

Measurement of cell membrane thermo-stability and leaf temperature for heat tolerance in maize (*Zea mays* L): Genotypic variability and inheritance pattern

Muhammad Naveed^{1,2*}, Muhammad Ahsan¹, Hafiz M Akram³, Muhammad Aslam¹, Nisar Ahmed⁴

¹Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Punjab-38040, Pakistan

²Pulses Research Institute, Ayub Agricultural Research Institute, Faisalabad, Punjab, Pakistan

³Plant Physiology Section, Agronomic Research Institute, AARI, Faisalabad, Punjab, Pakistan

⁴Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Punjab, Pakistan

*Corresponding author: E-mail: naveed1735@yahoo.com

Abstract

The rise in ambient temperature is intimidating sustainability of maize productions worldwide. To overcome heat stress effects, identification of potential genotypes and knowledge of inheritance pattern is necessary for developing thermo-tolerant cultivars. This investigation, consisted of four experiments, was conducted for assessing genetic variation followed by developing and evaluating breeding material in non-stressed and heat-stressed environments. The first experiment concerning reproductive stage heat tolerance comprised one hundred maize inbred lines, was conducted at mean day/night temperatures of 33°C/19°C (field) and 40°C/23°C (plastic tunnel), respectively. Variance analysis of absolute and relative data for leaf temperature and cell membrane thermo-stability revealed statistically significant ($P \leq 0.01$) genotypic variations. In second and third experiments, one heat tolerant (ZL-11271) and one susceptible (R-2304-2) lines were selected and crossed in a generation mean fashion to develop non-segregating (P_1 , P_2 , F_1) and segregating (BC_1 , BC_2 , F_2) plant populations. The fourth experiment involved appraisal of six basic generations in a factorial randomized complete block design replicated thrice with mean day/night temperatures of 33°C/20°C and 39°C/24°C. Data on leaf temperature and cell membrane thermo-stability recorded at reproductive phase were analyzed in a nested block design which suggested involvement of digenic epistatic interactions in controlling inheritance of both these traits. Generation variance analysis, however, revealed predominance of additive genes supported by higher estimates [$> 60\%$] of broad sense and narrow sense heritability (F_2 and F_{∞}). Future progress in plummeting leaf temperature and cell membrane thermo-stability of plant material is achievable through hybridization and rigorous selections in succeeding generations.

Keywords: heat tolerance, inheritance pattern, leaf temperature, cell membrane thermo-stability, maize

Introduction

The global rise in temperature due to increasing concentrations of atmospheric CO₂ and green house gases (GHGs) are slowly but gradually heating up the earth's temperature (Treut et al, 2007; IPCC, 2014). This phenomenon called global warming causes heat stress in plants resulting in reduced crop yields. Heat stress may make some crops grow faster, but at the same time could also reduce the productivity. The reason is that the time period necessary for seeds to fully develop is reduced, resulting in forced maturity, deterioration in quality and quantity of the crop product. High temperature has both favorable and outrageous effects on maize plant. Being a member of C4 plant community, it is capable of exploiting the solar energy and warmer environments, but to a certain level (Ashraf and Hafeez, 2004). Optimum day (25 - 32°C) and night (16.7 - 23.3°C) temperatures for maize plant lead to enhanced photosynthetic rate than respiration resulting in rapid plant growth. Maize plant growth affected severely when temperature de-

creases to 5°C or increases beyond 32°C (Steven et al, 2002). A temperature of 35°C during pollination and grain filling stages may reduce maize grain yield on a daily basis by 101 kg ha⁻¹ (Smith, 1996). Similarly, a temperature of 45 - 48°C during flowering and grain formation phases is dangerous to crop health ultimately affecting the grain yield (Ulukan, 2009).

Heat tolerance can be accomplished through genetic management approach as development of heat tolerant genotypes for once would be a cheap input technology that would play a vital role in lessening the harmful impacts of climate change on agricultural production of low income small land holding farmers (Saxena and Toole, 2002; Tester and Langridge, 2010). For this purpose, easy and effective selection criterion holds a key in improving heat tolerance of maize. A whole plant response predominantly at reproductive phases such as flowering and seed setting to high temperature is more appropriate for screening against thermo-tolerance. Identification of potential parents is the first step in breeding for high

temperature stress tolerance, however, the appropriate selection environment often not accessible (Ismail and Hall, 1999; Naveed et al, 2016a). Indirect selection strategies, therefore, may be employed to assess heat tolerance. Cell membrane thermo-stability (CMT) had been used extensively in cereals for screening against drought and heat stress tolerance (Blum and Ebercon, 1981; Agari et al, 1995; Ibrahim and Quick, 2001). Relative cell injury percentage (RCI %age) appraises the thermo-stability of cell membranes and may be used to determine heat tolerance in crop plants (Wahid et al, 2007; Azhar et al, 2009). In contrast, Hafeez et al (2004) suggested that there may be some other factors influencing the crop yield under heat stress, therefore discouraged the solitary use of cell membrane thermo-stability as selection criteria. Canopy temperature is another trait to look for heat tolerance, which gives an indication of transpirational cooling in crop plants. Higher transpiration rate lowers leaf temperature and improves stomatal conductance ultimately contributing to net photosynthesis and crop span (Ham et al, 1991; Moller et al, 2007; Araus et al, 2008). Canopy temperature is a reliable and a heritable parameter for assessing heat tolerance in cotton (Khan et al, 2014). The present research work investigates the genetic basis of heat tolerance by screening 100 maize genotypes for leaf temperature and cell membrane thermo-stability. These findings may be of some worth to corn breeders in developing heat tolerance in plant materials.

Materials and Methods

Studies on genetic basis and genotypic variability in leaf temperature and cell membrane thermo-stability as an appraisal of heat tolerance were conducted in the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan. For this purpose, one hundred maize inbred lines were collected. All these inbred lines were planted under normal field conditions during kharif season 2012. Self pollination was practiced to achieve the maximum uniformity.

Experimental plant materials and other details

The collected 100 inbred lines from various national and international research organizations were laid out in an alpha lattice design with three replications in two sets, concurrently during spring season 2013. One set of the experiment was conducted inside the plastic tunnel (heat-stressed) while the other in normal field (non-stressed) conditions. Fifteen seeds of each test entry were planted in each replication by maintaining 75 cm row-to-row and 25 cm plant-to-plant distances during screening phase. The recommended cultural practices of fertilizers, irrigations and insecticides were adopted according to the crop requirements of both the experiments.

The set of plant material raised in the tunnel was exposed to high temperature stress by covering

it with plastic sheet prior to the initiation of the reproductive period. Average day and night temperatures recorded for heat-stressed (plastic tunnel) and non-stressed (field conditions) experiments were 40°C/23°C and 33°C/19°C, respectively. The humidity inside the plastic tunnel was controlled by exhaust fans to prevent any possible disease outbreak.

Experiment 1: Measurement of leaf temperature and cell membrane thermo-stability

In non-stressed and heat-stressed experiments, leaf temperature (LT) was measured from fully exposed three leaves of each selected plant between 13.00 to 15.00 hours by using infrared thermometer (Model RAYPRM 30). In the screening phase, leaf temperature was recorded on 10 selected consecutive plants of each inbred line.

Cell membrane thermo-stability (CMT) was measured from both the experiments by following the procedure of Sullivan (1972). Using punch machine, round leaf discs of 0.75 cm in diameter were made after removing completely expanded uppermost leaves. In two sets of 50 ml glass tubes, 10 leaf discs were taken and washed slowly with de-ionized distilled water by changing it three times to remove surface adhered electrolytes. Then glass tubes were filled up to 10 ml of distilled water in order to submerge the washed leaf discs. Of the two sets, one set of test tube was placed in a water bath at 45°C for 1 hour. Then both the sets were exposed to 22°C temperature in an air conditioned room for an overnight. Very next day, electrical conductivity of each test tube sample was recorded with the help of LF 538 EC meter after shaking it well. Then kill the leaf tissues, both sets of test tube samples were autoclaved at 121°C temperature for 15 minutes at 15 lbs pressure, which were allowed overnight to cool down to 22°C temperature. Subsequently, electrical conductivity was recorded for the 2nd time. Under stress, the extent of membrane integrity permits a measure of membrane stability to electrolyte leakage. Relative cell injury percentage (RCI%) as an appraisal of cell membrane thermo-stability was worked out by using 1st and 2nd electrical conductivity readings and the following formula;

$$RCI\% = [1 - \{1 - (T1 / T2)\} / \{1 - (C1 / C2)\}] \times 100$$

where T & C indicate electrical conductivity (EC) of heat treated and controlled sets of test tube, and the subscripts 1 & 2 refer to 1st and 2nd EC readings, respectively.

Procedure for assessing heat stress tolerance

A procedure holds a key in assessing genotypic responses and further discriminating superior lines for heat tolerance. In the current studies, the genetic material was evaluated on the basis of absolute performances for leaf temperature and cell membrane thermo-stability under contrasting conditions. This is a useful criterion and had been used previously by various researchers for the identification of tolerant and susceptible genotypes (Azhar et al, 2005; Akhter

et al, 2007; Iqbal et al, 2011). The plant material was also assessed on the basis of relative performance in heat-stressed to non-stressed conditions multiplied by 100. This methodology had been used for discriminating drought, heat and salt tolerance in different field crops (Azhar and McNeilly, 1988; Azhar et al, 2009).

Experiment 2 and 3: Development of breeding populations

One hundred maize inbred lines were compared for absolute and relative performance of leaf temperature and cell membrane thermo-stability, which revealed genotypes ZL-11271 as heat tolerant and R-2304-2 as heat susceptible. Both these inbred lines were sown under normal field conditions during kharif 2013 and crosses were attempted to obtain F_1 seed. The F_1 seed along with parents were grown in field during spring and kharif seasons of 2014. Some F_1 plants were advanced to F_2 by selfing while a few F_1 plants were crossed with P_1 and P_2 to develop BC_1 and BC_2 generations, respectively.

Experiment 4: Evaluation of six basic generations for genetic studies

During spring season 2015, two sets each comprising same six basic generations (P_1 , P_2 , F_1 , F_2 , BC_1 , and BC_2) were planted simultaneously in heat-stressed (plastic tunnel) and non-stressed (field) conditions in factorial randomized complete block design with three replications. Experimental methodologies followed were same as in screening experiment. Thirty plants were planted each for parents, F_1 and sixty for back crosses while three hundred for F_2 generation in a replication. For the purpose of recording the observations in each replication, 20 (P_1 , P_2 , and F_1), 30 (BC_1 and BC_2) and 60 (F_2) plants were selected from non-segregating, backcrosses and segregating populations, respectively in non-stressed and heat-stressed environments. Mean temperatures recorded for heat-stressed and non-stressed treatments were 39°C/24°C and 33°C/20°C (day / night), respectively.

Statistical computations

The recorded data of absolute and relative ratios in non-stressed and heat-stressed conditions were subjected to statistical analysis of variance technique to find out significant differences among the inbred lines (Steel et al, 1997). For determining the genetic basis of heat tolerance, coefficients for dissecting sum of squares (SS) of six basic genera-

tions were generated (Little and Hills, 1978). The generation mean analysis (GMA) was performed using Mather and Jinks (1982) procedure. Means and variances of parents, BC_1 , BC_2 , F_1 , and F_2 generations used in the analysis were calculated from individual plant data pooled over replications. A weighted least square analysis was also performed on generation means beginning with simplest model using parameter m only. Further models of increasing complexity (md , mdh , etc.) were fitted. The best fit model was selected when estimate of χ^2 was non-significant with all significant parameters. For generation variance analysis, a weighted least square analysis of variances was also performed (Mather and Jinks, 1982). The data comprising six generations (parents, F_1 , F_2 , BC_1 , and BC_2) was analyzed using a computer program supplied by Dr HS Pooni, University of Birmingham. Models incorporating E, [D and E], [D, H, and E], [D, F, and E], and [D, H, F, and E] were tried. The best fit model was selected when χ^2 was non-significant with all significant parameters (Naveed et al, 2016b).

Estimates of the broad sense (Weber and Moor-
thy, 1952) and narrow sense (F_2 : Warner, 1952; F_∞ :
Mather and Jinks, 1982) heritability for leaf temper-
ature and cell membrane thermo-stability in non-
stressed and heat-stressed conditions were also cal-
culated using variances of parents (P_1 , P_2), F_1 and F_2
generations. Heritability in a broad sense (H^2) is the
measure of genotypic variance to phenotypic vari-
ance, whereas heritability in the narrow sense [h^2]
is the appraisal of additive genetic variance to the
phenotypic variance. Johnson et al (1955) classified
both H^2 and h^2 into low (< 30%), moderate (30 - 60%)
and high (> 60%). Broad sense heritability is based
on total genetic variance which comprises both fix-
able (additive) and non-fixable (dominance and epi-
static) variances. Traits exhibiting higher estimates of
broad sense heritability represent the least influence
of environment while lower values indicate higher
environmental influence, therefore, selection would
be inappropriate in these circumstances. In case of
narrow sense heritability, characters with higher val-
ues are governed by additive genes and the selec-
tion aimed at crop improvement would be rewarding
while characters with lower values are governed by
non-additive genes and hybrid breeding may be ef-
fective (Singh and Narayanan, 1993).

Table 1 - Mean squares of leaf temperature and cell membrane thermo-stability of 100 maize inbred lines grown under non-stress and heat stress conditions.

| Traits | DF | Leaf temperature | | Cell membrane thermo-stability | |
|------------------|-----|------------------|----------|--------------------------------|----------|
| | | Absolute | Relative | Absolute | Relative |
| Replications | 2 | 9.790 | 0.317 | 127.650 | 23.281 |
| Genotypes (G) | 99 | 6.300** | 7.119** | 1035.200** | 10.309** |
| Temperatures (T) | 1 | 2626.620** | - | 8233.100** | - |
| G \times T | 99 | 0.650** | - | 7.990** | - |
| Error | 398 | 0.140 | 0.273 | 1.790 | 2.504 |

DF: degrees of freedom, **: $P < 0.01$

Table 2 - Means of six basic generations for leaf temperature and cell membrane thermo-stability of *Zea mays* L.

| Traits | | P ₁ | P ₂ | F ₁ | F ₂ | BC ₁ | BC ₂ | LSD _(0.05) |
|--------------------------------|----|----------------|----------------|----------------|----------------|-----------------|-----------------|-----------------------|
| Leaf Temperature | NS | 31.733 | 41.007 | 34.833 | 34.008 | 33.578 | 35.016 | 0.671 |
| Cell membrane Thermo-stability | HS | 29.667 | 35.540 | 32.427 | 33.312 | 32.245 | 34.283 | 0.382 |
| | NS | 29.437 | 91.026 | 38.166 | 63.850 | 41.172 | 67.792 | 2.950 |
| | HS | 26.683 | 77.593 | 34.470 | 45.466 | 37.725 | 48.269 | 1.287 |

NS: non-stressed, HS: heat-stressed

Results

Mean squares acquired from variance analysis of absolute and relative ratios revealed statistically highly significant ($P \leq 0.01$) differences among the 100 genotypes (G) for leaf temperature and cell membrane thermo-stability (Table 1). Significant ($P \leq 0.01$) differences were also recorded in temperature (T) treatments and the interactions of $G \times T$ for leaf temperature and cell membrane thermo-stability which suggested that genotypes performed differently to heat stress.

Genotypic variability in leaf temperature

Genotypic values for leaf temperature were higher in non-stressed regime than heat-stressed conditions (Supplementary Table 1). Tukey test for mean values of 100 inbred lines indicated that differences among the genotypic responses were greater in heat-stressed in comparison to non-stressed conditions implying that comparison among the test entries is a useful technique for assessing tolerant, susceptible and intermediate groups. The reaction of ZL-11271 (No. 89), UM-2 (No. 53), HY-7 (No. 50), IC-654 (No. 51), and A-545 (No. 62) with lowest leaf temperatures of 29.53°C, 30.23°C, 32.23°C, 32.53°C, and 32.53°C to heat stress appeared to be tolerant while that of R-2304-2 (No. 5), B-42 (No. 33), N-18 (No. 36), G.P.F-9 (No. 28), OH-33-1 (No. 15), F-122 (No. 8), F-110 (No. 6), and F-160 (No. 4) with highest leaf temperatures (35.73 to 35.03°C) categorized as susceptible. The other genotypes were intermediate in their response to high temperature stress. Genotypes 89 (ZL-11271), 62 (A-545), 24 (W-10), 32 (B-34-2B), and 78 (Y-21) with highest relative ratios were more heat tolerant in contrast to 5 (R-2304-2), 91 (VL-1032), 80 (Y-11), 71 (Y-52), and 76 (Y-15) with lowest values.

Genotypic variability in cell membrane thermo-stability

Similar to leaf temperature, estimates of cell mem-

brane thermo-stability were higher in non-stressed in contrast to heat-stressed conditions (Supplementary Table 2). CMT ranged 29.07 to 91.57 in non-stressed and 26.67 to 77.15 in heat-stressed conditions. Relative cell injury percentage (RCI%) of ZL-11271 (No. 89), UM-2 (No. 53), ZL-11376 (No. 88), A-545 (No. 62), and VL-1033 (No. 92) was lowest among all the genotypes under heat-stressed conditions therefore regarded as heat tolerant. Entries R-2304-2 (No. 5), ZL111008 (No. 98), A-521-1 (No. 58), VL-0512420 (No. 97), VL-1029 (No. 96), N-48-94 (No. 37), 20-P2-1 (No. 44), VL107657 (No. 99), 150-P1 (No. 48), and ZL-111040 (No. 100) with RCI% > 70 were discriminated as heat susceptible. The RCI % for rest of the genotypes was in between of 33 to 69. Estimates of relative heat tolerance were highest (> 90) for entry no. 89, 93, 43, 62, 38, 41, 58, 39, 48, 40, 85, and 42 while lowest (< 85) for 5, 46, 86, 70, 100, 16, 45, and 71. The response of genotypes with highest relative ratios to high temperature stress was tolerant and vice versa.

Mean estimates of genetic materials

Prior to the initiation of reproductive phase, data recorded on leaf temperature and cell membrane thermo-stability for six basic generations were averaged over replications and used for genetic analysis of heat tolerance (Table 2). The estimates of LT and CMT for all the six generations were higher in non-stressed than heat-stressed environments. Significant differences were recorded among the non-segregating (P₁, P₂, F₁) and segregating (BC₁, BC₂, F₂) genetic materials for both these traits. Differences among the estimates of six populations for leaf temperature and cell membrane thermo-stability indicated variation in genotypic responses to heat tolerance.

Genetic basis of heat tolerance

Analysis of generation means indicated that genetic model comprising five parameters (mdhjl) was

Table 3 - Best fit model for leaf temperature and cell membrane thermo-stability in a cross of maize.

| Parameter | Leaf temperature | | Cell membrane thermo-stability | |
|---------------------|------------------|-------------|--------------------------------|-------------|
| | Non-stress | Heat stress | Non-stress | Heat stress |
| m±SE | 36.37±0.16 | 32.59±0.15 | 87.69±1.50 | 52.23±0.32 |
| [d]±SE | -4.64±0.16 | -2.87±0.14 | -30.79±0.22 | -25.49±0.33 |
| [h]±SE | -7.25±0.97 | 2.79±0.98 | -49.64±1.68 | -17.53±0.60 |
| [i]±SE | - | - | -27.49±1.53 | - |
| [j]±SE | 3.12±0.53 | - | 4.96±1.41 | 15.15±1.53 |
| [l]±SE | 5.72±1.09 | -2.96±1.09 | - | - |
| χ ² (df) | 0.551 (1) | 2.615 (2) | 6.039 (1) | 5.692 (2) |

m: mean, [d]: additive, [h]: dominance, [i]: additive-additive, [j]: additive-dominance, [l]: dominance-dominance, χ²: chi-square, df: degrees of freedom

Table 4 - Variance components for leaf temperature and cell membrane thermo-stability in a cross of maize.

| Traits | | [D] ± SE | [F] ± SE | [E] ± SE | χ^2 (df) |
|------------------|----|----------------|--------------|-------------|---------------|
| Leaf | NS | 21.93 ± 2.61 | -4.54 ± 1.87 | 2.22 ± 0.33 | 10.00 (3) |
| Temperature | HS | 24.42 ± 2.75 | - | 2.11 ± 0.31 | 12.57 (4) |
| Cell membrane | NS | 209.04 ± 18.94 | - | 3.58 ± 0.53 | 8.95 (4) |
| Thermo-stability | HS | 235.15 ± 22.20 | - | 7.15 ± 1.06 | 7.18 (4) |

NS: non-stressed, HS: heat-stressed, [D]: additive, [F]: additive-dominance, [E]: environmental, χ^2 : chi-square, df: degrees of freedom

found best fit to data of leaf temperature under non-stressed regime in comparison to four factors [mdh] model under heat-stressed environment (Table 3). Genetic models consisting of [mdhij] in non-stressed and [mdhj] under heat-stressed environments exhibited best fitness to the estimates of generation means from observed to the expected for cell membrane thermo-stability. Analysis of genetic variance for LT and CMT dissected total variance into additive [D], environmental [E], and additive-dominance [F] constituent components (Table 4). For this purpose, six basic generations comprising non-segregating and segregating populations were used for computing both genetic and environmental variance components. Non-significant χ^2 estimates were observed with genetic models comprising two [DE] and three [DEF] parameters for leaf temperature and cell membrane thermo-stability in non-stressed and heat-stressed environments. Additive variance [D] was greater in magnitude than the respective environmental [E] and interaction [F] variance components. The magnitudes of variances were higher under heat-stressed in contrast to non-stressed conditions for both these traits except that of [E] variance of leaf temperature in non-stressed environment.

Broad and narrow sense heritabilities were high (> 60%) for both leaf temperature and cell membrane thermo-stability (Table 5). The broad sense heritability for cell membrane thermo-stability (96.5 and 94.3) was greater than for leaf temperature (84.6 and 86.2) in conditions of non-stressed and heat-stressed, respectively. Similarly, narrow sense heritability of cell membrane thermo-stability for F_2 (76.9 and 82.7) and F_∞ (97.5 and 96.4) populations were higher in comparison to F_2 (78.4 and 69.3) and F_∞ (88.8 and 88.5) of leaf temperature in non-stressed and heat-stressed conditions, respectively. Estimates of narrow sense heritability for infinity generation were greater than for F_2 population under both the contrasting environments.

Table 5 - Estimates of heritability for leaf temperature and cell membrane thermo-stability in Zea mays L.

| Trait | | H^2 | h^2 | |
|------------------|----|-------|-------|------------|
| | | | F_2 | F_∞ |
| Leaf | NS | 84.6 | 78.4 | 88.8 |
| Temperature | HS | 86.2 | 69.3 | 88.5 |
| Cell membrane | NS | 96.5 | 76.9 | 97.5 |
| Thermo-stability | HS | 94.3 | 82.7 | 96.4 |

H^2 : broad sense heritability, h^2 : narrow sense heritability, F_2 : filial generation two, F_∞ : infinity generation

Discussion

Any crop improvement program including heat tolerance depends largely upon the existence of genetic variation and selection of potential parents. Presence of variability in genepool is the basis for the genetic improvement of a crop against any biotic and abiotic factors. Genetic variability for heat tolerance has been reported in all the field crops including cotton, mungbean, tomato, rice, wheat and so on (Collins et al, 1995; Farooq et al, 2011; Golam et al, 2012; Matsui and Omasa, 2002; Rehman et al, 2004). Potential variation in maize germplasm existed for drought and heat tolerance. Identification and classification of such variations are the most important steps in developing tolerant genotypes (Lu et al, 2011).

In the current investigation, the assessment of genotypic responses for leaf temperature and cell membrane thermo-stability was aimed for measuring the potential for heat tolerance of tested plant material and determining the inheritance pattern, thereafter. The results of the screening experiments implied that appraisal of leaf temperature and cell membrane thermo-stability at reproductive stage proved to be effective in differentiating 100 maize inbred lines for high temperature tolerance. An examination of all the genotypic data suggested general pattern of reactions to optimum and high temperatures indicating divergent responses to heat-stressed conditions. Both leaf temperature and cell membrane thermo-stability revealed existence of considerable variability among the experimental plant material for heat tolerance. Absolute and relative performances of genotypes for both these traits permitted the discrimination of heat tolerant and heat susceptible maize inbred lines. These findings are in agreement to the previously reported on canopy temperature (Wanjura et al, 2004; Khan et al, 2014) and cell membrane thermo-stability (Kumar and Sharma, 2007; Akbar et al, 2009).

From a plant breeding view point, exploitation of diverse / potential genetic resources could be possible only through selection followed by hybridization. In this perspective, the execution of any biometrical technique such as generation mean and variance analyses for dissecting total genetic variation into constituent components becomes very crucial. Generation variance analysis which partitions the total variance into components of additive (D), environmental (E), interaction (F), and dominance (H) has long been explored by research workers. The results revealed involvement of additive (D) component

largely in the inheritance of both leaf temperature and cell membrane thermo-stability under the contrasting conditions. However, appearance of interaction (F) component in case of leaf temperature under non-stressed regime complicated its inheritance pattern. Significant and greater magnitudes of additive (D) variance in comparison to other components for LT and CMT indicated transmission of both negative and positive alleles in the developed genetic material from the parents. Previous studies reported that additive variance controls the genetic variation in different plant traits (Rahman and Malik, 2008; Iqbal et al, 2015; Khan et al, 2016). The role of additive genetic variance in the inheritance of leaf temperature and cell membrane thermo-stability was supported by higher broad sense ($h^2_{b.s.}$) and narrow sense ($h^2_{n.s.}$; F_2 and F_{∞}) heritability estimates. This information may be helpful in determining response to selection and genetic gain in succeeding plant progenies. These results are encouraging for the maize breeders suggesting that plant material with inbuilt heat tolerance could be searched in this particular crossed population. The appearance of epistatic interaction [j, l] for leaf temperature in non-stressed conditions was confirmed by generation variance analysis (Sarwar et al, 2012; Khan et al, 2014). The use of generation mean analysis for analyzing abiotic plant stresses such as drought, heat and salinity is very extensive (Ahsan et al, 1996; Khan et al, 2014; Khan et al, 2016). Involvement of non-allelic (epistatic) interactions in leaf temperature and cell membrane thermo-stability validated the existence of genotypic variation in tested plant material. Occurrence of negative additive and dominance genetic effects for leaf temperature and cell membrane thermo-stability under both non-stressed and heat-stressed conditions suggested that these may be fixed at early segregating generations however manifestation of duplicate digenic interactions for both these traits intricated this situation. For this reason, development of multi-parent crossed material and maintaining larger plant populations followed by intermating among the desirable recombinants and recurrent selection practices could serve in increasing the frequency or accumulating the heritable additive genes. This procedure may ultimately break undesirable genetic linkages and generate viable transgressive segregants for exploitation.

Conclusion

Detection of variation in plant material for leaf temperature and cell membrane thermo-stability proved to be of potential use and heritable. The predominance of additive variance [D] along with higher magnitudes of heritability [h^2 and h^2] enhanced the scope for genetic improvement of maize against heat stress. The present understandings of genetic effects controlling heat tolerance may be exploited for developing heat resilient maize synthetics and hybrids.

References

- Agari S, Hanaoka N, Kubota F, Agata W, Kaufman PB, 1995. Measurement of cell membrane stability evaluated by electrolyte leakage as a drought and heat tolerance test in rice (*Oryza sativa* L.). J Agric Kyushu Univ 40: 233-240
- Ahsan M, Wright D, Virk D, 1996. Genetic analysis of salt tolerance in spring wheat. Cereal Res Commun 24: 353-360
- Akbar M, Saleem M, Ashraf MY, Hussain A, Azhar FM, Ahmad R, 2009. Combining ability studies for physiological and grain yield traits in maize at two temperatures. Pak J Bot 41: 1817-1829
- Akhter M, Ahmad M, Ramzan M, 2007. Effect of photoperiod sensitivity on yield and other economic traits of new strains of basmati rice (*Oryza sativa* L.). J Anim Plant Sci 17: 79-82
- Araus JL, Slafer GA, Royo C, Serret MD, 2008. Breeding for yield potential and stress adaptation in cereals. Crit Rev Plant Sci 27: 377-412
- Ashraf M, Hafeez M, 2004. Thermo tolerance of pearl millet and maize at early growth stages: growth and nutrient relations. Biologia Plantarum 48: 81-86
- Azhar FM, Ali Z, Akhtar MM, Khan AA, Trethowan R, 2009. Genetic variability of heat tolerance, and its effect on yield and fiber quality traits in upland cotton (*Gossypium hirsutum* L.). Plant Breed 128: 356-362
- Azhar FM, McNeilly T, 1988. The genetic basis of variation for salt tolerance in *Sorghum bicolor* (L.) Moench., seedlings. Plant Breed 101: 114-121.
- Azhar MT, Khan AA, Khan IA, 2005. Combining ability analysis of heat tolerance in *Gossypium hirsutum* L. Czech J Genet Plant Breed 41: 23-28
- Blum A, Ebercon A, 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci 21: 43-47
- Collins GG, Nie XL, Saltveit ME, 1995. Heat shock proteins and chilling sensitivity of mung bean hypocotyls. J Exp Bot 46: 795-802.
- Farooq M, Bramley H, Palta JA, Siddique KHM, 2011. Heat stress in wheat during reproductive and grain filling phases. Crit Rev Plant Sci 30: 491-507
- Golam F, Prodhan ZH, Nezhadahmadi A, Rahman M, 2012. Heat tolerance in tomato. Life Sci J 9: 1936-1950
- Hafeez FY, Safdar ME, Chaudhry AU, Malik KA, 2004. Rhizobial inoculation improves seedling emergence, nutrient uptake and growth of cotton. Aust J Exp Agri 44: 617-622
- Ham JM, Heilman JL, Lascano RJ, 1991. Soil and canopy energy balances of a row crop at partial cover. Agron J 83: 744-753
- Ibrahim AMH, Quick JS, 2001. Genetic control of high temperature tolerance in wheat as measured by membrane thermal stability. Crop Sci 41: 1405-1407
- IPCC, 2014. Intergovernmental Panel on Climate

- Change. In: Climate Change 2014: Impacts, Adaptation and Vulnerability. Geneva
- Iqbal J, Ahsan M, Saleem M, Ali A, 2015. Appraisal of gene action for indeterminate growth in mungbean [*Vigna radiata* (L.) Wilczek]. *Front Plant Sci* 6: doi: 10.3389/fpls.2015.00665
- Iqbal K, Azhar FM, Khan IA, Ullah E, 2011. Variability for drought tolerance in cotton (*Gossypium hirsutum*) and its genetic basis. *Int J Agric Biol* 13: 61-66
- Ismail AM, Hall AE, 1999. Reproductive stage high temperature tolerance, leaf membrane stability and plant morphology in Cowpea. *Crop Sci* 39: 1762-1768
- Johnson HW, Robinson HF, Comstock RE, 1955. Estimation of genetic and environmental variability in soybeans. *Agronomy J* 47: 314-318
- Khan NH, Ahsan M, Naveed M, Sadaqat HA, Javed I, 2016. Genetics of drought tolerance at seedling and maturity stages in *Zea mays* L. *Spanish J Agric Res* (Accepted)
- Khan N, Azhar FM, Khan AA, Ahmad R, 2014. Measurement of canopy temperature for heat tolerance in upland cotton: variability and its genetic basis. *Pak J Agri Sci* 51: 359-365
- Kumar A, Sharma SC, 2007. Genetics of excised-leaf water loss and relative water content in bread wheat (*Triticum aestivum* L.). *Cereal Res Commun* 35: 43-52
- Little TM, Hills FJ, 1978. *Agricultural Experimentation: Design and Analysis*. New York, NY: John Wiley & Sons, Inc
- Lu Y, Hao Z, Xie C, Crossa J, Araus JL, Gao S, Vivek BS, Magorokosho C, Mugo S, Makumbi D, Taba S, Pan G, Li X, Rong T, Zhang S, Xua Y, 2011. Large-scale screening for maize drought resistance using multiple selection criteria evaluated under water-stressed and well-watered environments. *Field Crops Res* 124: 37-45
- Mather K, Jinks JL, 1982. *Biometrical Genetics*. London, UK: Chapman and Hall Ltd
- Matsui T, Omasa K, 2002. Rice (*Oryza sativa* L.) cultivars tolerant to high temperature at flowering: anther characteristics. *Ann Bot* 89: 683-687
- Möller M, Alchanatis V, Cohen Y, Meron M, Tsipris J, Naor A, Ostrovsky V, Sprintsin M, Cohen S, 2007. Use of thermal and visible imagery for estimating crop water status of irrigated grapevine. *J Exp Botany* 58: 827-838
- Naveed M, Shafiq M, Rafiq CM, Naeem MK, Amin M, 2016a. Grain yield stability of newly evolved desi chickpea strains under rainfed conditions. *J Anim Plant Sci* 26: 481-486
- Naveed M, Ahsan M, Akram HM, Aslam M, Ahmed N, 2016b. Genetic effects conferring heat tolerance in a cross of tolerant × susceptible maize (*Zea mays* L.) genotypes. *Front Plant Sci* 7: 729. doi: 10.3389/fpls.2016.00729
- Rahman S, Malik TA, 2008. Genetic analysis of fibre traits in cotton. *Int J Agric Biol* 10: 209-212
- Rehman H, Malik SA, Saleem M, 2004. Heat tolerance of upland cotton during the fruiting stage evaluated using cellular membrane thermo stability. *Field Crop Res* 85: 149-158
- Sarwar M, Khan IA, Azhar FM, Ali A, 2012. Generation means analysis in cotton (*Gossypium hirsutum*) for drought tolerance. *Pak J Nut* 11: 941-945
- Saxena NP, O'Toole JC, 2002. Field screening for drought tolerance in crop plants with emphasis on rice. In: *Proc of an International Workshop on Field Screening for Drought Tolerance in Rice*. Patancheru: ICRISAT India
- Singh P, Narayanan SS, 1993. *Biometrical Techniques in Plant Breeding*. Kalyani Publishers, New Delhi
- Smith KL, 1996. *Corn Production*. Ohio Agronomy Guide, Bulletin 472. Ohio state Univ, USA
- Steel RGD, Torrie JH, Dicky DA, 1997. *Principles and Procedures of Statistics. A Biometrical Approach*. New York, USA: McGraw Hill Book Company
- Sullivan CY, 1972. Mechanisms of heat and drought resistance in grain sorghum and methods of measurement, pp. 247-264. In: *Sorghum in the Seventies*. Oxford and IBH Publishing Co
- Tester M, Langridge P, 2010. Breeding technologies to increase crop production in a changing world. *Science* 327: 818-822
- Treut Le, Somerville H, Cubasch U, Ding Y, Mauritzen C, Mokssit A, Peterson T, Prather M, 2007. Historical Overview of Climate Change. In: *Climate change 2007: The Physical Science Basis*. Cambridge Univ Press, New York
- Ulukan H, 2009. Environmental management of field crops: A case study of Turkish agriculture. *Int J Agric Biol* 11: 483-494
- Wahid A, Gelani S, Ashraf M, Foolad MR, 2007. Heat tolerance in plants: An overview. *Environ Exp Bot* 61: 199-223
- Wanjura DF, Maas SJ, Winslow JC, Upchurch DR, 2004. Scanned and spot measured canopy temperatures of cotton and corn. *Comp Electron Agri* 44: 33-48
- Warner NJ, 1952. A method for estimating heritability. *Agron J* 44: 427-430
- Weber CR, Moorthy BR, 1952. Heritable and nonheritable relationships and variability of oil content and agronomic characters in the F₂ generation of soybean crosses. *Agron J* 44: 202-209