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Research Article

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Integrative screening of maize genotypes for heat tolerance based on biomass, NDVI, and cell membrane thermostability traits

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ABSTRACT

Heat stress is a major threat to sustainable maize production especially during the spring season as high temperature causes tassel blast, pollen sterility, poor seed setting and barren cobs. The adoption of heat stress mitigation strategies is the prerequisite to reduce income risks for farmers and to ensure food security under changing climatic scenarios. Therefore, this study was designed to characterize maize germplasm under heat stress. For this purpose, a set of 125 indigenous maize genotypes were screened for key heat-responsive traits i.e., cell membrane thermostability (%), shoot and root weight (g), NDVI and chlorophyll content (SPAD units). Significant variation was observed among genotypes for all traits, with strong associations between CMT and biomass-related parameters under heat stress. Genotypes maintaining higher membrane stability exhibited better growth performance, indicating the importance of cellular integrity in stress adaptation. Based on combined trait performance and multivariate analysis DR-02, DR-150, DR-93, DR-139, EL-147, EL-234, and EL-36 were identified as heat tolerant genotypes, whereas EL-267, EL-94 and UML-1 were classified as heat sensitive. These findings highlight the utility of CMT and associated morpho-physiological traits for early-stage screening of heat tolerance in maize. The identified genotypes can serve as valuable parental material for breeding programs aimed at developing climate-resilient maize cultivars.

Keywords: Maize, Heat stress, Cell membrane thermostability, Heat tolerance, Germplasm screening

INTRODUCTION

Heat stress is a major threat to maize production as a sudden increase in temperature significantly impacts plant physiology, particularly affecting cell membrane thermostability, chlorophyll content and reduced biomass accumulation. Heat stress causes inactivation of cell membrane functionality, as a physiological response to high temperature (Blum, 1996).

Cell membrane damage persists for a longer time, it might reduce the transport of water, ions, and water-soluble proteins within plant cell membranes, thus affecting carbon assimilation, transport and storage (Kumar et al., 2011). Prolonged exposure to temperature above 35 °C is considered detrimental to crop growth and development, with temperature above 40 °C during flowering and grain-filling stage causing a significant reduction in yield (Shiferaw et al., 2011).

Additionally, heat stress affects the maize seedling growth, leading to poor shoot and root development, reduced biomass accumulation, chlorophyll degradation and alteration in cell structure (Himani et al., 2022; Wahid et al., 2007). Chlorophyll content is a crucial trait for plant health and photosynthetic efficiency. Heat stress can lead to significant decline in chlorophyll and carotenoid content in wheat and Arabidopsis seedlings, which is associated with heat sensitivity in plants (Kaur & Thind, 2017; Xue et al., 2010).

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Cell membrane thermostability has been widely used as an indirect selection criterion for heat tolerance, as it reflects the ability of plant cells to maintain structural integrity under stress. The research reports indicated that genotypes exhibiting higher cell membrane thermostability tend to relate with lower relative cell injury under heat stress conditions. For instance, the tolerant genotypes of wheat and sugarcane exhibited higher cell membrane thermostability and exhibited less relative cell injury, while the contrasting sensitive genotypes displayed lower cell membrane thermostability and higher relative cell injury (Ashfaq et al., 2022; Mehdi et al., 2023).

Understanding the relationship between physiological stability and growth performance is important for incorporating reliable selection traits into breeding programs.. Therefore, the present study aimed to evaluate indigenous maize germplasm under heat stress conditions and to classify genotypes into heat-tolerant and heat-sensitive groups based on key morpho-physiological traits.

Material and methods

Plant Material

The germplasm used in this study was obtained from Maize and Millet Research Institute (MMRI) Yousafwala, Sahiwal and Maize Research Station (MRS), Ayub Agricultural Research Institute (AARI), Faisalabad Pakistan. A total of 125 maize genotypes were included to classify their response under heat stress conditions.

Experimental Design and Growth Conditions

The experiment was laid out in a split-plot arrangement under a completely randomized design (CRD) with three replications. Seedlings were raised in 4 × 4 inch plastic pots containing peat moss (Peltracom) under greenhouse conditions, maintaining day/night temperature of approximately 28/23 °C for the control treatment. At 21 days after germination, when the second leaf was fully expanded, seedlings were transferred to heat stress conditions in a glasshouse for one week, with temperature maintained at approximately 42/30 °C (day/night). The temperature fluctuations were continuously monitored using Elitech RC-5 temperature data logger (Figure 1). Data was recorded at 28 days after germination using five plants per genotype per replication under both control and heat stress treatments.

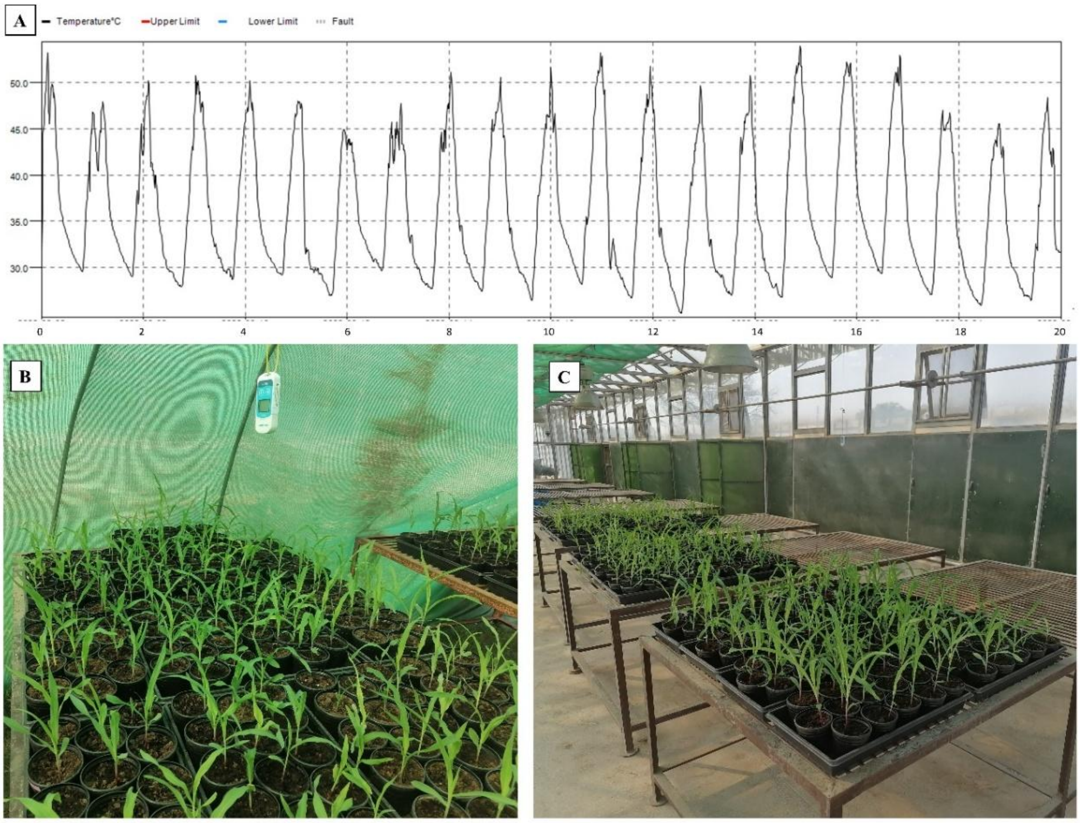

Figure 1. Experimental conditions and temperature monitoring during heat stress screening. (A) Daily temperature profile recorded at 15-minute intervals during the heat stress treatment, showing day and night fluctuations. (B) Seedlings grown under control conditions in the greenhouse. (C) Seedlings exposed to heat stress conditions in the glasshouse environment.

Fresh Shoot Weight (g)

To measure fresh shoot weight, the roots of the plants were cut with the help of scissors, and the shoots were weighed in the analytical weight balance. The average data of five plants per replication per genotype was recorded.

Dry Shoot Weight (g)

To measure dry shoot weight, the roots of the plants were cut with the help of scissors, and the shoots were placed in dry oven at 65 °C for 72 hours and weighed in the analytical weight balance. The average data of five plants per replication per genotype was recorded.

Fresh Root Weight (g)

To measure fresh root weight, the roots of the plants were cut with the help of scissors and washed thoroughly. To remove dust and soil particles, surface water was absorbed by tissue paper and weighed in the analytical weight balance. The average data of five plants per replication per genotype was recorded.

Dry Root Weight (g)

To measure dry root weight, the roots of the plants were placed in dry oven at 65 °C for 72 hours and weighed in the analytical weight balance. The average data of five plants per replication per genotype was recorded.

Cell Membrane Thermostability (%)

Cell membrane thermostability (CMT) was determined following the method of (Sullivan, 1972) from both control and heat-stressed plants. CMT was calculated using the formula:

$CMT (\%) = 1 - (E_1/E_2) \times 100$

Where: E_1 represents initial electrical conductance and E_2 represents final conductance after autoclaving.

Table 1. List of maize germplasm evaluated for heat stress tolerance.

Sr.#	Genotype	Sr.#	Genotype	Sr.#	Genotype	Sr.#	Genotype	Sr.#	Genotype
1	IL-17	**26**	DR-116	51	EL-185	**76**	EL-29	**101**	EL-122
2	DR-52	**27**	DR-40	52	EL-208	**77**	EL-24	**102**	EL-275
3	DR-18	**28**	DR-180	53	EL-202	**78**	EL-373	**103**	EL-231
4	DR-04	**29**	DR-113	54	EL-265	**79**	EL-30	**104**	IL-01
5	DR-07	**30**	DR-194	55	EL-123	**80**	EL-397	**105**	IL-02
6	DR-02	**31**	DR-152	56	EL-171	**81**	EL-140	**106**	IL-03
7	DR-150	**32**	DR-27	57	EL-195	**82**	EL-205	**107**	IL-04
8	DR-29	**33**	DR-49	58	EL-98	**83**	EL-176	**108**	IL-05
9	DR-23	**34**	EL-228	59	EL-227	**84**	EL-138	**109**	IL-06
10	DR-14	**35**	EL-267	60	EL-200	**85**	EL-75	**110**	IL-07
11	DR-87	**36**	EL-230	61	EL-212	**86**	EL-10	**111**	IL-08
12	DR-93	**37**	EL-302	62	EL-39	**87**	EL-145	**112**	IL-09
13	DR-88	**38**	EL-197	63	EL-97	**88**	EL-72	**113**	IL-10
14	DR-189	**39**	EL-76	64	EL-270	**89**	EL-228	**114**	IL-11
15	DR-11	**40**	EL-196	65	EL-203	**90**	EL-123	**115**	IL-12
16	DR-162	**41**	EL-366	66	EL-27	**91**	EL-413	**116**	IL-15
17	DR-184	**42**	EL-198	67	EL-94	**92**	EL-36	**117**	UML-1
18	DR-161	**43**	E-236	68	EL-199	**93**	EL-211	**118**	UML-2
19	DR-64	**44**	EL-293	69	EL-37	**94**	EL-149	**119**	UML-3
20	DR-96	**45**	EL-268	70	EL-138	**95**	EL-170	**120**	UML-4
21	DR-67	**46**	EL-269	71	EL-21	**96**	EL-77	**121**	UML-5
22	DR-139	**47**	EL-166	72	EL-226	**97**	EL-316	**122**	UML-6
23	DR-145	**48**	EL-278	73	EL-153	**98**	EL-414	**123**	UML-7
24	DR-167	**49**	EL-147	74	EL-23	**99**	EL-318	**124**	UML-8
25	DR-76	**50**	EL-234	75	EL-215	**100**	EL-194	**125**	UML-9

Chlorophyll Content (SPAD unit)

Chlorophyll content was measured using hand-held chlorophyll content meter Minolta SPAD-502 (Minolta Camera Co. Ltd, Japan).

Normalized Difference Vegetation Index (NDVI value)

NDVI measurements were taken with the help of GreenSeeker™ Handheld Optical Sensor Unit (Trimble Navigation Limited, US).

Statistical Analysis

Data was subjected to analysis of variance (ANOVA) to assess the variation among genotypes, treatments and their interaction (Steel et al., 1997). Correlation was assessed to determine a relationship among various traits. Mean trait values were further used to construct biplots for identifying heat-responsive genotypes (Yan & Kang, 2002).

Results

Screening of Maize Genotypes under Heat Stress

The present study evaluated 125 maize genotypes at the seedling stage under heat-stress and non-stress conditions based on set of morphological and physiological traits. The aim was to identify genotypes with stable performance under elevated temperature. Traits recorded included fresh root weight (g), dry shoot weight (g), dry root weight (g), chlorophyll content (SPAD units), NDVI and cell membrane thermostability stability (%). The analysis of variance indicated a highly significant effect ($p \leq 0.01$) of treatments, genotypes and their interaction for all measured traits, demonstrating substantial variability among genotypes under contrasting temperature (Table 2).

Table 2. Analysis of variance (ANOVA) showing mean square values for morpho-physiological traits under control and heat stress conditions.

SOV	Df	CMT	CC	NDVI	FSW	DSW	FRW	DRW
Replications	2	135	3.2	0.00227	1.1	0.00	4.39	6.59
Treatments (A)	1	119807**	3286**	0.51498**	3177.5**	763.8**	2849.92**	700.25*
Error A	1	15	0.1	0.00008	0.1	0.05	0.1	0.35
Genotypes (B)	124	228**	27.2**	0.00360**	26.3**	9.14**	30.78**	7.10**
A×B	124	182**	7.2**	0.00121**	6.1**	1.30**	4.94**	0.92**
Error B	248	2	0.5	0.00004	0.2	0.10	0.13	0.00

Note: * and ** indicate significance at $p < 0.05$ and $p \leq 0.01$, respectively.

CMT = Cell membrane thermostability; CC = Chlorophyll content (SPAD units); NDVI = Normalized Difference Vegetation Index; FSW/DSW = Fresh/Dry shoot weight (g); FRW/DRW = Fresh/Dry root weight (g).

Fresh Shoot Weight (g)

The data for fresh shoot weight (g) was found to be highly significant showing diversity among the genotypes (Table 2). Under non-stress conditions, the genotype DR-18, EL-211, EL-24, IL-17, and DR-93 showed higher fresh shoot weight whereas EL-302, EL-269, and DR-18 showed lowest fresh shoot weight. Under heat-stress, DR-139, DR-02, DR-93, EL-211 and DR-04 maintained comparatively higher weight, while DR-18, UML-3 and UML-1 showed reduced performance. Across both conditions, EL-234, DR-139 and DR-02 displayed relatively stable performance, whereas UML-5, UML-4 and EL-267 remained consistently low (Figure 2A).

Dry Shoot Weight (g)

The data for dry shoot weight (g) was found to be highly significant showing diversity among the genotypes. Under control conditions, DR-184, UML-05, DR-02, EL-122, and EL-230 exhibited higher dry shoot weight, whereas IL-11, EL-196, and DR-18 showed lowest dry shoot weight. Under heat stress, DR-139, DR-02, EL-147, DR-150 and DR-93 performed better, while EL-94, UML-1 and EL-212 showed reduced dry shoot weight. Across both conditions, EL-234, DR-139 and DR-04 maintained relatively stable performance, whereas UML-5, IL-06 and EL-94 showed poor performance (Figure 2B).

Fresh Root Weight (g)

Fresh root weight also differed significantly among genotypes (Table 2). Under non-stress condition, the genotype UML-4, DR-04, DR-93, IL-17, and DR-139 exhibited higher fresh root weight, while EL-302, IL-09, and EL-275 showed lower fresh root weight. Under heat stress, DR-139, DR-93, DR-02, EL-147 and DR-150 performed better, whereas DR-11, IL-09 and DR-67 exhibited reduced root weight. Genotype DR-02, EL-147 and DR-139 showed consistent better performance across both conditions, while EL-94, UML-1 and EL-267 remained among the poor performers (Figure 2C).

Dry Root Weight (g)

Dry root weight revealed significant genotypic variations (Table 2). Under non-stress condition, DR-07, IL-15, DR-139, UML-4, and DR-02 showed higher dry root weight, whereas DR-14, EL-200, and EL-176 were lower performers. Under heat-stress condition, DR-139, DR-02, EL-36, EL-147 and EL-93 maintained higher root weight, while EL-94, DR-87 and EL-138 exhibited reduced DRW. DR-14, EL-200 and DR-139 showed consistent performance across both environments, while EL-373, EL-267 and EL-94 showed poor performance (Figure 2D).

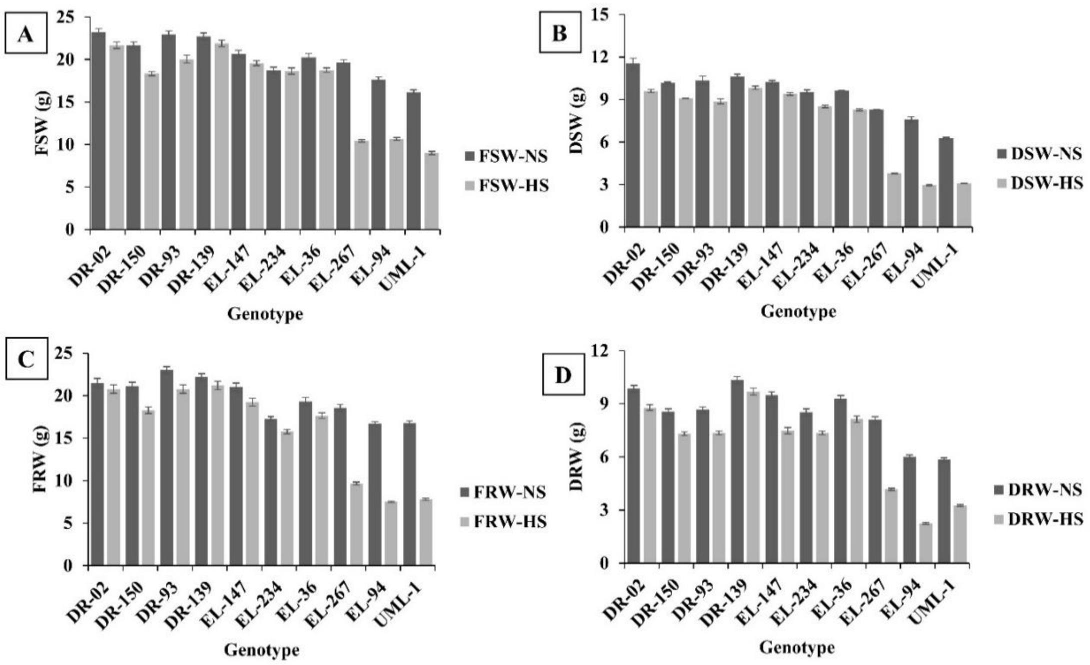

Figure 2. Comparison of biomass traits under non-stress and heat stress conditions in selected maize genotypes.(A) Fresh shoot weight (FSW), (B) dry shoot weight (DSW), (C) fresh root weight (FRW), and (D) dry root weight (DRW) measured under non-stress (NS) and heat stress (HS) conditions. Values represent mean performance of selected genotypes, showing variation in biomass accumulation under contrasting temperature regimes.

Cell Membrane Thermostability (%)

Significant variation in CMT was observed between treatments and among genotypes ($p \leq 0.01$). Under non stress condition, the genotype UML-4, UML-5, DR-04, DR-23 and 36 (EL-230) showed higher CMT, while EL-196, EL-269, and EL-98 recorded lower values. Under heat stress conditions, DR-139, EL-36, EL-234, DR-02 and EL-147 exhibited higher CMT, whereas EL-94, EL-267 and UML-1 showed lower stability. Across environments, EL-234, EL-147 and EL-36 showed stable behavior, on the other hand, UML-1, IL-06 and EL-267 remained poor performer (Figure 3A).

Chlorophyll Content (SPAD units)

Chlorophyll content differed significantly among genotypes under both environments ($p \leq 0.01$). Under non-stress condition, UML-4, IL-04, EL-30, and EL-293 showed higher values of CC, whereas EL-196, and EL-176 showed lower CC. Under heat-stress, IL-04, DR-139, EL-24, EL-293 and DR-02 maintained higher chlorophyll content, while UML-1, EL-94 and EL-10 exhibited reduced values. EL-234, EL-366 and IL-17 showed stable performance across control as well as heat treatment, while UML-4, EL-94 and 86 EL-10 showed poor performance (Figure 3B).

Normalized Difference Vegetation Index (NDVI value)

NDVI showed highly significant variation among genotypes ($p \leq 0.01$). Under control conditions, EL-30, DR-04, EL-226, DR-23, and DR-02 had higher NDVI values, whereas EL-138, EL-205, and IL-07 showed lower values. Under heat-stress, DR-02, EL-147, DR-139, DR-152, and EL-234 performed better among the total genotypes under study, while EL-94, UML-1, and EL-138 showed reduced NDVI. DR-02, EL-147, and DR-93 exhibited stable performance across both conditions, whereas EL-30, UML-1, and EL-230 remained lower (Figure 3C).

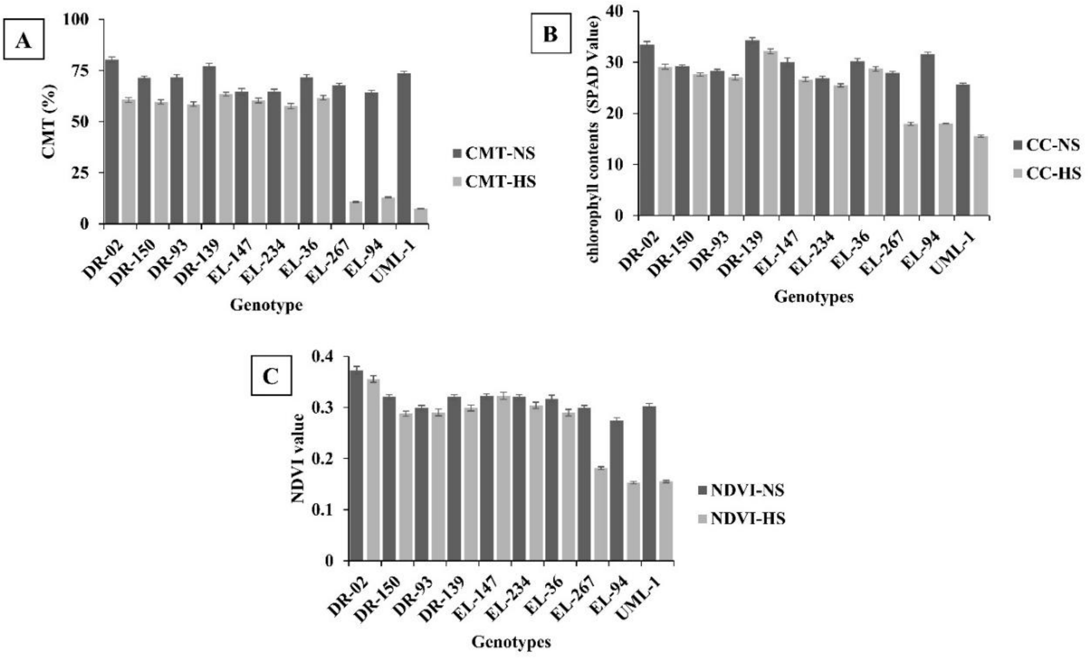

Figure 3. Physiological responses of selected maize genotypes under non-stress and heat stress conditions. (A) Cell membrane thermostability (CMT), (B) chlorophyll content (SPAD units), and (C) normalized difference vegetation index (NDVI) measured under non-stress (NS) and heat stress (HS) conditions. Values represent mean performance of selected genotypes, highlighting variation in physiological traits under contrasting temperature regimes.

Correlation Analysis under Non-stress and Heat Stress Conditions

Correlation analysis indicated positive correlations for all traits studied under control and heat stress conditions, but the strength of the correlations differed between both conditions. Under control conditions, many traits associated with biomass showed moderate to strong correlations with one another; for instance, fresh shoot weight (FSW) had a strong correlation to dry shoot weight (DSW) (r = 0.68) and fresh root weight (FRW) (r = 0.73), while there was a moderate correlation between FSW and dry root weight (DRW) (r = 0.49). Similarly, dry shoot weight (DSW) had a positive response in relation to fresh root weight (FRW) (r = 0.58) and dry root weight (DRW) (r = 0.44). Cell membrane thermostability (CMT) also had moderate positive responses towards dry shoot weight (DSW) (r = 0.51) whilst displaying weak correlations with respect to fresh shoot weight (FSW) (r = 0.46) and fresh root weight (FRW) (r = 0.41). NDVI and chlorophyll content (CC) were positive but weakly correlated with biomass related traits, with CC showing correlations ranging from r = 0.28 to 0.36. These results indicate coordinated growth under favorable conditions, with relatively lower dependence on physiological stress-related traits.

Under heat stress conditions, the strength of relationship was increased across most trait combinations. FSW exhibited a very strong correlation with DSW (r = 0.84) and FRW (r = 0.82), exhibiting strong association between biomass related traits. FRW also showed strong associations with DSW (r = 0.80) and CMT (r = 0.68), while DSW remained positively correlated with CMT (r = 0.65) and DRW (r = 0.56). Notably, CMT showed consistently stronger correlations with all biomass traits under heat stress conditions, including FSW (r = 0.72) and FRW (r = 0.68), compared with non-stress conditions. Chlorophyll content and NDVI maintained positive associations with biomass related traits, although their association remained moderate (r ≈ 0.38-0.50). A comparison (non-stress vs heat-stress) revealed a clear strengthening of trait associations under heat stress. For instance, correlations between FSW and DSW increased from 0.68 to 0.84, similarly between FSW and FRW from 0.73 to 0.82, and likewise CMT with FSW from 0.46 to 0.72. This change indicates that under heat stress, plant performance becomes more tightly linked with membrane stability and biomass accumulation. Overall, biomass related traits (FSW, DSW, FRW, and DRW) showed strong interdependence in both environments, while the contribution of physiological traits, particularly CMT, became more pronounced under heat stress. These patterns of correlation suggest that traits such as CMT, FRW, and DSW provide a reliable indicator for selecting heat-tolerant genotypes under elevated temperature.

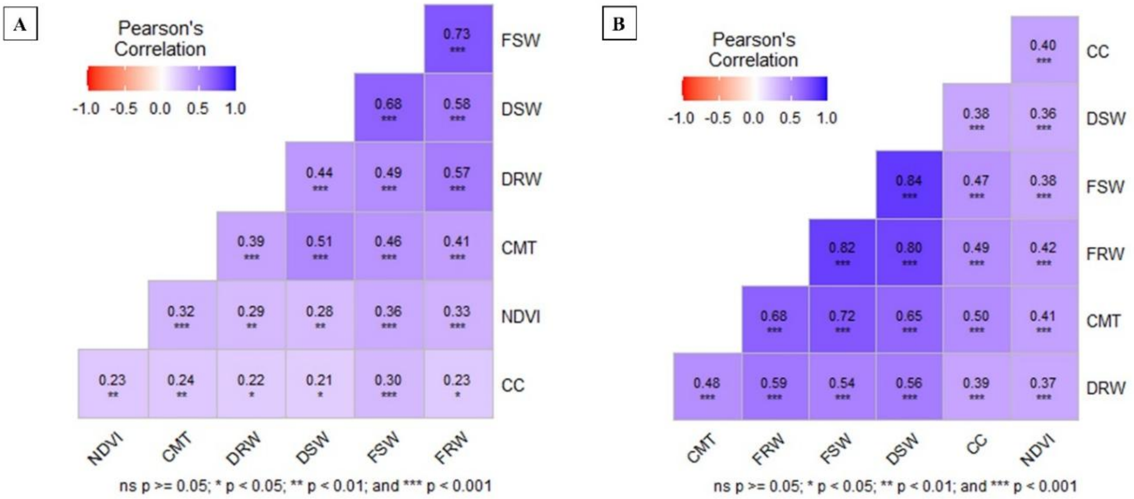

Figure 4. Pearson correlation analysis of morpho-physiological traits under contrasting temperature conditions**.** (A) Correlation matrix under non-stress conditions. (B) Correlation matrix under heat stress conditions. Color intensity represents the strength and direction of correlation (-1 to +1). Asterisks indicate levels of significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Biplot Analysis for Selection of Heat-tolerant and Sensitive Genotypes

Biplot analysis was performed to examine the multivariate relationship among genotypes and morpho-physiological traits under both non-stress and heat stress conditions (Figure 5). The first two principal components (PC1 & PC2) explained a substantial proportion of the total variation. Traits under both environments were clearly separated along the principal components. Most of the heat stress-associated traits (FSW.HS, FRW.HS, DRW.HS, DSW.HS, NDVI.HS, CC.HS and CMT.HS) were clustered on the positive side of PC1, indicating strong positive associations among these traits under stress conditions. In contrast, traits recorded under non-stress conditions (FSW.NS, FRW.NS, DSW.NS, NDVI.NS, CMT.NS, and CC.NS) were positioned relatively closer to the origin or distributed along a different vector orientation, reflecting variation in trait contribution between environments. The direction and length of trait vectors indicated their relative contribution to total variation. Biomass-related traits under heat stress, particularly FSW.HS and FRW.HS, showed longer vectors, suggesting a greater role in discriminating against genotypes under stress conditions. Similarly, CMT.HS was positioned distinctly, indicating its importance as a physiological indicator associated with heat tolerance. Genotype distribution in the biplot revealed clear separation based on performance. Genotypes located in the direction of heat stress trait vectors and away from the origin exhibited superior performance under stress conditions. In this context, DR-02 (6), DR-150 (7), DR-93 (12), DR-139 (22), EL-147 (49), EL-234 (50), and EL-36 (92) were positioned closer to key heat-responsive traits, indicating their stable and superior performance across traits under elevated temperature. Conversely, genotypes located opposite the direction of major trait vectors or closer to the negative side of PC1 showed poor performance. Accordingly, EL-267 (35), EL-94 (67), and UML-1 (117) were identified as heat-sensitive genotypes due to their distant positioning from key contributing traits. Overall, the biplot analysis highlighted that biomass traits (FSW, FRW, DSW) and cell membrane thermostability (CMT) played a central role in differentiating genotypes under heat stress, and their combined contribution can be effectively used for selecting heat-tolerant maize lines.

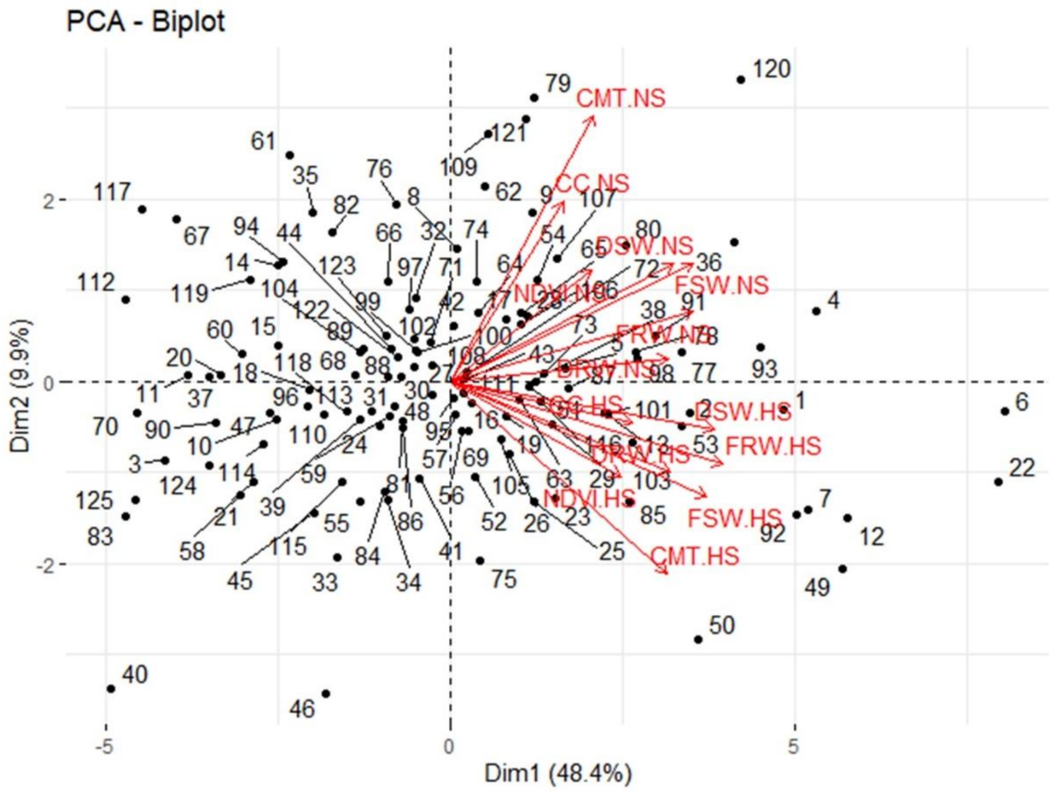

Figure 5. Principal component biplot showing the relationship among genotypes and morpho-physiological traits under non-stress and heat stress conditions. The first two principal components (Dim1 = 48.4% and Dim2 = 9.9%) explain the major proportion of variation. Vectors represent trait contributions, while points indicate individual genotypes. Traits measured under non-stress (NS) and heat stress (HS) conditions are shown separately. Genotypes positioned in the direction of trait vectors exhibit higher values for the corresponding traits, facilitating the identification of heat-tolerant and heat-sensitive genotypes.

DISCUSSION

Genetic improvement through selection is the most economic, long-term and sustainable approach to cope with adverse impact of abiotic stresses on crop productivity (Naveed et al., 2016; Zafar et al., 2020). In maize, the development of climate-resilient cultivars has been reported to increase yield stability by 5-20% under stress prone environments (Cairns & Prasanna, 2018). Harnessing genetic variation already exists among the maize germplasm for improving thermotolerance might be a suitable option (Alam et al., 2017). However, direct selection merely based under stress conditions is often unreliable due to its complex inheritance and strong environmental effect (Edmeades et al., 1993). In this context, the use of secondary traits with stable association to yield offers a more effective strategy (Alam et al., 2017).

In the present study, a diverse set of indigenous maize genotypes was evaluated at seedling stage to identify heat-responsive genotypes. The screening was performed based on cell membrane thermostability, chlorophyll content, NDVI and biomass related traits. The observed significant variation among genotypes and strong genotype × environment interaction indicates substantial genetic diversity for heat response, which can be utilized in breeding programs. Cell membrane thermostability has been widely used as a reliable indicator of heat tolerance across crop species (Blum et al., 2001; Khajuria et al., 2016; Wahid et al., 2007). The positive association observed in this study between CMT and biomass traits (FSW, FRW, DSW, and DRW), particularly under heat stress, suggests that genotypes maintaining membrane integrity are better able to sustain growth under elevated temperature. Similar relationship has been observed in wheat and rice, where reduced membrane injury was linked with improved stress tolerance and yield stability (Khajuria et al., 2016; Khakwani et al., 2012; Kumari et al., 2009). The enhanced correlation of CMT with biomass traits under stress conditions further indicates that membrane stability becomes a limiting factor for growth under high temperature. This response is often associated with improved antioxidant capacity and efficient reactive oxygen species (ROS) scavenging, which helps maintain cellular homeostasis (Hasanuzzaman et al., 2019).

Heat stress is known to impair chlorophyll synthesis and accelerate pigment degradation, leading to reduced photosynthetic capacity. In agreement with previous studies, a decline in chlorophyll content was observed across genotypes under heat stress. However, tolerant genotypes exhibited relatively smaller reductions, indicating better maintenance of photosynthetic apparatus (Iqbal et al., 2020; Ristic et al., 2007; Yousaf et al., 2022). The moderate positive association between chlorophyll content and biomass traits under stress conditions suggests that genotypes capable of sustaining chlorophyll levels are able to maintain higher growth rates. Similar findings have been reported in which biomass accumulation in maize is closely linked with chlorophyll content under temperature variation (Sun et al., 2016). This suggests that chlorophyll content is closely related to biomass accumulation in maize plants under heat stress.

NDVI, as a rapid and non-destructive indicator of canopy vigor and stay-green characteristics, also showed positive associations with biomass traits. The relatively strong relationship between NDVI and biomass under heat stress suggests that NDVI can serve as a useful alternate for screening heat tolerance. Previous studies have demonstrated that genotypes with higher NDVI values tend to maintain photosynthetic activity and exhibit delayed senescence under stress conditions (Cerrudo et al., 2017). According to reports, genotypes that exhibit tolerance to high temperatures show a longer retention of chlorophyll compared to genotypes that are sensitive to such conditions. This observation has been documented in studies conducted on rice and creeping bent grass (Sohn & Back, 2007).

Moreover, correlation analysis revealed a strong relationship between biomass-related traits (FSW, FRW, DSW, and DRW) and physiological traits (CMT, CC and NDVI) under both environments, while strong associations were observed under heat stress. Notably, the relationship between CMT and biomass traits increased substantially when subjected to heat stress, indicating that physiological stability and biomass accumulation are more closely associated with under adverse environmental conditions. Therefore, selection based on combined physiological and biomass traits would be more effective than relying on individual traits alone.

The biplot analysis provided an integrated view of genotype performance across multiple traits and contrasting temperature regimes. The clustering of stress-associated traits, along with their relatively strong vector contributions, highlights their key role in differentiating genotypic response. Genotypes including DR-02, DR-150, DR-93, DR-139, EL-147, EL-234, and EL-36 were positioned in close proximity to major heat responsive traits, indicating stable and superior performance under heat-stress conditions. In contrast, EL-267, EL-94, and UML-1 were located distant from these vectors, indicating relatively weaker adaptation to heat stress. These results demonstrate that genotypes closer to the origin are more stable, whereas those located along trait vectors show better performance for the respective traits (Yan & Kang, 2002).

Collectively, the findings suggest that cell membrane thermostability, biomass accumulation, chlorophyll content, and NDVI are closely associated and contribute to heat tolerance in maize. Incorporating these traits into selection frameworks may increase the effectiveness of breeding programs focused on developing heat-resilient cultivars. Moreover, the identified genotypes offer promising genetic resources for improving performance under elevated temperature environments.

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