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Nature of Inheritance to Heat Stress and Selection of Inherent Genotypes in Tropical Maize

Chiseche Mwanza[1], Mebelo Mataa[2] and Langa Tembo[2*]

[1]Syngenta Zambia Limited, Private Bag 33088, Lusaka, Zambia.
[2]Department of Plant Science, School of Agriculture Sciences, University of Zambia, P.O. Box 32379, Lusaka, Zambia.

Authors' contributions

This work was carried out in collaboration among all authors. Author CM undertook the research performed the statistical analysis, interpreted the data and writing of the manuscript. Authors MM and LT helped in planning for execution of research, guided the analyses and interpretation process and proof read the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Maize is an important cereal in sub-Saharan Africa. Its production is however hampered by both biotic and abiotic factors. Among the abiotic factors, heat stress has been reported to cause yield losses. The objective of this study was therefore to identify tolerant genotypes to heat stress and determine the type of gene action conditioning heat tolerance in tropical maize. To achieve these objectives, five maize inbred lines (L2 [P1]; DTS 6,36 [P2]; L5527 [P3]; DTS 6,6 [P4] and DTS 6,92 [P5]) were mated in a 5 x 5 half diallel. Their progeny were evaluated at a heat prone site (Lusitu) and at the University of Zambia (UNZA), a control site. The experiment was laid as a randomised complete block design with two replications in each site. Highly significant differences ($P \leq 0.01$) were obtained among genotypes in Lusitu with regards to all measured parameters. The crosses[P2 (DTS 6,36) x P4 (DTS 6,6)] and [P4 (DTS 6,6) x P5 (DTS 6,92)]were identified as tolerant genotypes to heat stress. Further analysis showed that the general combining ability (GCA) effects for parent P4 (DTS 6, 6) and P3 (L5527) were positively and negatively significantly different ($P \leq 0.01$) from zero respectively with regards to all measured parameters. On the other hand, crosses [P1 (L2) x P3 (L5527)] & [P4 (DTS 6,6) x P5 (DTS 6,92)]were found to possess desirable significant ($P \leq 0.05$) specific combining ability (SCA)effects from zero. The results of baker's ratio

**Corresponding author: E-mail: langatembo@yahoo.com;*

obtained for responseto heat stress for all secondary traits measured were found to be greater than 0.88. This implied that additive gene action was more important in conditioning the response of these traits to heat tolerance.

Keywords: Heat tolerance; combining abilities; gene action; inbred lines.

1. INTRODUCTION

Maize (*Zea mays* L.) is an important cereal grain in the sub-Saharan Africa. A major portion of maize produced worldwide is used for animal and human consumption [1]. Each part of the maize plant may be put to one or the other use [2,3]. The green plant, made into silage, has been used with much success in the dairy and beef industries. The dried leaves and upper part are used to provide relatively good forage for ruminant animals owned by many small farmers in developing countries.

In sub-Saharan Africa, maize yield among small scale farmers remain low, averaging 1.2 t ha^{-1} due to biotic and abiotic stresses [4]. Among the abiotic factors, heat stress causes maize yield losses ranging from 1 to 1.7 % per day for every rise in temperature above 30°C and has become a major concern that needs attention [5]. Increased exposure to high temperature causes permanent tissue injury to developing/young leaves and the injured tissues dry out quickly [6,7,8].

It has been suggested that selection and utilisation of heat tolerant genotypes is the most feasible way to reduce yield losses in maize [9]. Selection of desirable genotype, where one or two variables (traits) are involved, has been achieved using visual selection and or by genotypic trait analysis [10,11]. On the other hand, multivariate analysis such as principle component analysis (PCA) have been used where genotypic selection relies on genotypic response of several traits [12]. It has been determined that an effective approach in breeding for heat tolerance in maize is to utilize indirect selection through secondary traits such as chlorophyll concentration, plant height, curling etc. as they are highly heritability compared to direct selection such as yield [13,14]. However, it must be noted that an important step in developing heat tolerant 'climate smart' maize varieties requires identification of an appropriate breeding strategy. In that regard, it is vital to investigate the type of gene action conditioning the transfer of trait under-study in order to understand the type of breeding strategy to employ [15]. Computation of GCA and SCA effects helps the breeder to further understand the performance of each genotype and identify heat tolerant lines that can be used as parents in the breeding program. The objective of this study was therefore to identify tolerant genotypes to heat stress and determine the type of gene action conditioning heat tolerance in tropical maize.

2. MATERIALS AND METHODS

2.1 Germplasm Used and Conduct of Experiment

Five inbred lines [L2 (P1); DTS 6,36 (P2); L5527(P3); DTS 6,6 (P4) and DTS 6,92 (P5)] with varying tolerance to heat stress were mated following a 5 x 5 half diallel, generating 10 crosses. Development of crosses were carried out as by Ndeke and Tembo [16]. These were provided and previously evaluated for heat tolerance by Golden Agriculture Research Trust (GART) (Latitude $14^0$40' S; longitude $25^0$01'E) maize breeding programme. The F_1 crosses were evaluated for heat tolerance in Lusitu (Latitude 16˚ 08'S;longitude 28˚50'E) as done by Phiri [17] and a control experiment was conducted at University of Zambia (UNZA) (Latitude15˚23'S; longitude 28˚20'E).Both experimental sites have a sandy loamy type of soil.

The 10 generated progeny, together with the check XH3506 hybrid were evaluated for heat stress in Lusitu and another set of the same crosses were planted at UNZA field station. The experiment was laid out following randomized complete block design with two replications in both site. Standard cultural practices such as weeding, and appropriate fertilizer applications were followed. The highest mean temperature for the entire cropping season was approximately recorded as 33.5°C and 30°C for Lusitu and UNZA respectively.

2.2 Data Collection

Heat stress phenotypic data was obtained following the standard procedure as done by ZaidandCairns [18]. Vigour was recorded 3 weeks after germination following a scale rating

of 1 - 5. Where 1 = not vigorous, 2=slightly vigorous, 3= moderately vigorous, 4= vigorous, 5=very vigorous. Chlorophyll concentration index (CCI) was measured using a chlorophyll meter (model SPAD-502, Japan). The chlorophyll meter was calibrated to zero every time before taking on a new measurement. Then CCI measurements were obtained at 6 weeks after germination. Curling was recorded at 7 weeks after germination following a scale rating of 1 – 5. Where 1 = Very curled, 2 = curled, 3= moderately curled, 4= slightly curled, 5= not curled. Plant height was measured from the soil surface to the base of the tassel (excluding tassel length) at 6 weeks. Final maize yield was not measured in Lusitu owing to a 4-week dry and hot spell experienced in the month of December- January 2019, which caused a total crop failure in most parts, south of Zambia [19].

2.3 Data Analysis

A 2 tailed paired t-test was done in excel to compare mean genotypic performance in two locations. Analysis of data on heat stress was done on secondary traits using analysis of variance (ANOVA), assuming a fixed model. Obtained means of total biomass, root biomass, shoot biomass, vigour, curling, plant height and CCI were separated using the fisher protected Least Significant Difference (LSD) method, at a significant level of α= 0.05. Multivariate analysis was done using Principle Component Analysis (PCA) using correlation matrix.

Half diallel analysis was performed using Griffing's method IV, fixed model I, using regression analysis. Genotypic GCA and SCA variance components and effects were determined as done by Singh and Chaudhary [20]. The relative contribution of GCA and SCA was estimated using Baker's ratio [21].

$$2\delta^2gca/ (2\ \delta^2gca + \delta^2sca)$$

Whereσ^2gca and σ^2sca are the variance components due to GCA and SCA respectively.

All data analysis was performed using Genstatstatistical software [22].

3. RESULTS

3.1 Genotypic Responses Performance of Measured Parameters

A comparison of mean performance of measured parameters at UNZA and Lusitu site revealed highly significant differences (P ≤ 0.01) (Table 1) between the two location with regards to total biomass, shoot biomass, root biomass, plant height, CCI and vigour.Further analysis revealed that highly significant differences (P ≤ 0.01) (Table 2) were obtained among genotypes evaluated at Lusitu with regards to total biomass, curling of leaves, CCI, root length, vigour and plant height. Phenotypic difference in curling and CCI were clearly observed in the field (Plate 1). However, at the control environment (UNZA), no significant differences (P> 0.05) were obtained among genotypes with regards to grain yield, CCI, plant height, root length and biomass.

Mean separation revealed that (P2 x P4) and (P4 x P5) (Table 3) were good performing genotypes under heat stress with regards to total biomass, shoot biomass, root biomass, chlorophyll concentration index, curling, plant height and vigour.

3.1.1 Application of multi-variate analysis in evaluating genotypes

Hybrids (P2 x P4) and (P4 x P5) clustered together and were identified as better performers across all measured traits (Fig. 1). PC1 and PC2 explained 91.5% and 3.8% of the phenotypic variation respectively.

Table 1. Comparisons of mean performance of similar parameters measured at the University of Zambia (UNZA) and Lusituduring 2018/2019 cropping season using paired t- test

Parameter	Mean^X	Mean^Y	P-Value
Total biomass(g)	7154.09	297.5	< 0.001
Shoot biomass(g)	7091	276	< 0.001
Root biomass(g)	62.87	21.63	< 0.001
Plant height(cm)	223	57.3	< 0.001
CCI	47.7	21.17	< 0.001
Vigour	4	2.64	=0.01

X - meanvalue measured at UNZA, Y-mean value at Lusitu, CCI -chlorophyll concentration Index. P- level of probability, g- grams, cm- centimeter

Table 2. Mean squares for 5x5 half diallel for heat tolerance evaluated at Lusitu with regards to all measured during the 2018/ 2019 cropping season

SOV	df	TBM	VIG	RL	RBM	SBM	CCI	Cur	PH
Rep	1								
Cross	9	15910 ***	1.66 **	36.27 **	126.59***	13209 ***	60.34 **	1.91***	213.97 **
gca	4	32910 ***	3.56**	70.56 **	257.5***	27345 ***	120.35 ***	3.94 ***	417.14 **
sca	5	2311 **	0.13	8.83	21.86**	1899 **	12.34	0.29	51.44
Error	9	408.1	0.25	6.42	3.52	349.75	8.65	0.136	37.53

***, *** data significantly different from zero at P≤0.05, P≤0.01, P≤0.001 respectively and SOV – source of variation. TBM-total biomass, VIG-vigour, RL-root length, RBM-root biomass, SBM-shoot biomass, CCI-Chlorophyll concentration index, PH-plant height and Cur – Curling*

Plate 1. Genotypic differences in curling and chlorophyll concentration among the genotypes in Lusitu

A - (DTS 6,36 x DTS 6,6 [P2 x P4]) was identified as a heat tolerant line while B - (L2 x L5527 [P1 x P3]) was identified as a susceptible genotype

Table 3. Genotypic mean performances for measured parameters under heat stress at Lusitu with regards to all measured during the 2018/ 2019 cropping season

Genotype	TBM	SBM	RBM	CCI	CUR	PH	VIG
P2 x P4^{x}	493	453.5	39.52	32.79	5	83.1	5
P4 x P5^{x}	483.7	444.5	39.16	30.68	5	66.5	4
XH3506^{z}	385.8	362.4	24.41	24.15	3	63.75	3
P1 x P4	352.7	326.9	25.75	28.72	4	79.7	3.5
P3 x P4	341.2	314.2	26.99	25.04	4	53.75	3
P2 x P5	283.6	263.7	19.88	11.78	3	50	2.5
P1 x P2	240.2	222.5	17.66	18.7	2	50.7	2.5
P1 x P3	185.8	172.7	13.11	16.07	2	48.75	1.5
P3 x P5	183.2	173.1	10.08	17.58	1	41.65	1
P1 x P5	171.6	160	11.61	14.11	2	45.3	1.5
P3 x P2	151.7	142	9.72	13.2	2.5	47.05	1.5
LSD (α =0.05)	60.39	55.91	6.04	8.79	1.1	18.42	1.58

LSD- Fishers protected least significant difference test performed at α=0.05; x- identified tolerant genotype z-Control. TBM- Total biomass; SBM- Shoot biomass; CCI -Chlorophyll concentration index; CUR- Curling; PH-Plant height; VIG- Vigour; P1- L2; P2 - DTS 6,36; P3 - L5527; P4 - DTS 6,6; P5 - DTS 6,92

3.2 Nature of Inheritance for Heat Tolerance

Further analysis showed that the P4 and P3 (Table 4) had a positive and negative significant (P ≤ 0.01) GCA effects for all parameters measured. Genotypes (P1 x P3) and (P4 x P5) had positive significant (P ≤ 0.01) SCA effects whereas (P1 x P4), (P1 x P5) & (P3 x P2) had negative significant (P ≤ 0.01) SCA effects (Table 5) with regards to total biomass, root biomass and shoot biomass.

3.2.1 Evaluation of barker's ratio

An estimation of the Barker's ratio for response of total biomass, vigour, root biomass, shoot biomass, CCI, curling, and plant height to heat stress was found to be 0.90, 0.93, 0.88, 0.90, 0.94, 0.93 and 0.94 respectively.

4. DISCUSSION

Heat stress leads to low crop productivity and in turn production. This was evident from the results of the t-test (Table 1) that showed that overall plant productivity was low in a heat prone area (Lusitu). Further analysis of mean separation revealed that (P2 x P4) and (P4 x P5) were the best performing genotypes with regards to total biomass, vigour, CCI, plant height, shoot biomass, curling and root biomass under heat stress (Table 3). These two genotypes performed better than the control, XH3506 which is an already released hybrid on the market. This was verified by principle component analysis (Fig. 1).

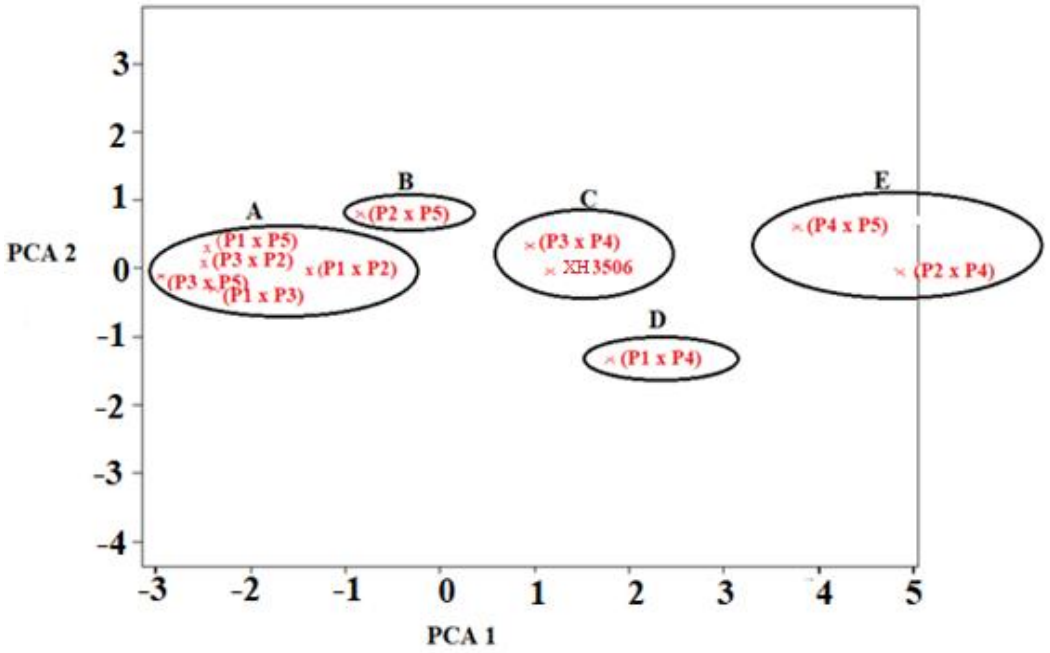

Fig. 1. Principle component analysis exhibiting genotypes in clusters

Phenotypic variation explained by PC1 and PC2 explain variation of 91.5% and 3.8% respectively. Group A, B, C, D, E shows genotypes that exhibited similar performance with regards to measured parameters. With E displaying best tolerant genotypes to heat stress

Table 4. GCA effects of parental lines used in the study for all measured parameters evaluated in Lusitu during the 2018/ 2019 cropping season

PC	TBM	VIG	RL	RBM	SBM	CCI	Cur	PH
P1	-68.16***	-0.47	-4.16*	-5.76***	-62.4***	-1.96	-0.73**	-0.70
P2	4.64	0.37	1.99	0.46	4.20	-2.34	0.10	1.40
P3	-97.56***	-1.13**	-4.66**	-8.5***	-89.1***	-3.86*	-0.9**	-11.80**
P4	171.94***	1.70***	7.14***	15.34***	156.6***	11.25***	1.93***	18.84***
P5	-10.86	-0.47	-0.31	-1.56	-9.30	-3.11	-0.40	-7.73*
SE	10.43	0.26	1.31	0.97	9.16	1.52	0.19	3.16

, **, * Data significantly different from zero at P≤0.05, P≤0.01, P≤0.001 respectively. PC- Parental code, TBM-total biomass, VIG-vigour, RL-root length, RBM-root biomass, SBM-shoot biomass, CCI-Chlorophyll concentration index, PH-plant height, Cur - curling and SE-standard error of the effect. P1- L2; P2 - DTS 6,36; P3 - L5527; P4 - DTS6,6; P5 - DTS 6,92*

Table 5. SCA effects of crosses used in the study of all significant parameters evaluated in Lusitu during the 2018/ 2019 cropping season

Genotype	TBM	RBM	SBM
P1 x P2	15.05	1.60	13.42
P1 x P3	62.85**	6.01**	56.85**
P1 x P4	-39.82*	-5.19**	-34.65*
P1 x P5	-38.08*	-2.43*	-35.62*
P3 x P2	-43.98*	-3.59*	-40.38*
P2 x P4	27.75	2.37	25.42
P2 x P5	1.18	-0.38	1.55
P3 x P4	-21.85	-1.20	-20.65
P3 x P5	2.98	-1.22	4.18
P4 x P5	33.92*	4.02*	29.88*
SE	14.28	1.33	12.55

**, ** data significantly different from zero at P≤0.05, P≤0.01 respectively. TBM-total biomass, RBM-root biomass, SBM-shoot biomass, and SE-standard error of effects and P1- L2; P2 - DTS 6,36; P3 - L5527; P4 - DTS6,6; P5 - DTS6,92*

which clustered the same genotype (P2 x P4)and (P4 x P5) in the same group (E), with a higher PC1 score of 5.This implies that group E was the best performer as compared to other groups. Genotypic clustering approaches are more reliable when molecular markers are utilised as they are independent of the environmental effect [23]. However, the finding of same genotypes, as been tolerant to heat stress using analysis of variance and principle component analysis entails reliability of the finding. In that regard, the selected crosses [(P2 x P4) and (P4 x P5)] may undergo a multi-location evaluation to determine the possibility of releasing it as a variety or utilising it as a parent in further breeding. The reliability of principal component analysis using several morphological is as reliable as its accuracy in phenotypic scoring. The higher combined value for phenotypic variation explained (95.3%) by PC1 and PC2 implies that the generated clusters are reliably more informative (more genetic than environmental effect at play) and can easily be regenerated in the same way when re-evaluated under similar conditions. The fact that PC1 had a much higher value (91.5%) compared to PC 2 (3.8%) imply that genotypic responses for each trait evaluated were likely to be similar [24]. This means that using only one specific trait to evaluate for heat tolerance among genotypes may produce reliable results.

In this study, significant positive GCA effect of an inbred line entailed that it contributed favourable alleles in all possible combination. Therefore, P4 contributed favourable alleles towards heat tolerance whereas P3 contributed unfavourable alleles towards heat tolerance with regards to total biomass, curling of leaves, CCI, root length, vigour and plant height (Table 4). Thus, P4 can be crossed with P3 to create mapping population to use in identifying associated QTL's to heat tolerance with respect to total biomass, curling of leaves, CCI, root length, vigour and plant height [25]. Similar results were reported in previous studies were only desirable GCA effects among utilised germplasm with regards to height response to heat stress was obtained [26,27]. This implies that height is a high heritable trait which is less likely to be influenced by the environment when utilised for selection to heat stress.

The barker's ratio of heat stress to total biomass, vigour, root biomass, shoot biomass, CCI, curling, and plant height traits was found to be 0.90, 0.93, 0.88, 0.90, 0.94, 0.93 and 0.94 respectively. This implies that additive gene action was more important in conditioning these secondary traits associated to biomass for heat tolerance.

Previous authors results, have contradicted on the type of gene action conditioning heat stress with regards to vigour of seed, curling (rolling of leaf), leaf senescence, CCI, plant height, 100-grain weight, yield and biomass. While some have attributed to non-additive gene action as important, others have attributed it to both additive and non-additive effect [28,29,30]. The differences observed could be attributed to different germplasm used and type of environment under study.

5. CONCLUSION

The Genotypes (P2 [DTS 6, 36] x P4 [DTS 6,6]) and (P4 [DTS 6,6] x P5 [DTS 6,92])were

identified as tolerant genotype with regards to total biomass, vigour, root biomass, root biomass, CCI, curling, and plant height. The inbred line P4 (DTS 6,6) had desirable general combining ability with regards to total biomass, shoot biomass vigour, root biomass, CCI, curling, and plant height. Whereas, the crosses (P1[L2] x P3 [L5527]) & (P4 [DTS 6,6] x P5[DTS 6,92]) had desirable specific combining ability with regards to total biomass, root biomass and shoot biomass. The results of baker's ratio obtained for response of total biomass, vigour, root biomass, shoot biomass, CCI, curling, and plant height to heat stress was found to be 0.90, 0.93, 0.88, 0.90, 0.94, 0.93 and 0.94 respectively. These results revealed that additive gene action was more important in conditioning the response of those traits to heat tolerance. Therefore, recurrent selection methods for general combining ability could be employed in population improvement as a breeding strategy for heat tolerance where selection is focused on total biomass, vigour, CCI, plant height, shoot biomass, curling and root biomass.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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