantly between the different treatments with the lowest maturity time being shown by plants in treatment T7 (group ‘f’) and the highest by plants in treatment T4 (group ‘e') (96.33 days). The earlier T4 and T7 highlighted that it has a heat escape mechanism that ensures the grain filling process is complete before terminal heat stress is a bigger concern, which is beneficial for harvest management and protection in regions where heat stress may impact yield.

3.2. Mean Performance of Sunflower Treatments Under Heat Stress Conditions

The number of seeds per head is a significant genotypic difference under heat stress conditions (Figure 2A). The seed counts varied from genotype T6 (545) to genotype T7 (1181.67). High-performance group (classified as ‘a ' group) comprised of genotypes T7, T4, T2, T9, and T5 with seed count ranging from 976.67 to 1181.67, indicating high floret fertility and continued grain development under thermal stress. The second level performance (group ‘b’) consisted of T1, T8, T3, and T10 with seed counts ranging from 766.67 to 911.67. The lowest seed count (545) was produced by genotype T6 and was a group of seed counts that did not perform well ('c'). Means separated by a least significant difference (LSD) test (205.30 at $p \leq 0.05$) showed that those Genotypes with the same alphabetical designations were statistically the same, and those with different alphabetical designations were significantly

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different in the ability to set seed. The error bars were those for replicates, and T6 had a significantly higher number of error bars, suggesting that it was not performing consistently well under stress. The results showed the high potential of these elite genotypes (T7, T4, and T2) for direct cultivation in hot areas or as parents in breeding programs that would lead to reproductive resilience in sunflower.

The genotypic differences were also significant with regard to the capacity to fill the seed (Figure 2B) and partitioning of assimilates to the seed (Figure 2C), which were measured in terms of 1000-seed weight. The variation of the traits ranged from 53.67g (T6) to 78.33g (T7). The genotype T7 gave the highest seed weight, which was statistically significantly different from the other genotypes (' a'), and the genotypes T4, T9, and T2 gave intermediate seed weight ranging from 70.00 to 72.00 g and were grouped together as a performance level (' b'). Genotypes T1 and T5 formed group 'c' (65.00–66.67 g), while T8 and T3 shared group ' d ' (56.67–60.00 g). Treatments T10 and T6 (53.67-55.00 g) had the lowest weight out of the various treatments, and both were in the 'e' group. The LSD ($p \leq 0.05$) at the 0.05 level showed that the seed mass accumulation of the genotypes having the same alphabetical designation is not significantly different at the same level, but it is different at different alphabetical designations. The results suggest that the T7 genotype has a superior grain genotype, holds a high sink strength, and is a resource-saving genotype, which can be used to increase the grain quality and yield stability in the sunflower agroecosystems under thermal stress conditions.

The differences in seed yield per plant (reproductive productivity) were observed among the evaluated genotypes (Figure 2C). The seed yield ranged from least to most for the genotypes, with genotype T6 giving the least seed yield (66.67g) and genotype T7 giving the highest seed yield (127.0g). The highest yield was obtained in genotype T7 with a statistically significant top group ('a '), which was followed by genotype T4 (120.0 g, 'b'). The performance of genotypes T2 and T9 was found to be similar (108.67 g and 106.67 g, respectively), grouped in group 'c'. T5 and T1 were grouped between (group ' d ' and 'e '), respectively. The lowest yields were observed in T8 (80.0 g, 'f'), T3 (71.67 g, 'g'), T10 (70.0 g, 'g'), and T6 (66.67 g, 'h'), with T3 and T10 showing no significant difference. Genotypes having the same alphabetical designation are statistically equivalent, as mean separation at a $p \leq 0.05$ LSD guarantees this. Results from these studies have shown that these 2 high yielding, elite genotypes have potential for good yield under heat stress conditions, and they are expected to be used in heat stress agroecosystems immediately.

Oil percentage analysis (Figure 2D) revealed genotypic differences in the ability to store oil. The oil content varied from 35.4% (in genotype T6) to 51.93% (in genotype T4). The highest oil contents (51.93% and 50.43% respectively) were yielded by the two best performing genotypes, that is, genotype T4 and genotype T7, which formed the superior performance group ‘a’. A second genotype (group 'b') comprised genotypes T9, T2, T5, T8, and T1 with oil content ranging from 40.07% to 45.6%, which were not significantly different from one another. 'Oil accumulation was the least with genotype T3, T10, and T6 (35.4–37.33%) in one group (c). The results of the mean separation test with LSD (6.26) at $P \leq 0.05$ show that within the four alphabetic designations of the genotypes, the means are not significantly different from each other, but the difference between the different alphabetic designations in the oil synthesis efficiency is highly significant. The findings showed that T4 and T7 are a high oil genotype with potential to maintain the rate of lipid biosynthesis during thermal stress and can be considered as an elite option for the breeding programs to enhance oil quality and climate resilience.

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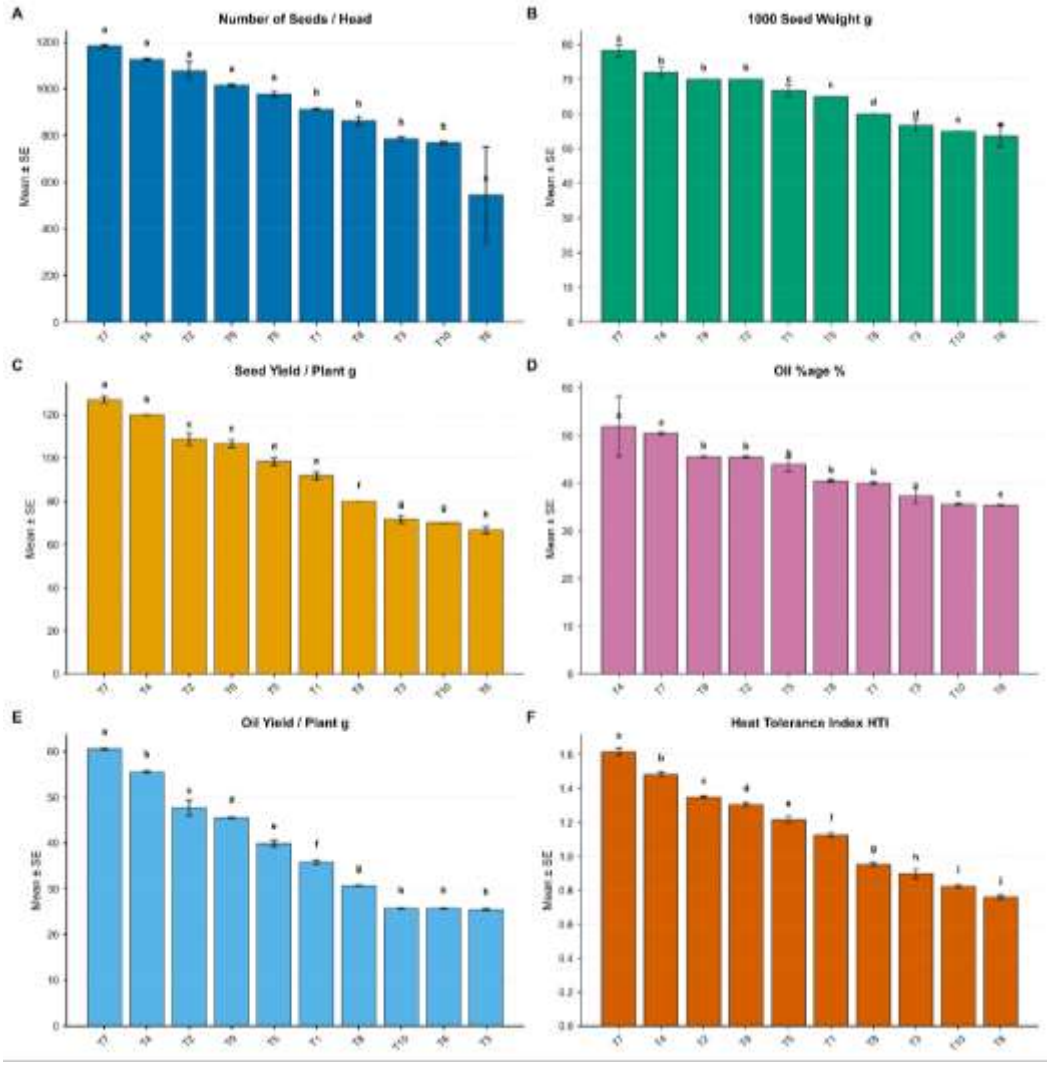


Figure 2. Mean performance of sunflower treatments under heat stress conditions

The differences in performance of the genotypes were also demonstrated by the production potential of oil (Figure 2E). The lowest yield of the oil obtained was 25.4 g in genotype T3, and the highest genotype T7 yielded 60.5 g. The highest oil yield was obtained by genotype T7, while T4 and T2 produced T2 (47.63 g,'c') and (55.5 g, 'b') respectively, which were grouped statistically as 'a ', 'b', and 'c', respectively. On the other hand, the genotype T10, T6, and T3 had the lowest oil production, which ranged from (25.4 to 25.7 g), and were grouped together under 'h' with no significant difference among them. The mean separation performed at $p \leq 0.05$ with an LSD value of 1.60 showed that the means of the oil accumulation of the same alphabetical designation did not differ significantly, and the means of the oil accumulation of different alphabetical designations differed significantly. The results showed that genotypes T7 and T4 are good oil producers under thermal stress conditions, and hence their utilization in direct cultivation in heat-prone areas is

recommended, and also as elite parental lines in a breeding program for breeding oil productivity improvement of sunflower.

Heat Tolerance Index (HTI) (Figure 2F) was used to test for stability in physiological performance and yield among the genotypes. The minimum value of HTI was 0.760 for genotype T6, while the maximum value of HTI was 1.617 for genotype T7. All ten genotypes were found to be in completely different performance groups, and the differences among the entries were highly significant and non-overlapping at $p \leq 0.05$ (a to j) according to the LSD 0.0305. The genotype having the highest value of HTI was T7 with a value of 1.583 (group ' a '), and was closely followed by T4 with a value of 1.483 (group 'b'), and T2 with a value of 1.350 (group 'c'), showing the highest heat tolerance. Genotypes with good heat stress tolerance and metabolic efficiency in a hot climate are some of the best-performing genotypes for minimizing heat stress effects on yield. However, the genotypes that showed the lowest HTI values were T6 (0.760, 'j'), T10 (0.823, 'i '), and T3 (0.897, 'h'), showing a high degree of heat sensitivity. All of the genotypes were completely separated by this statistical separation, further supporting the potential of HTI to be a very discriminative and reliable germplasm screening method in heat stress environments.

3.3 Stress Tolerance Indices and Yield Stability Under Heat Stress

The relative tolerances of evaluated genotypes to thermal stress were quantified by Stress Susceptibility Index (SSI) (Figure 3A). The range of SSI values was from a minimum of 0.407 (genotype T7) to a maximum of 1.287 (genotype T6), and all 10 entries with an LSD value of 0.0515 at $p \leq 0.05$ were statistically different from each other. Plants with T6, T10, and T3 had SSI>1.0 (1.287, 1.180, and 1.123, respectively) and were found to be very vulnerable to yield loss from heat. By contrast, seven other genotypes had SSI values less than 1.0, meaning that they were less prone to stress.

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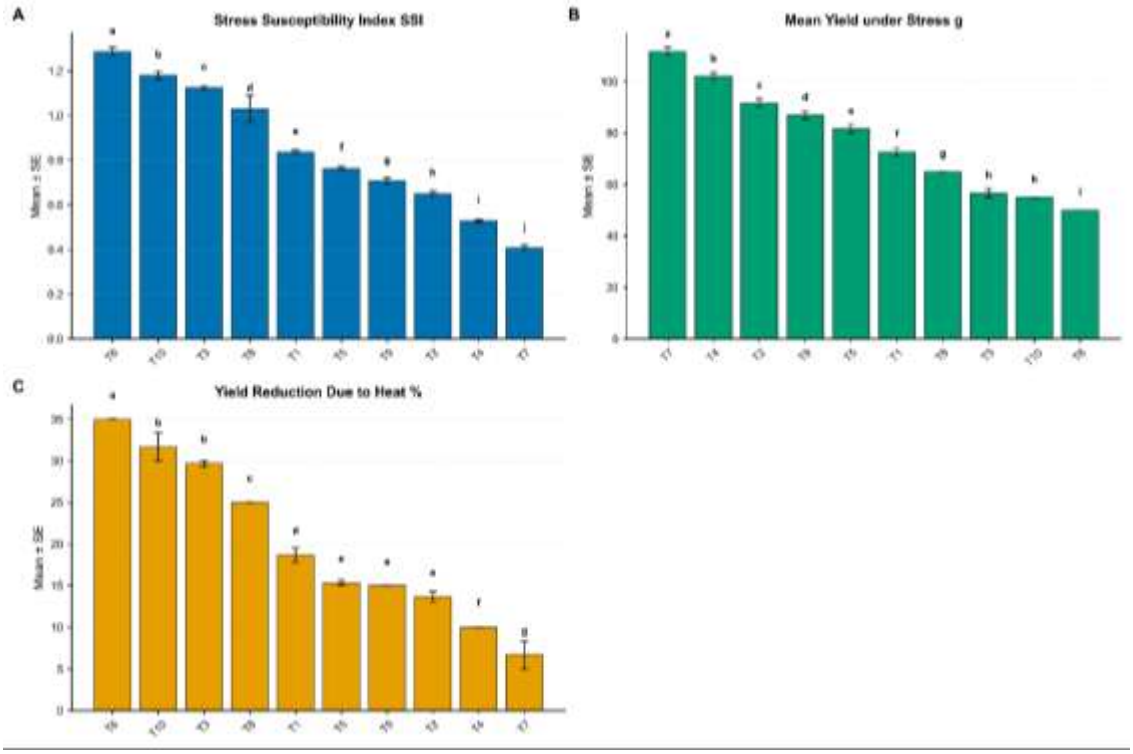


Figure 3. Different letters above bars indicate significant differences among treatments according to the LSD test at $P \leq 0.05$. Within the tolerant group, T7 had the lowest yield penalty under stress (SSI = 0.407, group 'j'), followed by T4 (SSI = 0.527, group 'i ') and T2 (SSI = 0.650, group 'h'). From an agronomic point of view, an SSI below 1.0 indicates that a genotype is better adapted to stable yield than would be expected based on the overall level of stress. This may be due to better osmotic adjustment, improved antioxidant level, or better source-sink functions. They are highly tolerant and are considered to be great candidates for direct use in thermal-sensitive agroecosystems or as parental lines in breeding for thermal resilience.

The seed production per plant was also analyzed under heat stress to understand absolute production under challenging conditions (Figure 3B). There were significant differences in genotypic means between the cultivars, ranging from 50.0g to 111.67g. The genotype T7 produced the highest mean yield of 111.67g under stress conditions, with genotype T4 (102.0g) and genotype T2 (91.67g) following. However, the T6 genotype exhibited the lowest yield (50.0 g), whereas the T10 (55.0 g) and T3 (56.67 g) had a low ability to tolerate stress. Performance groups (a–i) above each bar were determined statistically by an LSD of 2.77 at a $p \leq 0.05$ level, and groups with the same letter (T3 and T10, both labeled ‘h’) did not differ significantly in yield potential, but groups with different letters were significantly different. Overall, these results confirm that T7, T4, and T2 are high-yielding and heat-tolerant, and could be used directly in the production of heat-stress agroecosystems.

The differences in the genotype vulnerability (Figure 3C) were further emphasized by the percentage yield reduction due to heat stress. Significant differences were observed between the different genotypes as far as heat stress sensitivity is concerned (6.67% to 35%). The genotype T6 showed the highest loss in yield of 35%, followed

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by T10 (31.67%) and T3 (29.67%), which thus show high susceptibility. The genotype T7 showed the least loss of yield (6.67 %), followed by T4 (10 %) and T2 (13.67 %), which means that it is more tolerant to heat stress. The genotypes were classified according to mean separation, LSD value 2.38 at $p \leq 0.05$ to highly susceptible (T6, T10, T3), moderately susceptible (T8, T1), intermediate response (T5, T9, T2), and heat-tolerant (T4, T7) categories. Based on the results, the genotypes T7 and T4 had very high mechanisms of heat tolerance with minimal risk of yield loss, implying their high suitability for cultivation under hot environmental conditions and as parents for breeding sunflower heat-tolerant varieties.

Trait Correlations

The Pearson correlation heatmap in Figure 4 shows the linear relationships between the 15 morpho-physiological, reproductive, yield, and stress tolerance traits of sunflower genotypes under heat stress. The color gradient from dark blue (large negative correlations, r is close to −1.00) to dark red (large positive correlations, r is close to +1.00) represents three different types of relationships. The seed yield traits are strongly correlated with each other (r = 0.82–0.99), and the oil yield traits are similarly highly correlated, with vegetative vigor, yield, and resilience highly correlated, indicating that a genotype with high performance in one type of yield is likely to perform well in the other. Second, there is a clear physiological trade-off, that the two highest performing traits are very negatively correlated with the stress penalty indicators, stress susceptibility index (SSI), and percentage yield reduction due to heat (r = −0.83 to −0.98). Third, a strong positive correlation exists between phenological traits (days to flowering and days to maturity; r = 0.95), whereas the relationship between phenological traits with yield and tolerance indices is negative, suggesting that a longer developmental cycle may be a negative factor on the heat resilience of this germplasm.

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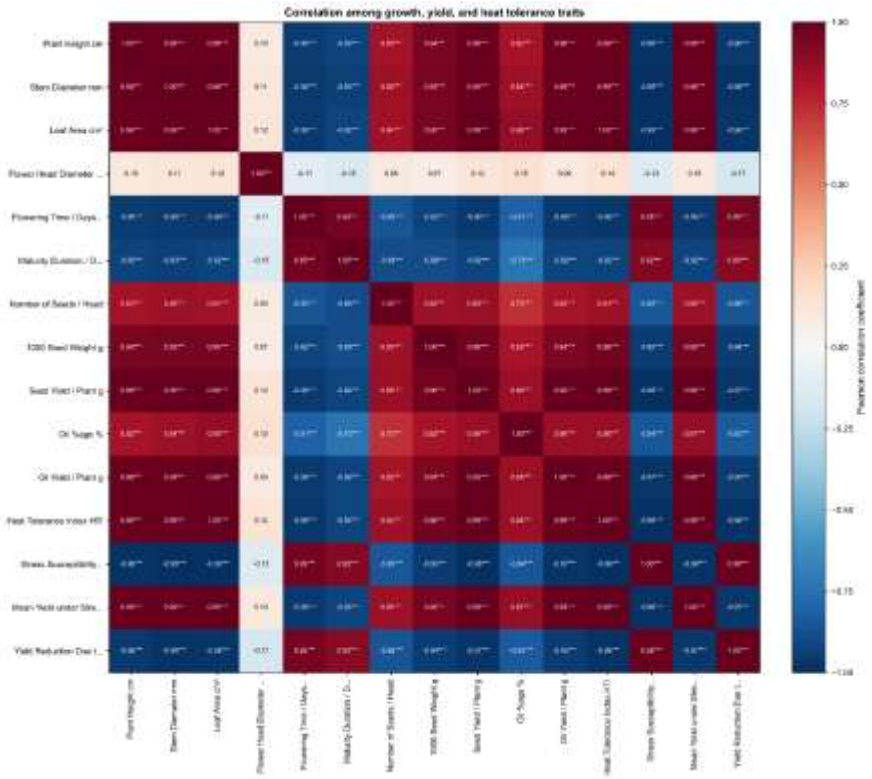


Figure 4. Pearson correlation heatmap among growth, yield, and heat tolerance traits in sunflower treatments. Values inside cells represent correlation coefficients. *, **, and *** indicate significance at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

There is a weak correlation with nearly all other variables with flower head/disc diameter ($|r|<0.20$); it is a morphological determinant that is largely independent of the major yield-stress gradient. All these correlations support the biological consistency of the dataset, underline trait interactions and trade-offs at thermal stress, and provide a sound statistical foundation for the development of multi-trait selection indices and predictive breeding models for the development of climate change-resistant sunflower ideotypes.

3.3 Principal Component Analysis (PCA) of Heat Stress Responses

Phenotypic variance is 98.39% (PC1 = 91.87% and PC2 = 6.52%), which was projected by PCA score plot in order to project the ten sunflower genotypes onto the first two PCs (Figure 5A). The PC1 axis is clearly divided into 3 distinct and separate clusters of genotypes with no overlapping, suggesting a good gradient of heat stress response and overall agronomic performance. The negative region of PC1 in Table 3 (Cluster 1: green; T6, T10, T3, T8, T1) is associated with lower expression of traits, reduced productivity, and increased heat susceptibility. Cluster 2 (orange: T4, T7) is at the extreme positive end of PC1, with high vegetative vigor, high yield potential, and good heat tolerance. Cluster 3 (purple: T5, T9, T2) is concentrated around the origin along PC1, with relatively adapted genotypes, including T5, which has a markedly positive deviation along PC2, suggesting a distinct phenotypic signature,

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which may include combinations of traits or physiological responses, not captured in the main PC1 gradient. The absence of spatial overlap and spatial segregation of the clusters facilitates efficient genotype separation in terms of integrated morpho-physiological interactions with thermal stress and provides a statistically valid and biologically relevant genotype ranking, selection, and breeding prioritization framework for heat-prone agroecosystems.

Figure 5B shows the contribution of the traits measured in the multivariate separation of the sunflower genotypes along the first two principal components. The diameter of the flower head (disc) was the most important morphological feature contributing to the differentiation among the genotypes in the PCA space, accounting for about 0.95–1.00 of the space, suggesting that this trait is very strongly determined by the heat stress. The other nine traits (e.g., yield loss due to heat, 1000-seed weight, oil yield, plant height, stem diameter, seed yield, mean yield under stress, flowering time, and stress susceptibility index (SSI)) contribute at a low and similar level (<0.10). Variation in the head diameter trait was strongly associated with most of the phenotypic variance in the PC1-PC2 space, suggesting a considerable variance between the head diameter trait and the other agronomic, yield, and stress-index traits. Biologically, this means that head diameter is a very good screening character for rapid genotype stratification of heat-stressed sunflower populations. However, further examination of PCA axes that are more meaningful with respect to yield and tolerance suggests that the multivariate approach to selection, such as higher-dimensional PCA axes or other multivariate selection strategies, may be necessary to fully capture the genetic architecture of adaptation to climatic stress and stability of yield.

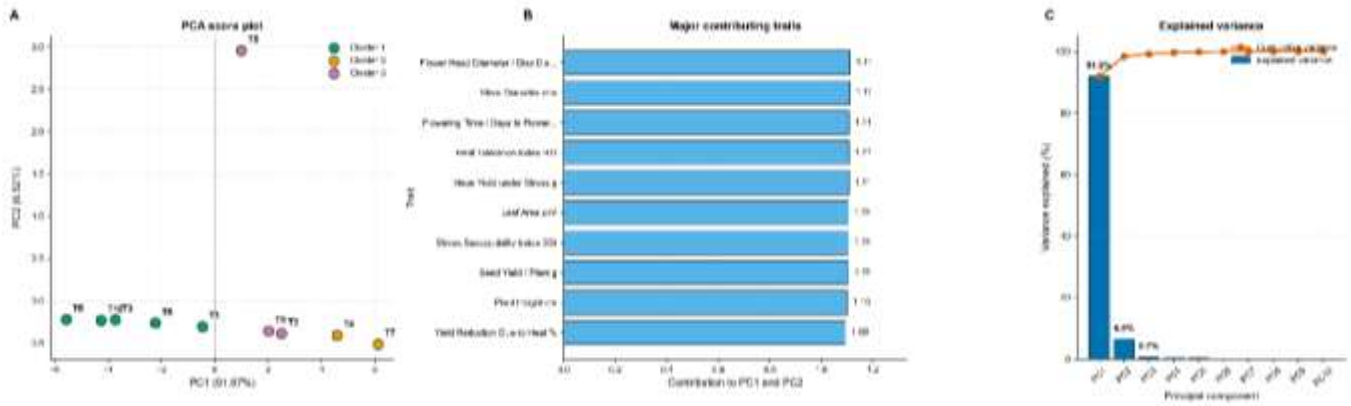


Figure 5. Principal component analysis of sunflower treatments under heat stress. A: PCA score plot showing the distribution of treatments according to PC1 and PC2 with cluster groups. B: PCA loading plot showing the most influential traits contributing to treatment separation. C: Explained and cumulative variance of principal components.

The scree plot and cumulative variance curve (Figure 5C) indicate the contribution of each PC to the total phenotypic variance. The first principal component (PC1) was responsible for almost all of the observed trait covariation (91.87%), indicating that

most of the observed trait covariation can be explained by a common physiological gradient, likely involving integrated plant vigor, biomass gain, and/or stress-resilience capacity. The remaining 6.52% is accounted for by the second component (PC2), which accounts for 98.39% of the variance. This rapid convergence demonstrates that it is possible to reduce the multivariate data to just two dimensions and that little loss of meaning takes place. The contribution from higher order PCs (PCs 3-10) is also small (all<0.75%) and reaches 100% by PC9, meaning that PCs higher than this do not contain a strong signal from biologically relevant patterns, but from random noise or small measurement variation. Thus, the two-dimensional PCA framework used for the discrimination of genotyped plants and the ranking of plants according to their heat tolerance in this study is statistically valid and is also biologically reasonable because only two dimensions of the PCA were used to interpret, cluster, and connect the plants to the traits.

3.4 Hierarchical Clustering, Trait Profiling, and Genotype Ranking

The hierarchical clustering of sunflower genotypes based on their multivariate trait profiles (Euclidean distance, phenotypic similarity) is shown in Figure 6A. This analysis shows a noticeable bifurcation at about 14.5, dividing the genotypes into two main clusters, which correspond to different adaptation strategies in response to heat stress. The left cluster (orange branches) consists of genotypes T1, T8, T6, T10, and T3 together in a group with low Euclidean distance and may share similar physiological characteristics with high stress tolerance and lower agronomic performance. Within this group, T10 and T3 are the two most similar phenotypically (distance ~0.8), suggesting that they share the same stress-response mechanisms and yield-limiting constraints, and T1 and T8 are the two most similar phenotypically (distance ~2.0), indicating that they share similar stress-response mechanisms and yield-limiting constraints. The other cluster (green branches), however, has T4, T7, T5, T2, and T9, which exhibit better heat tolerance and higher productivity potential. The distances from T2 to T9 are the shortest (~0.7) and the distances from T4 to T7 are the shortest (~1.8), it is notable. T5 is in this tolerant, but related, adaptive category, but at a moderate distance ~4.1. As expected, the two dominant branches are well separated vertically, and the two genotypic classes (stressed and non-stressed) were strongly discriminated in these two analyses (PCA and SSI), strongly supporting the spatial segregation observed. This clustering architecture can be applied to identify a breeding program strategy that maintains the stability of desirable trait combinations within each cluster and maximizes heterosis and breeding for more rapid development of new varieties that are adapted to climate change between clusters.

The standardized (z-score) phenotypic values for the 15 morphological, reproductive, and stress-tolerance traits were plotted as a heat map for the 10 genotypes (Figure 6B). It goes from dark blue (-2 standard deviations) to bright red/yellow (+2 standard deviations) with respect to the population mean. In the case of Genotype T7, all the traits like: seed yield, oil yield, oil percentage, 1000 seed weight, heat tolerance index (HTI), mean yield under stress have consistent positive deviations (bright red) while at the same time stress susceptibility index (SSI) and Yield reduction due to heat have strong negative deviations (dark blue). It's a clear indication

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of its high potential productivity and thermal resistance. On the other hand, standardized values for the genotypes T6, T3, T10, T8, and T1 are mostly negative with high stress indices for yield and growth, indicating heat-susceptible entries. Genotype T5 is an extreme morphological outlier with a very high signal for flower head/disc diameter, indicating a very large size of reproductive sink that seems to be independent of its stress response profile and overall yield. The visualization shows the relationship between traits within the system: The agronomic output and HTI cluster are correlated, with tolerant genotypes showing positive correlation, while SSI and yield penalty are correlated, with a negative correlation. A widely used heatmap to provide a rapid multi-trait diagnosis for genotype stratification, which provides information on trade-offs between traits, and helps to inform parent selection in sunflower breeding programs aiming for the development of ideotypes for climate-smart production.

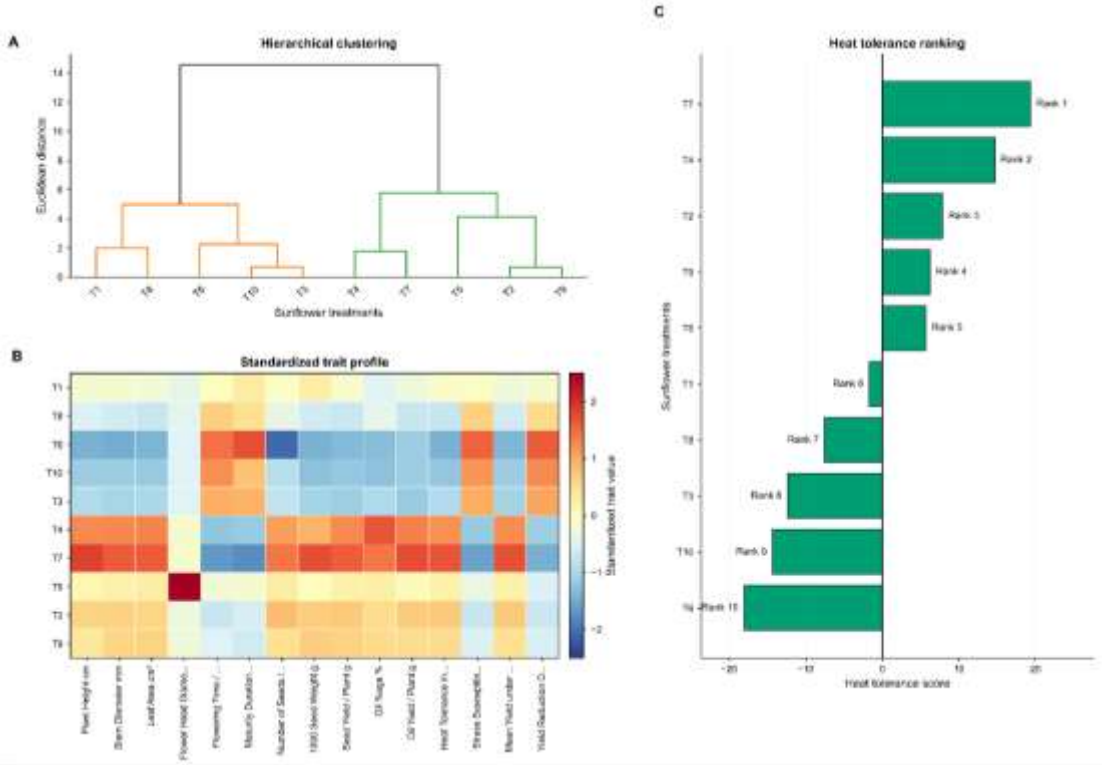


Figure 6. Cluster classification, standardized trait profile, and heat tolerance ranking of sunflower treatments. A: Hierarchical clustering dendrogram based on standardized trait values. B: Standardized trait profile heatmap ordered according to dendrogram clustering. C: Ranking of treatments based on the combined heat tolerance score.

Finally, the 10 sunflower genotypes were ranked according to their heat tolerance score determined here (Figure 6C), providing an immediate numerical stress adaptation potential assessment. The scoring scale is well polarised, with positive scores

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for entries that did not lose physiological function significantly under thermal stress and maintained it, and negative scores for entries that significantly lost physiological function under thermal stress. All of the heat-tolerant groups have positive scores, with genotype T7 being the most heat-tolerant with a score of ~19.5, followed by T4 and T2 with scores of ~14.8 and ~7.8, respectively, T9 with a score of ~6.5, and T5 with a score of ~5.8. Genotype T1 is somewhat intermediate and has a moderately negative score (~−2.0), meaning that it is intermediate/borderline in regard to stress adaptation. The four other studied genotypes (T8, T3, T10, and T6) have increasingly negative scores ranging from ~−7.5 to −18.0, with T6 showing the most negative score in the studied set, which means it is highly susceptible to yield loss and physiological disturbance under high temperatures. The spatial segregation shown in the PCA biplot is supported by the ranking, inverse SSI values, and the standardized trait heatmap, as well as the robustness and internal consistency of the multivariate selection framework. From an agronomic point of view, the difference between positive and negative scorers enables a quick selection of parental candidates for breeding programs to develop sunflower climate-resilient ideotypes T7 and T4 and the identification of T6 and T10 as reference scorers for stress-physiology studies or targeted agronomic mitigation in heat-sensitive production areas.

Heat stress, particularly at the reproductive phases (anthesis and grain filling), is a critical problem for the productivity of sunflower (Helianthus annuus L.) as it can influence the rate of phenology, decrease the viability of the pollen, and influence the partitioning of assimilates. The present study was also able to point out the negative impact due to heat stress on late sowing as a significant genotypic difference, and the loss of overall yield was seen between the various treatments evaluated. Early phenology was one of the major parameters that could be used to mitigate heat stress in this study, particularly in the two elite genotypes, T7 (DRSH-1) and T4 (KBSH-44). Days to 50% flowering and Days to Physiological maturity of these genotypes were significantly earlier, and are reflecting a strong ‘heat escape' strategy, because they are completing many of the important reproductive processes before high levels of terminal heat stress. This aligns with the recognized standards in agriculture, which call for aligning their activities with the best climate-smart practices in the environment. Moreover, the vegetative growth parameters of T7 and T4 are also higher, including plant height, stem diameter, and leaf area, which indicates better canopy architecture and the ability to capture light. The morphological traits are known to lead to higher biomass production and suggest higher transvascular efficiency and structural strength, which are essential to ensure water and nutrient flow with increasing thermal-driven evaporative demand (Xu et al., 2026).

There is also a difference between the reproductive sink strength and partitioning of assimilates of truly tolerant and early maturing genotypes, in addition to phenological escape. Both high and low temperature stress environments, genotypes T7 and T4 were outstanding for seed set per head and 1000-seed weight, indicating a capacity to maintain floret fertility and efficient source to sink translocation under thermal stress. Curiously, genotype T5 (PAC-36) had an unusually large flower

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head diameter, suggesting a very large potential reproductive sink. The average yield and stress tolerance scores, however, suggest that the yield of a sink is insufficient to guarantee yield under stress if there are no mechanisms for stress resilience (Buenrostro-Jáuregui et al., 2025). That is, the non-stabilizing effect head diameter had on overall yield was also seen in the PCA, with the primary axis being dominated by head diameter, but without it being one of the most important traits in the final composite rank. This decoupling further highlights the importance of the interactions among the traits. Furthermore, the high oil content and high oil yield per plant of T4 and T7 are related to their high oil biosynthesis efficiency, which is frequently substantially compromised under heat stress conditions in their heat stress susceptible counterparts, e.g., the T6 and T10.

Stress tolerance indices were introduced (Stress Susceptibility Index—SSI and Heat Tolerant Index—HTI) to get a quantitative value of stress tolerance and quantify yield stability. The very low SSI coupled with the very high HTI of T7 and T4 indicates their heat-induced yield loss resistance, which may be associated with their efficient osmotic adjustment, strengthened antioxidant defense mechanisms, or stomatal control. The positive values of vegetative vigor, yield components, and resilience variables, accompanied by their strong negative correlation with SSI and percentage yield reduction, also validate the data's biological consistency. These correlation networks clearly reflect the physiological compromise of the genotype – those with strong vegetative and reproductive efforts also have a powerful metabolic reserve to deal with thermal injury (Dey et al., 2026). The negative correlation between phenological duration and yield in stress conditions, however, further reinforces the penalty that is paid for late-maturing and sensitive genotypes (T6, T10, and T3), which are exposed to longer thermal stress of their florets and developing seeds, resulting in an increased yield and quality penalty.

Untangling the complex and multidimensional heat stress responses was helped by the use of multivariate statistical procedures, of which Principal Component Analysis (PCA) and Hierarchical Cluster Analysis were very useful. The first principal component (PC1) explains over 91% of the total variance, indicating that heat stress is a strong and consistent selection pressure, leading to a large physiological gradient within the germplasm. The univariate results confirm the segregation of the genetically susceptible and susceptible group (T6, T10, T3, T8, T1) from the elite tolerant group (T4, T7), which provides a good visual and univariate statistical validation of genotypic grouping. Furthermore, the creation of a Composite Heat Tolerance Score broke down these multivariate relationships into one useful measure, which could be used for action. An integrated approach that avoids the potential problems that might arise from selection for one superior desirable characteristic (e.g., big head size in T5) and ensures that an ideotype is fully balanced and has a well-developed phenotype. The heatmap was also standardized to provide a rapid diagnosis of the system and confirmed the inverse relationship between agronomic output and stress penalty metrics, consistent systemically.

Elite genotypes (T7 or DRSH-1 and T4 or KBSH-44) have been identified as heat-tolerant, high-yielding, stable, and having

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good quality of oil, which will have a considerable impact on sunflower breeding programs. The recommended cultivars or lines for cultivation in agroecosystems under high heat stress conditions may be these genotypes, as well as lines from which thermal resilience can be introgressed. Highly susceptible genotypes (particularly T6, EC-68414, and T10, CMS-108B) are valuable sensitive checks for physiological studies and/or contrasting donors for mapping populations for heat stress-sensitive regions of the genome. This is a very comprehensive phenotypic characterization and multivariate heat tolerance analysis, but it requires more research to understand the molecular and biochemical mechanisms (Hasanuzzaman et al., 2013). A validation of the physiological basis for the tolerance observed will require a combination of transcriptomic profiling, metabolomic analysis, and targeted (phenological) physiological assays (including chlorophyll-fluorescence, antioxidant enzyme kinetics, and canopy temperature depression). Furthermore, testing such elite genotypes in multiple and diverse multi-environment trials (METs) will be necessary to validate their heat-tolerant characteristics for different climatic conditions, and speed up the process of generating climate-smart sunflower ideotypes for the future agricultural systems (Sheta et al., 2024).

This study has been able to identify elite phenotypes and the morpho-physiological traits associated with heat tolerance, and future research should focus on identifying the molecular mechanisms related to heat tolerance. These contrasting genotypes (e.g., T7 vs. T6) will be key for the identification of candidate genes and genome-wide association studies (GWAS) related to the "heat escape" phenology, vascular robustness, and lipid biosynthesis stability under thermal stress. The use of marker-assisted selection of sunflower ideotypes with genomic advances will enable faster breeding of more climate-smart varieties to minimize the risk of climate change on global oilseed security.

4. CONCLUSION

In this study, the ability to identify elite sunflower phenotypes was achieved, as well as the ability to distinguish the morpho-physiological characteristics associated with heat tolerance. Under terminal heat stress, genotypes T7 (DRSH-1) and T4 (KBSH-44) showed better performance with respect to heat tolerance (early phenology, ‘heat escape’), yield stability, and stress tolerance. These genotypes can be used as genetic resources for sunflower breeding programs targeting the development of climate-smart ideotypes. Their performance, however, needs to be validated by multi-environment trials (METs) where they are grown in various environments and over different seasons to ensure that they are stable and stable with respect to adaptation before they can be recommended for direct cultivation.

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