

EXPLORING THE GENETIC OF HEAT STRESS TOLERANCE FOR YIELD ATTRIBUTING TRAITS IN TROPICAL MAIZE (*Zea mays* L.)

ABSTRACT

Heat stress severely affects maize yield and productivity, making it essential to identify and select heat-tolerant genotypes. Three hundred seven testcrosses derived from a biparental population were evaluated during summer at two distinct environments to know their performance and association of traits under heat stress conditions. Analysis of variance revealed significant variability among the testcrosses, indicating substantial genetic diversity. Correlation analysis identified strong positive correlations between grain yield and related traits like plant height and ears per plant, highlighting these as vital selection criteria in breeding programmes. Additionally, Best Linear Unbiased Predictors (BLUPs) were utilized to evaluate the *per se* performance of the testcrosses, identifying the top-performing ones with superior heat tolerance. These top testcrosses achieved an average grain yield of 3.47 t ha⁻¹, surpassing the population mean of 3.13 t ha⁻¹. The top 10% of selected testcross families exhibited a yield gain of 0.34 t ha⁻¹, representing a 9.79% improvement over the population under heat stress conditions. In order to create resilient maize hybrids, the study effectively isolated 18 elite testcrosses with improved heat tolerance that outperformed the superior check P1844. This provides a mitigation method for reducing the negative effects of climate change on maize productivity.

Keywords: Heat stress, Testcrosses, Correlation, BLUPs, MetaR

1. Introduction

Maize (*Zea mays* L.), known as the 'Queen of Cereals,' is an important crop contributing to food security after rice and wheat due to its high physiological and photosynthetic efficiency and superior yield. Under ideal circumstances, it is quite productive, although it is susceptible to heat stress and drought. A temperature that is higher than the threshold and persists long enough to harm crop growth and development irreversibly is referred to as heat stress. In India, maize production is 35.67 million tonnes with productivity of 3032 kg per hectare from 9.47 million hectare cultivated area. In Karnataka, 1.6 million hectares of area is under the cultivation of maize and production of 3.55 million tonnes and productivity of 2755 kg per hectare [1].

Maize is highly susceptible to drought and heat stress, especially during the reproductive stage [2]. Under heat stress, plants open their stomata to cool leaves through transpiration, but in combined drought and heat conditions, they keep stomata closed to conserve water, increasing leaf temperatures and yield losses. For instance, a temperature rise above 30°C leads to a 1% reduction in yield under optimal conditions, 1.7% under drought, and over 40% under combined stress [3]. Meseka et al. [4] reported yield losses of 77% from combined stresses.

Comment [SK1]: the study provides valuable insights into maize heat tolerance, but there are some limitations related to environmental scope, trait diversity, and methodology transparency. It over emphasis yield, lack information about heritability

With climate change becoming a reality, the need for heat-tolerant cultivars is essential. Hosmani et al. [5] assessed genetic gains in maize testcrosses from two heat-tolerant heterotic multi-parental synthetic populations (MPS 1 and MPS 2). The top 10% of $F_{2:3}$ families were selected for further genetic gain analysis, showing significant improvements in grain yield (58.55%), anthesis (-2.05%), silking (-2.24%), ASI (-3.35%), plant height (4.25%), and ear height (5.49%) in MPS 1 compared to MPS 2. These findings suggest elite heat-tolerant lines with strong testcross performance. Vinutha [6] worked on genetic analysis for heat stress tolerance in hybrids developed from heat resilient doubled haploid lines of tropical maize. Basavarajeshwari [7] have also contributed to heat tolerance research in tropical maize through genetic analysis and QTL studies. A key step in developing 'climate-smart' maize is identifying germplasm tolerant to abiotic stresses [8].

There are limited reports on improving maize for both drought and heat stress simultaneously. In this paper, we are reporting performance of testcrosses derived from heat and drought tolerant biparental population evaluated under heat stress condition.

2. Materials and Methods

A biparental population (BIP1) was developed at International Maize and Wheat Improvement Center (CIMMYT), c/o ICRISAT, Patancheru, Hyderabad, Telangana by crossing a heat-tolerant line, CAL 182, with a drought-tolerant line, CAL 1514, both belonging to heterotic group A. The resulting 307 early inbred lines were crossed with a tester from the opposite heterotic group, CML 451 during *khariif* 2023. The testcrosses obtained were evaluated for heat stress at two locations *i.e.*, Main Agricultural Research Station, Raichur and Agriculture College, Bheemaranagudi during summer 2024. Sowing was carried out in the second fortnight of March 2024 to ensure that the flowering period would align with the peak of summer (around mid-may), thereby subjecting the crops to heat stress under field conditions.

The experiment was conducted using an alpha lattice design with two replications, including four checks (P1844, P1855, DKC9162, DKC9108). Observations were recorded on various quantitative traits, such as days to 50% anthesis (DA), days to 50% silking (DS), anthesis-silking interval (ASI), plant height (PH), ear height (EH), SPAD at 60 DAS (SPAD₆₀), SPAD at 90 DAS (SPAD₉₀), ears per plant (EPP), and grain yield (GY).

Grain yield being a complex trait is affected by many genetic and non-genetic factors. In order to identify the independent variables like yield attributes, association studies serve as an effective tool. The analysis of variance for alpha lattice and predicted BLUPs were carried out in MetaR software and correlation coefficients were computed as suggested by Pearson in R software using metan package.

3. Results and Discussion

3.1. Analysis of variance (ANOVA) for yield and yield attributing traits in testcrosses under heat stress conditions across locations

The results of ANOVA for morphological and yield traits in testcrosses from the BIP1 population under heat stress are shown in Table 1. The mean sum of squares due to genotypes were highly significant for most traits, including days to 50% anthesis, days to 50% silking, anthesis-silking

Comment [SK2]: the methodology has strong points in terms of breeding strategy, statistical methods, and trial design, but could be improved by broadening environmental scope, increasing replication, and providing more detailed data collection, equipment used, and selection criteria.

Comment [SK3]: Justification for using BLUPs is not sufficiently explained, and the genetic architecture underlying the observed traits is not discussed. Lastly, the significance of variances is inconsistently reported, which could cause confusion in interpreting the results.

interval, SPAD at 90 DAS, ears per plant and grain yield. In the present study, we did not observe the leaf firing and tassel blast symptoms.

Significant Genotype × environment interactions observed for several traits indicated varying testcross behavior across locations. Environmental and Environmental × Replication variances were significant, reflecting the diverse conditions of the evaluation sites. The highly significant differences among environments were possibly a result of the different soil and climatic conditions prevailing in the different environments. Block and replication variances were also significant, indicating the importance of proper experimental design to control field variability. Residual variance was relatively low, suggesting good model fit. Hosmani et al. [5], Nelimor et al. [8], Alam et al. [9], and Teng et al. [10] also observed comparable outcomes for all traits under conditions of heat stress.

Table 1: Analysis of variance for morphological and yield related traits in testcrosses derived from BIP1 across locations under heat stress condition

Source of variance	DA	DS	ASI	PH	SPAD_90	EPP	GY
Genotype Variance	38.07**	23.33*	1.796***	166.04	520***	149.20***	194.10**
Environmental Variance	0.49*	6.43*	10.464**	2781.96**	3598.1***	290.941**	210.71*
Genotype × Environmental Variance	3.09*	1.02	0.557	2926.40**	8.5*	121.224**	201.93**
Block × Replication Variance	109.49**	108.66**	0.0016	226.51	188.1***	1.652	118.4
Environmental × Replication Variance	31.49*	22.44*	0.765*	892.8*	119.8*	8.075*	524.7***
Residual Variance	8.43	7.85	0.2287	232.09	18.5	5.138	752.49

*, ** and *** Significant at 0.05, 0.01 and 0.001 level of probability, respectively.

DA = Days to 50% anthesis (d), DS = Days to 50% silking (d), ASI = Anthesis-silking interval(d), PH = Plant height (cm), SPAD_90 =SPAD at 90 DAS, EPP = Ears per plant, GY = Grain yield (t ha⁻¹)

3.2. *Per se* performance of testcrosses under heat stress conditions for selected traits across locations

The *per se* performance of the testcrosses was estimated using Best Linear Unbiased Predictors (BLUPs). BLUPs were determined for each genotype by applying a mixed-effects model, where the environments were treated as fixed effects and the genotypes as random effects. The current study investigated the performance of testcrosses from the BIP1 maize population under heat stress conditions in environments characterized by moderate to high vapour pressure deficit (VPD). The results revealed a significant genotype × environment (G × E) interaction, influencing grain yield variations across the two environments.

Across locations, *per se* performance of testcrosses derived from BIP1 population for grain yield ranged from 2.53 (Z1821-254) to 3.60 (Z1821-17) t ha⁻¹ with a population mean of 3.13 t ha⁻¹ under heat stress conditions. The top 10 per cent BIP1 population demonstrated superior yield performance (Table 2), with grain yields ranging from 3.39 to 3.60 t ha⁻¹ and mean of 3.47 t ha⁻¹.

Several testcrosses performed better for grain yield than the best check hybrid P1844 (3.42 t ha⁻¹). These top 10 per cent selected testcross families provided a gain of 0.34 t ha⁻¹ representing 9.79 per cent improvement over the population under heat stress conditions.

The findings of this study are consistent with those reported by Vinayan et al. [11], who emphasized that relative humidity and VPD are key environmental covariates contributing to the large G × E interactions observed in grain yield under heat stress. These environmental factors can significantly alter the physiological responses of maize plants, leading to variations in yield outcomes depending on the specific conditions encountered. As a result, maize crops exposed to heat stress at different locations may respond differently, depending on the level of VPD at maximum temperature (Tmax). This underscores the importance of considering environmental variability when assessing the performance of potential maize varieties under stress conditions.

Grain yield, being a quantitatively inherited trait, is highly sensitive to environmental changes, making it challenging to determine the true genetic value of potential varieties. As highlighted by Sserumaga et al. [12], significant G × E interactions necessitate the careful sampling of target environmental conditions during the breeding process. This ensures that the varieties released for commercial production are well-adapted to the environments in which they will be grown. Consequently, it is crucial to evaluate maize trials across multiple environments to capture the full extent of G × E interactions and accurately assess the performance of breeding lines.

The comparison of average grain yield performance between the top 10 per cent of entries from the BIP1 population and commercial checks revealed that the heat stress breeding pipeline produced superior or at least comparable results. The selected testcross families achieved an average grain yield of 3.47 t ha⁻¹, surpassing or equaling the yields of commercial hybrids like P1844 (3.42 t ha⁻¹), P1855 (3.34 t ha⁻¹), DKC9162 (3.26 t ha⁻¹) and DKC9108 (3.07 t ha⁻¹). These findings align with those of Vinayan et al. [11], who reported that top entries from heat-stressed breeding programmes performed better than commercial checks, particularly in moderate-yielding locations.

Eighteen testcrosses (Z1821-17, Z1821-6, Z1821-14, Z1821-263, Z1821-37, Z1821-50, Z1821-24, Z1821-104, Z1821-84, Z1821-91, Z1821-113, Z1821-205, Z1821-280, Z1821-126, Z1821-303, Z1821-96, Z1821-9, Z1821-143) outperformed the superior check P1844, which had a grain yield of 3.42 t ha⁻¹ (Table 2). Similarly, Sserumaga et al. [12] found that the top 15 testcross hybrids provided a 5.9% yield advantage over the best commercial check, further validating the effectiveness of targeted breeding under heat stress conditions. Overall, this study highlights the importance of evaluating testcrosses under diverse environmental conditions to identify superior genotypes that can maintain high yields under heat stress.

Table 2: BLUPs for top 10 per cent of the selected testcrosses of maize across locations under heat stress conditions

Sl. No.	Entries	GY	DA	DS	ASI	PH	EH	EPP
1	Z1821-17	3.60	58	61	2	159.53	89.67	0.76
2	Z1821-6	3.57	59	62	2	161.44	88.84	0.72

3	Z1821-14	3.56	61	63	2	157.79	85	0.72
4	Z1821-263	3.56	61	62	2	158.02	86.63	0.75
5	Z1821-37	3.55	61	63	2	156.93	86.29	0.73
6	Z1821-50	3.54	60	62	2	158.89	87.34	0.71
7	Z1821-24	3.53	60	62	2	159.64	91.13	0.77
8	Z1821-104	3.53	60	61	2	159.81	85.94	0.73
9	Z1821-84	3.52	60	62	2	159.28	85.69	0.7
10	Z1821-91	3.52	61	63	2	156.5	86.85	0.73
11	Z1821-113	3.51	60	62	2	157.08	88.47	0.72
12	Z1821-205	3.51	60	62	2	159.43	89.74	0.72
13	Z1821-280	3.5	61	63	2	158.65	85.82	0.68
14	Z1821-126	3.49	60	62	2	158.38	87.59	0.72
15	Z1821-303	3.48	60	62	2	157.52	86.24	0.75
16	Z1821-96	3.47	58	61	3	160.13	87.82	0.75
17	Z1821-9	3.47	60	62	2	154.02	90.66	0.72
18	Z1821-143	3.43	61	64	3	153.71	85.76	0.68
19	Z1821-298	3.42	60	62	2	155.12	84.39	0.71
20	Z1821-64	3.42	60	62	2	156.29	84.52	0.7
21	Z1821-225	3.41	60	62	2	158.5	86.34	0.75
22	Z1821-180	3.41	60	63	3	156.13	86.06	0.69
23	Z1821-119	3.41	60	62	2	156	87.55	0.71
24	Z1821-212	3.40	60	62	2	158.89	84.69	0.73
25	Z1821-246	3.40	60	62	2	159.59	85.24	0.7
26	Z1821-53	3.40	60	62	2	156.38	86.27	0.72
27	Z1821-166	3.40	60	62	2	156.71	90.54	0.73
28	Z1821-205	3.40	60	63	3	154.67	89.34	0.71
29	Z1821-196	3.39	60	63	3	159.4	91.93	0.72
30	Z1821-88	3.39	61	63	2	157.67	84.25	0.68
31	Z1821-274	3.39	61	63	2	160.9	87.46	0.72

	Mean	3.47	60.09	62.25	2.16	157.83	87.22	0.72
	P1844 (C)	3.42	58	60	2	164.96	93.62	0.71
	P1855 (C)	3.34	57	59	2	162.65	81.95	0.72
	DKC9162 (C)	3.26	55	58	2	160.04	78.67	0.67
	DKC9108 (C)	3.07	57	60	2	167.89	91.4	0.75

GY = Grain yield (t ha⁻¹), DA = Days to 50% anthesis (d), DS = Days to 50% silking (d), ASI = Anthesis-silking interval (d), PH = Plant height (cm), EH = Ear height (cm), EPP = Ears per plant

3.3. Association among yield and its attributing traits in testcrosses under heat stress condition

The results of correlation analysis (Table 3) showed that grain yield had a significant positive correlation with several yield-attributing traits: plant height (0.22), ears per plant (0.60), and SPAD at 90 DAS (0.34), all at the 1% level of significance. This suggests that improving these associated traits would enhance grain yield (Fig 1).

The correlation analysis revealed that traits such as grain yield, plant height, SPAD at 90 DAS and ears per plant had significant positive associations with each other under heat stress condition. In contrast, traits such as days to 50 per cent anthesis and days to 50 per cent silking were significantly negatively correlated with all the other traits. The same trend was earlier noticed by the findings of Elmyhun et al. [13], Zaidi et al. [14], Tandzi et al. [15] and Longmei et al. [16].

Table 3: Estimates of correlation between grain yield and its attributing traits in testcrosses derived from BIP1 population

	DA	DS	ASI	SPAD_60	SPAD_90	PH	EH	EPP	GY
DA	1								
DS	-0.25*	1							
ASI	-0.45*	-0.36*	1						
SPAD_60	-0.14	-0.34	0.06	1					
SPAD_90	0.02	-0.13	0.19	0.56	1				
PH	-0.09*	-0.21*	0.12*	0.08	0.18**	1			
EH	-0.12	-0.09	0.06	0.1	0.18**	0.19**	1		
EPP	-0.24	-0.23	0.1	0.12*	0.28**	0.15**	0.09	1	
GY	-0.36**	-0.39*	0.06	0.34**	0.02	0.22**	0.09	0.60**	1

DA = Days to 50% anthesis (d), DS = Days to 50% silking (d), ASI = Anthesis-silking interval(d), PH = Plant height (cm), SPAD_90 =SPAD at 90 DAS, EPP = Ears per plant, GY = Grain yield (t ha⁻¹)

Under heat stress conditions, grain yield was positively associated with plant height. However, as heat stress intensifies, plant height tends to decrease due to decreased internodal elongation, leading to a significant reduction in grain yield. This result was in agreement with the report of Kaur et al. [17] and Jodage et al. [18]. Dinesh et al. [19] and Jodage et al. [18] reported that plant height and ears per plant were positively associated with grain yield under heat stress condition. Therefore, it can be concluded that selecting for greater plant height and ears per plant could lead to improved grain yield under heat stress conditions.

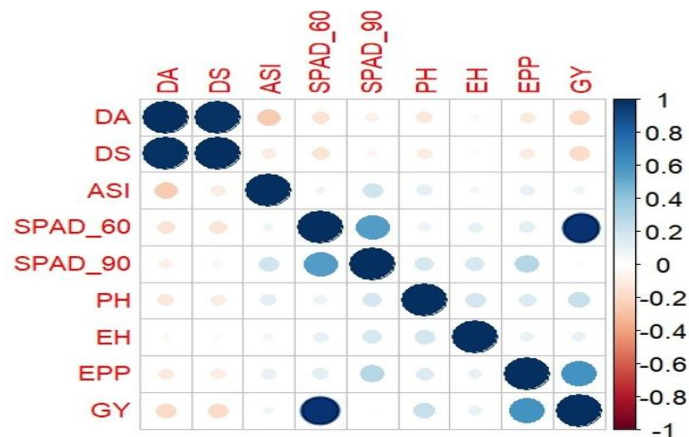


Fig 1. Correlogram depicting estimates of correlation coefficients between yield and its component traits among testcrosses of BIP1 population

4. Conclusion

The study on the genetic basis of heat stress tolerance in tropical maize has provided significant insights into the genetic variability and environmental interactions affecting yield-related traits under heat stress conditions. Through comprehensive ANOVA and correlation analyses, the research identified key traits such as plant height and ears per plant, which have strong positive associations with grain yield. The findings underscore the importance of these traits as selection criteria in breeding programmes aimed at developing heat-tolerant maize varieties. The evaluation of testcrosses across different environments revealed substantial genotype-by-environment interactions, highlighting the critical role of environmental factors in trait expression. The study successfully identified 18 elite testcrosses with improved heat tolerance that outperformed the superior check P1844 and holds potential for developing heat-tolerant maize hybrids and creating new cycles of inbred lines with enhanced yield potential.

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