

Elucidating genetic diversity of heat tolerance contributing physiological traits in Maize at two temperature regime in Pakistan

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Abstract

An experiment was performed to determine the genetic diversity of heat tolerance contributing physiological traits in sixty maize genotypes at National agricultural research centre, Islamabad and research field of Plant Breeding and Genetics Department, Gomal University, D.I. Khan during the autumn season, 2017 in Randomized Complete Block design with three replications. These genotypes were evaluated for grain yield, chlorophyll contents, membrane stability index, leaf relative water content and proline content were recorded in five randomly selected plants in each replication. Genetic characterization of studied attributes revealed genotypes were containing marked variation. Higher genetic coefficient values, heritability along with genetic advancement were observed for grain yield. Chlorophyll contents, leaf relative water content and membrane stability index also showed higher values of heritability. Genotype 60, 30 and 21 performed well at both locations for studied attributes which can be utilized further for developing heat tolerant hybrid.

Keywords: cell membrane thermo stability, chlorophyll contents, genetic diversity, leaf relative water content, morpho-physiological traits, proline contents

1. Introduction

Globally elevated thermal condition is considered as high threatening production limitations to maize globally. Rise in temperature at important stages of crop development cause sterility and crop yield reduction [1]. In under developed countries, crop yield is vulnerable to temperature deviation due to low adoptive strategies. Climatic unevenness is a major threat for Pakistan. Production capabilities can be deliberately affected at a greater magnitude in near future. Heat stress effects impact can be lessen by adapting optimum agronomic practices but it cannot eliminate all deleterious impacts [2]. Increased heat tolerance in cereal is very essential to enhance and stabilize crop productivity in the country [3]. Screening upon physiological basis and variability determination play vital role in future breeding program [4]. Plant genetic resources are vital for agricultural system, evaluation, maintenance and available germplasm improvement is necessary [5]. Efficient methods should be devised to improve maize yield for meeting the growing population requirement [6]. Potential of plants for improved efficiency and role in breeding can be determined through genetic diversity in available gene pool. Cell membrane thermo-stability test is a favourable test to detect tolerance. Cell membrane stability can be reduced due to alteration in secondary and tertiary membrane structure in the result of higher loss of electrolytes [7]. Photosynthetic system of maize is prone to high temperature. Photosynthesis lessens above 38°C and severe hindrance occurs due to abrupt rise in temperature [8]. Heat stress also adversely affects many other physiological processes of plant like decreasing the process of photosynthesis and variation in metabolism of protein [9]. It may reduce the water content of leaves due to decreased stomatal conductance or owing to hydraulic conductance reduction resulting in a decrease water absorption. High proline content tended to be high in the

yield components [10, 11]. Heat stress deleteriously affect pollen viability due to failure in pollination [12]. The current research study was thus performed to assess the material for genetic diversity and heat tolerance by performing biochemical analysis.

2. Materials and Methods

2.1 Experimental Site

The field research trial was performed during autumn season at National agricultural research centre, Islamabad, Pakistan to evaluate the material for heat tolerance and genetic diversity. For this purpose sixty genotypes of maize were grown in randomized complete block (RCB) design in which plant material was replicated thrice. Plant material was sown by keeping 25cm plant*plant distance and 75cm row*row distance. Three seeds hill⁻¹ were sown followed by immediate irrigation. All the recommended practices required for agronomic and plant protection purpose were performed throughout cropping season.

2.2 Data Collection

a. Grain yield

Grain yield was recorded by weighing the grains after cobs shelling of three randomly selected plants and average was taken.

b. Membrane stability index (MSI)

MSI was measured as proposed by [13]. Leaves were cut in the form of discs and made two sets filled with 10ml of distilled water. One set of test tubes were kept at 40°C for half an hour and other at 100°C for 15 minutes. Stability of membrane to electrolytes can be measured by the extent of membrane integrity particularly under heat stress conditions. Electrical Conductivity (E.C₁ and E.C₂) measured with E.C meter and value was put in following formula:

$$MSI = (1 - (E.C_1/E.C_2)) \times 100$$

c. Relative Leaf water content (RLWC)

RLWC was calculated as suggested by [14]

$$RWC = (FW - DW) / (TW - DW) \times 100$$

Where, FW, DW and TW = fresh, dry and turgid weight

d. Proline content (PC)

As per protocol given by [15] plant sample (0.1 g) was crushed with 5ml 3.0% sulfosalicylic acid, 5ml distilled water, glacial acetic acid and acid ninhydrin. The content in the tubes was kept in water bath for 1hr at boiling temperature. Test tubes were then allowed to cool and 10 ml of toluene added in separating funnel. Toluene layer absorbance was noted at 520nm through spectrophotometer.

e. Pollen viability (PV)

Pollens were collected at early morning and glass slides were prepared by using 1 % acetocarmine (1 drop). Prepared slides were observed under fluorescent microscope.

f. Chlorophyll Content (CC)

Chlorophyll contents were measured using SPAD 502DL Plus at maturity stage at three times a day via, early morning, mid-day and evening from 40 days old plant and average result was taken.

3. Statistical Analysis

The recorded data were subjected to test the level of variability by means of the ANOVA approach suggested by [16] using statistical software Statistics 8.1. The genotypic and phenotypic variances were found out by the technique of [17]. The method of [18] was applied for the estimation of

genotypic and phenotypic coefficients of variance. Genetic advance was assessed following the methodology of [19]. Heritability in broad sense was calculated by the procedure of [20].

4. Results & Discussion

4.1 Grain yield

It was proposed that significant differences were found among studied accessions. Genotype*location interaction was also significant as shown in Table 1. The mean for grain yield (92.8 & 82.43g) was recorded at tasseling stage in Islamabad & D.I. Khan. It was recommended from fig. a. that the genotype 60 showed highest grain yield followed by 46, while the lowest grain yield was genotype 45 at Islamabad region. In D.I. Khan, highest grain yield was recorded by genotype 60 followed by genotype 31, while lowest value observed by genotype 57. While the lowest grain yield was recorded as 47 in Islamabad by genotype 45 and in D.I. Khan genotype 57 as showed in Fig 4.1a. The higher estimate of heritability (99%) and genetic advance (88.41%) were found for grain yield per plant (Table 2). The higher estimates of heritability and genetic advance designated that grain yield per plant may be useful to increase productivity. [21, 22] reported similar results.

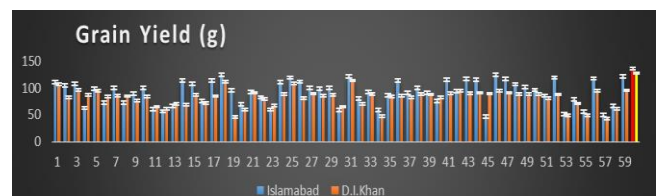


Fig 1a: Comparison of grain yield in 60 maize accessions

Table 1: Analysis of variance for studied grain yield and physiological traits

Source	DF	GY (g)	CC	MSI	LRWC	PC	PV
Rep	2	702.8	166.2	336.9	442.82	9.443	26.32
Genotype	59	1731.2**	42.7*	97.7 ^{N.S}	96.78*	5.637 ^{N.S}	188.35**
Location	1	1848.7 *	114*	1364.9**	825.07*	34.163**	390.62*
Genotype*location	59	1115.1**	36.7*	80.2*	75.83*	4.081 ^{N.S}	144.16**
Error	238	536.4	36.4	96.8	85.88	4.064	68.34

Table 2: Genetic parameters of studied traits

Parameters	Vg	GCV%	Ve	ECV%	Vp	PCV%	h ² bs%	GA% Mean
GY	9	42.9	1.00	14.29	9.00	42.86	99.00	88.41
CC	10.3	13.4	0.90	3.95	11.23	13.97	91.99	26.50
MSI	15.4	20.6	4.00	10.53	19.33	23.14	79.31	37.86
LRWC	10.11	9.35	2.00	4.16	12.11	10.24	83.49	17.63
PC	5	24.9	6.00	27.22	11.00	36.85	45.45	34.56
PV	3.21	29.88	2.00	23.57	5.21	38.06	61.64	48.40

4.2 Chlorophyll content

Highly significant differences were measured among studied accessions. Genotype*location interaction was also significant as shown in Table 1 The mean for chlorophyll contents (37.9& 37.1 mg g-1 fr. wt.) was measured in Islamabad and D.I.Khan, respectively. Chlorophyll contents showed higher values for heritability (91.94%) and genetic advance (26.5%) as shown in Table 2. It is revealed from previous studies that increment in photosynthetic rate higher

values of chlorophyll contents, which is responsible for increase in plant biomass ultimately higher grain yield. It was suggested from the figure 4.2b. that the highest chlorophyll contents were estimated for hybrid followed by genotype 21 followed by hybrid in Islamabad and genotype 49 in D.I. Khan. While the lowest chlorophyll content was 14 and 05 in Islamabad and D.I.Khan, respectively. High temperature tolerant genotypes showed more chlorophyll contents [23]. Supported the present findings.

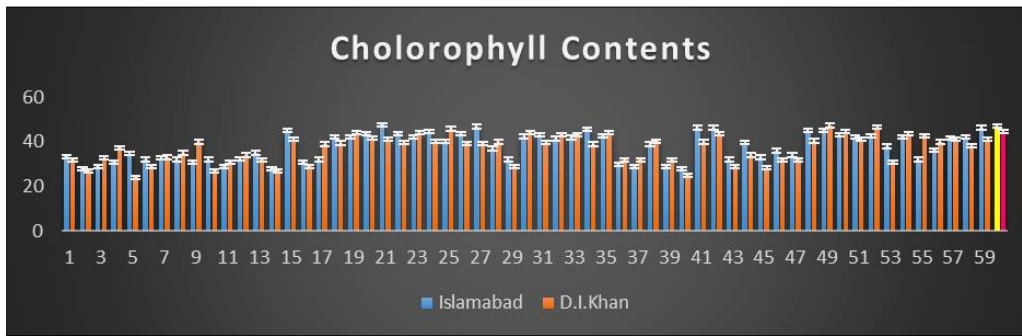


Fig 2b: Comparison of chlorophyll contents in 60 maize accessions

4.3 Membrane stability index Assay

Significant differences were found among studied genotypes. Genotype*location interaction was also significant as shown in Table 1. The mean for membrane stability index (41.8 & 39.4%) was recorded at tasseling stage in Islamabad and D.I.Khan, respectively. The higher values of heritability (79.31%) and genetic advance (37.36%) were found for membrane stability index (Table

2). It was suggested from the figure 4.3c. That the highest membrane stability index was recorded for hybrid followed by genotype 50 in Islamabad and hybrid followed by genotype 21 in Islamabad & hybrid followed by genotype 57 in D.I.Khan. While the lowest value of membrane stability index was 45 and 44 in Islamabad and D.I.Khan, respectively. Findings were found similar to [24].

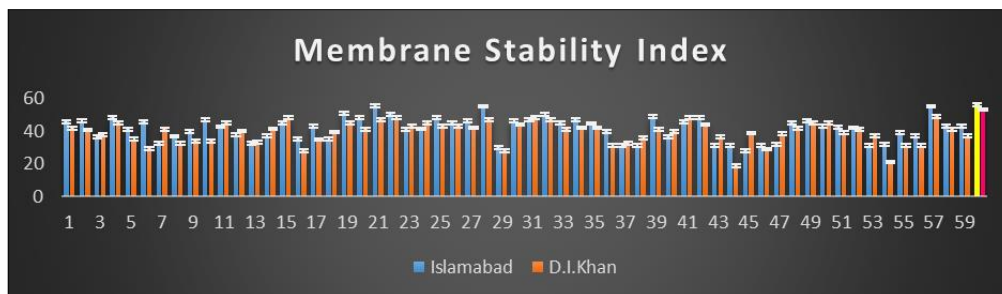


Fig 3c: Comparison of membrane stability index in 60 maize accessions

4.4 Leaf Relative Water Content%

Significant differences were found among studied genotypes. Genotype*location interaction was also significant as shown in Table 1. The mean for leaf relative water content (46.3 & 42.9%) was recorded at tasseling stage in Islamabad and D.I.Khan, respectively. The higher estimate of heritability (83.5%) and genetic advance

(17.6%) were found for leaf relative water content (Table 2). It is suggested from the figure 4.4d. that the highest leaf relative water content was recorded for hybrid followed by genotype 34 and 21 in Islamabad and D.I.Khan, respectively. While the lowest value of leaf relative water content was 47 and 05 in Islamabad and D.I.Khan, respectively. [25, 26] supported present findings.

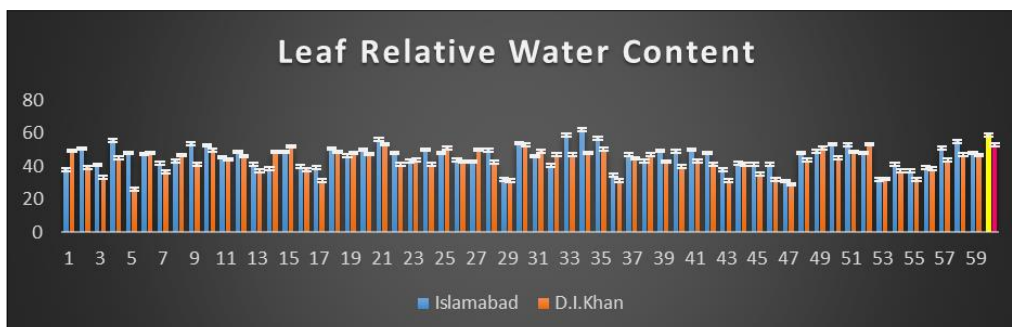


Fig 4d: Comparison of leaf relative water content in 60 maize accessions

4.5 Proline Contents ($\mu\text{mole/gFW}$)

Significant differences were observed among studied genotypes. Genotype*location interaction was non-significant as shown in Table 1. The mean for proline contents i.e. 3.48 & 3.46 ($\mu\text{mole/gFW}$) was recorded at tasseling stage in Islamabad and D.I.Khan, respectively. The moderate estimate of heritability (45.5%) and genetic advance (34.6%) were found for proline contents (Table 2). It was suggested from the figure 4.5e. that the highest

proline contents was recorded for hybrid followed by genotype 12 & 30 in Islamabad and genotype 30 followed by hybrid and genotype 32 in D.I.Khan. While the lowest value of proline contents was 45 and 02 in Islamabad and D.I.Khan, respectively. The selection of higher grain yielding maize genotypes on the basis of proline contents may be helpful in producing heat tolerant maize genotypes. Present findings were in close accordance with [10, 11].

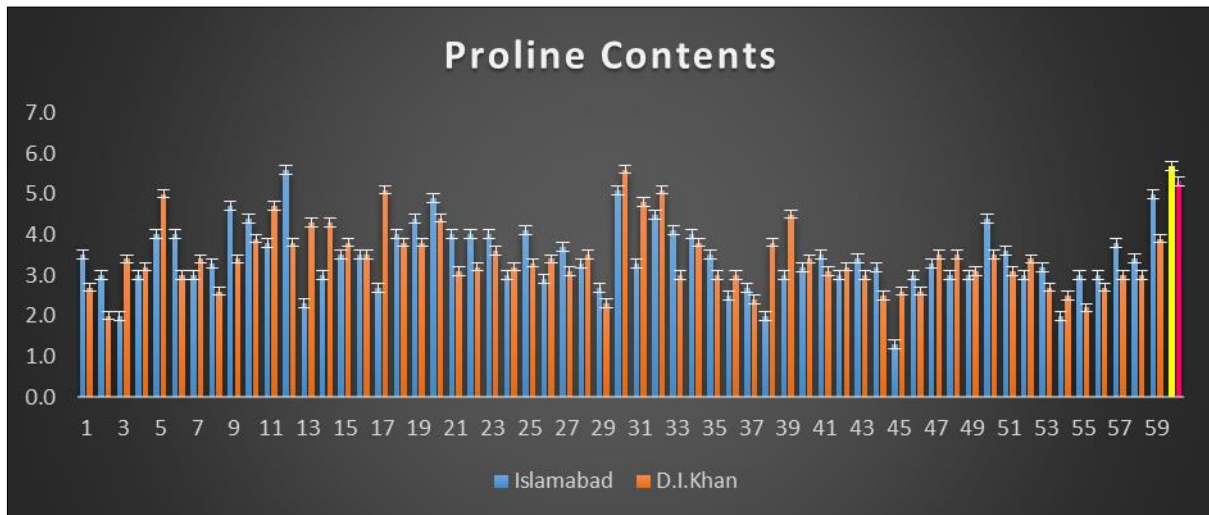


Fig 4.5e. Comparison of proline contents in 60 maize accessions

4.6 Pollen viability (%age)

Significant differences were found among studied accessions. Genotype*location interaction was highly significant as shown in Table 1. The mean for pollen viability (71.6 & 67.9%) was recorded at tasseling stage in Islamabad and D.I.Khan, respectively. The higher values of heritability (61.6%) and genetic advance (48.4%) were estimated for pollen viability (Table 2). The higher pollen

viability indicated higher seed size and volume. It was suggested from the figure f. that the highest pollen viability was recorded for genotype 18 followed by hybrid in Islamabad and hybrid followed genotype in 15 in D.I.Khan. While the lowest value of pollen viability was 13 and 39 in Islamabad while genotype 06 performed lowest in D.I.Khan as shown in Fig 4.6f. [27] reported similar findings.

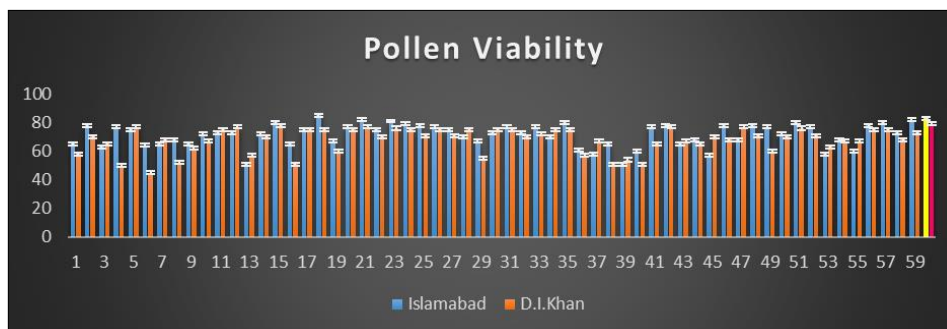


Fig 6f: Comparison of pollen viability percentage in 60 maize accessions

5. Conclusions

The Genotypic and phenotypic coefficient variances were estimated highest while ECV estimates were less, portrayed that the studied traits are genetically governed having very minute effect of environment. The higher heritability was estimated for grain yield, chlorophyll content, leaf water content and membrane stability index. Higher heritability and genetic advance were found for grain yield. Higher heritability estimates along with genetic advance designated the best performing parents to develop hybrids may be helpful to grain yield improvement. Higher genetic advance coupled with higher heritability might be due to the additive genetic effects for the studied attributes. The screened genotypes 60, 30 and 21 performed well at both locations for studied attributes that will help to develop heat tolerant maize hybrids in future breeding programs.

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7. References

1. McKeown JW, McDonald MR. Long-term marketable yields of horticultural crops in southern Ontario in relation to seasonal climate. *Canadian Journal of Plant Science*, 2005; 85:431-438.
2. Chen J, Xu W, Burke JJ, Xin Z. Role of phosphatidic acid in high temperature tolerance in maize. *Crop Sciences*, 2010; 50:2506-2515.
3. Khan MI, Mohammad T, Subhan F, Amin M, Shah ST. Agronomic evaluation of different bread wheat *Triticum aestivum* L. genotypes for terminal heat stress. *Pakistan Journal of Botany*, 2007; 397:2415-2425.
4. Reif JC, Xia XC, Melchinger AE, Warburton ML, Hoisington DA, Beck D, Bohn M, Frisch M. Genetic diversity determined within and among CIMMYT maize populations of tropical, subtropical, and temperate germplasm by SSR markers. *Crop Sciences*, 2004; 44:326-334.
5. Ortiz R, Crossa J, Franco J, Sevilla R, Burgueno J. Classification of Peruvian highland maize races using plant traits. *Genetic Resources and Crop Evolution*, 2008; 55:151-162.

6. Riffat A, Ahmad MSA. Alleviation of adverse effects of salt stress on growth of maize *Zea mays* L. by sulfur supplementation. *Pakistan Journal of Botany*, 2020; 523:763-773.
7. Wahid A, Gelani S, Ashraf M, Foolad MR. Heat tolerance in plants: An overview. *Environ. Experimental Botany*, 2007; 61:199-223.
8. Steven J, Brandner C, Salvucci M. Sensitivity of photosynthesis in C₄ maize plant to heat stress. *Journal of Plant Physiology*, 2002; 129:1773-1780.
9. Ristic Z, Momcilovic I, Bukovnik U, Prasad PVV, Fu J, DeRidder BP, *et al.* Rubisco activase and wheat productivity under heatstress conditions. *Journal of Experimental Botany*, 2009; 60:403-404.
10. Sultan K, Atilla LT. The investigation on accumulation levels of proline and stress parameters of the maize *Zea mays* L. plants under salt and water stress. *Acta Physiologiae Plantarum*, 2010; 32:541-548.
11. Sinay H, Arumingtyas EL, Harijati N, Indriyani S. Proline content and yield components of local corn cultivars from Kisar Island, Maluku, Indonesia. *International Journal of Plant Biology*, 2015; 6:6071.
12. Uribealarea M, Cárcova J, Otegui ME, Westgate ME. Pollen production, pollination dynamics, and kernel set in maize. *Crop Sciences*, 2002; 42:1910-1918.
13. Sairam RK, Deshmukh PS, Shukla DS. Increased antioxidant enzyme activity in response to drought and temperature stress related with stress tolerance in wheat genotypes, Abstract: National Seminar ISSP, IARI, New Delhi, 1997, 69.
14. Barrs HD, Weatherley PE. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Sciences*, 1962; 15:413-428.
15. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. *Plant and Soil*, 1973; 39:205-207.
16. Steel RGD, Torrie JH, Dickey DA. Principles and procedures of statistics; a biometrical approach. 3rd edition. McGraw-Hill, Boston, 1997.
17. Lush JL. Intrasine correlation and regression of offspring on dams as a method of estimating heritability of character. *American Society of Animal Nutrition*, 1940; 32:293-301.
18. Burton GW. Quantitative inheritance in grasses. *International Grassland Congress*, 1952; 1:277-283.
19. Falconer DS. Introduction to Quantitative Genetics 3rd ed. Logman Scientific and Technical, Logman House, Burnt Mill, Harlow, Essex, England, 1989.
20. Hanson CH, Robinson HF, Comstock RE. Biometrical studies on yield in segregating population of Korean lespedeza. *Agronomy Journal*, 1956; 48:268-272.
21. Yousafzai, F, Al-Kaff N, Moore G. The molecular features of chromosome pairing at meiosis: the polyploidy challenge using wheat as a reference. *Functional and Integrative Genomics*, 2009; 10:147-156.
22. Monneveux P, Sanchez C, Tiessen A. Future progress in drought tolerance in maize needs new secondary traits and cross combinations. *Journal of Agricultural Sciences*, 2008; 146:287-300.
23. Havaux M. Stress tolerance of photosystem II in vivo. *Plant physiology*. 1992; 100:424-432.
24. Ibrahim AMH, Quick JS. Genetic control of high temperature tolerance in wheat as measured by membrane thermal. *Crop Science*, 2001; 41:1405-1407.
25. Dekov I, Tsonev T, Yordanov I. Effect of water stress and high temperature stress on the structure and activity of photosynthetic apparatus of *Zea mays* and *Helianthus annuus*. *Photosynthetica*. 2000; 383:361-366.
26. Kumar A, Bernier J, Verulkar S, Lafitte HR, Atlin GN. Breeding for drought tolerance: direct selection for yield, response to selection and use of drought-tolerant donors in upland and lowland-adapted populations. *Field Crops Research*, 2008; 107:221-231.
27. Sinsawat V, Leipner J, Stamp P, Fracheboud Y. Effect of heat stress on the photosynthetic apparatus in maize *Zea mays* L. grown at control or high temperature. *Environmental and Experimental Botany*, 2004; 52:123-129.
28. Ezgi Yazici, Oğuz Bilgin. Heritability estimates for milling quality associations of bread wheat in the northwest turkey. *Int. J Res. Agron.* 2019;2(2):17-22. DOI: 10.33545/2618060X.2019.v2.i2a.18