The Journal of Animal & Plant Sciences, 24(4): 2014, Page: 1141-1145
ISSN: 1018-7081

PHYSIOLOGY OF HIGH TEMPERATURE STRESS TOLERANCE AT REPRODUCTIVE STAGES IN MAIZE

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ABSTRACT

Maize is a dynamic cereal of world's agriculture community and is grown both in spring and autumn seasons in Pakistan. In case of spring sowing (February sowing) both pistillate and staminate flowers face high temperature stress and ultimately results in poor seed setting because of increased silk dryness and pollen desiccation. Maize accessions were identified on the basis of their performance at high temperature stress against the indicators like cell membrane thermostability (CMT), leaf temperature (LT), pollen viability %age (PV%), pollen production (PP), pollen germination %age (PG%), pollen grain size (PGS), pollen moisture contents %age (PMC%), and pollen tube length (PTL). Significant differences were observed among the genotypes for high temperature tolerance related indicators which provided wide range of option for selection and opportunity to improve tolerance level against high temperature stress. Principle component analysis (PCA) proved PV%, PP, PG% and PTL as best physiological standards for evaluation of germplasm against high temperature stress. Genotypes viz. B-321, EV-323, POP-209, B-308, B-316, F-127, B-236, EV-340, F-143 and SAWAN-3 showed high level of variability and performed well under heat stress. Genotypes showing high level of adaptability in response to imposed stress could be a good genetic source carrying wide diversity of genes responsible for high temperature tolerance and could be used in breeding program to breed for high temperature tolerance in maize.

Key words: Maize, high temperature stress, pollen and cell membrane physiology.

INTRODUCTION

Maize (*Zea mays* L.) is one of the significant cereals after wheat and rice, grown all around the world. Maize has C4 pathway of photosynthesis therefore use water and carbon dioxide more efficiently. It is cultivated both in spring and summer seasons in Pakistan. It is naturally a monoecious plant with pistillate and staminate flowering pattern. The reproductive stage of spring maize crop faces high temperature stress which results in increased anthesis silking interval and poor seed setting. High temperature stress disturbs the proper plant growth and yield by inhibiting photosynthesis and affecting pollination due to silk desiccation and pollen abortion in maize. Leaf temperature above 38ºC inhibits net photosynthesis because of thermal inactivation of enzymes. Temperature higher than 30ºC progressively inactivates the Rubisco (Steven *et al*., 2002) which efficiently reduces photosynthesis. The optimum temperature for maize plant in day and night times ranges from 22 to 32ºC and 16.7 to 23.3ºC, respectively. On these temperatures the photosynthetic rate become rapid than respiration resulting in increased plant growth. Plant growth is affected badly when the temperature decreases below 5ºC or rises above 32ºC.

Heat stress and low humidity can dry up the pollen grains when released from anthers and silks due to thin outer layer (Sinsawat *et al.*, 2004). Degree of damage depends upon the duration and intensity of high temperature spell. The temperature up to 35ºC or higher during pollination and grain filling decreases the corn yield by 101 kg/ha every day. The grain yield production in Pakistan was 1.59 times lesser than the average grain yield of the world due to high temperature and other abiotic stresses like drought, salinity and low temperature (Anonymous, 2006).

In 2020, the maize requirements will reach up to 504 million tones for the developing countries (IFPRI, 2000). Improvement in maize germplasm against different types of biotic and abiotic stresses will gradually increase the yield and ensure food supply for growing population of the world. Hence, knowledge about genetic makeup for high temperature tolerance is essential for the development of tolerant synthetics and hybrids for sustainable agriculture. Spring season maize exposed to high temperature stress at reproductive stage during the months of April and May, results in wilting, top firing, stunted growth, delay silking, pollen desiccation and abortion which ultimately leads towards poor yield. To avoid heat shock spell at reproductive stage, the maize crop cannot be grown earlier in the first week of January because suboptimal temperature disturbs the normal emergence and early seedling development. Therefore, the production of such hybrids/synthetics that can tolerate heat stress without disturbing their yield is the principal concern of breeders.

MATERIALS AND METHODS

Maize germplasm was collected from Ayub Agricultural Research Institute (AARI) Faisalabad, Maize and Millet Research Institute (MMRI) Sahiwal and National Agricultural Research Centre (NARC) Islamabad. Total 70 accessions were evaluated against high temperature stress related standards like cell membrane thermostability (CMT), leaf temperature (LT), pollen viability %age (PV%), pollen production (PP), pollen germination %age (PG%), pollen grain size (PGS), pollen moisture contents %age (PMC%), and pollen tube length (PTL) in the University of Agriculture, Faisalabad, Pakistan. All the entries were grown by following triplicated randomized complete block design.

For pollen collection, tassels were covered with kraft paper bags one day before the start of anthesis. Pollen grains were collected during October 2011 at 6-9 am with average temperature of 33^{o}C on the daily basis from tassels, separated from anthers by sieve (0.5 mm aperture), packed in labeled zipper bags, placed in ice box and then stored at -80^{o}C.

Pollen production (mg) and viability (%): In maize pollen shedding continues for 7-9 days. Fresh Pollens were collected from tassels of three representative plants in each entry on daily basis until pollen shedding stopped. To measure pollen production, the zipper bags with pollens belonging to same accession were pooled, weighed and average was calculated. Pollen viability was observed by following methodology outlined by Dafni (1992).

Pollen Production = Weight of collected pollens with bag – weight of zipper bag.

Pollen germination (%): Pollen germination was done in laboratory by spreading almost 1000 pollen grains per genotype per petri dish containing a semi solid nutrient medium proposed by Cook and Walden (1965). Petri dishes were placed in an incubator chamber for three hours at 28^{o}C and 100% relative humidity. After three hours, petri dishes were stored at 4^{o}C to stop enzymatic activity and germination was calculated by counting germinated pollen tubes under light microscope. Pollen grains having 70µm pollen germ tube were considered as germinated pollen.

Pollen tube length (µm): Randomly 10 samples of germinated pollens were taken and pollen tube length was measured by visualizing germinated pollen grains under light microscope. Ocular micrometer was standardized to minimize error chances. The length of pollen tube was measured by using 12.5X ocular lens with 40X objective.

Pollen grain size (µm): Random selection of 10 pollen grains per entry was made and used to measure size. Pollen grain size was measured by using standardized microscope with 40X objective and 12.5X ocular lenses. Calculations were made by using formula given below.

$$\text{Area of grain} = \pi r^2$$

Pollen moisture content (%): Pollen moisture content was measured in laboratory by following procedure proposed by Inagaki and Kazi (1994).

Cell membrane thermostability: Relative cell injury percentage (RCI %), an indicator of cell membrane thermostability (CMT) was measured following the method proposed by Sullivan (1972).

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

Whereas,
T1 = EC of sap of treated discs (50°C) before autoclaving
T2 = EC of sap of treated discs (50°C) after autoclaving
C1 = EC of sap of treated discs (25°C) before autoclaving
C2 = EC of sap of treated discs (25°C) after autoclaving

Leaf temperature: Leaf temperature of three leaves fully exposed to sun was measured from selected plants in the field at 13.00-14.00 pm. Data was recorded with infrared thermometer (RAYTEK PM PLUS)**.**

Statistical analysis: Data recorded for different traits under study were statistically analyzed by using analysis of variance and principal component analysis (Sneath and Sokal, 1973).

RESULTS

Mean square comparison (Table-1) exhibited highly significant differences among genotypes for all the traits under study i.e. cell membrane thermostability (CMT), leaf temperature (LT), pollen viability %age (PV%), pollen production (PP), pollen germination %age (PG%), pollen grain size (PGS), pollen moisture contents %age (PMC%), and pollen tube length (PTL). Accessions POP-209, BF-238 and EV-7009 had the highest mean leaf temperature whereas F-113, B-305 and F-147 showed lowest mean leaf temperature values. The accessions EV-343, MT-1 and F-136 responded well and had highest cell membrane thermostability whereas accessions EV-310, F-113 and B-308 responded poorly and showed lesser stability. Assessment for pollen viability exhibited that B-321, B-316 and POP-209 showed highest pollen viability whereas SH-139, MF-2 and EV-1097 showed lowest values for pollen viability. For pollen germination, the accessions POP-209, B-308 and B-316 had the highest germination level while EV-7009, F-150 and EV-1097 had the lowest germination level. The maize accessions B-305, EV-310 and B-313 exhibited highest mean pollen production whereas maize accessions SH-139, B-313 and B-314 showed lowest mean pollen production. Regarding pollen grain size results showed that UAF-2, F-142 and F-143 had the highest mean pollen grain size but B-312, B-305 and B-

314 had lowest mean pollen grain size. In a pollen tube length comparison, the highest values for F-143, B-321 and UAF-1 and lowest values for F-150, F-111 and EV-7009 accessions were observed. Accessions VB-06, EV-1097 and EV-330 had the highest mean values whereas UAF-5, EV-79 and SAWAN-3 showed lowest mean values for pollen moisture content.

Principal component analysis (PCA) exhibited that only three components showed eigen values greater than one and considered for further analysis. First three PC's contributed about 65.4% of the total variability and were given prime importance for evaluation of accessions. The first principal component (PC1) exhibited 34.6%, second principal component (PC2) showed 16.2% and third principal component (PC3) had 14.6% variability among the genotypes for traits under study. PC1 showed highest variability for pollen viability %age, pollen germination %age, pollen tube length (PTL) and pollen production (PP). PC2 found pollen grain size (PGS) and pollen production as most variable traits and PC3 described that leaf temperature (LT), pollen grain size and pollen germination %age (PG%) as traits with highest variability. Assessment of three principle components (PC1, PC2 and PC3) proved that pollen viability %age, pollen germination %age, pollen tube length and pollen production as the characteristics of highest variability. All other PC's excluding first three PC's provided less option of selection for the improvement of traits because of low proportion of variability they contributed in total variability (Table-2).

Principal component biplot explained that variables were super imposed on the plot as vector and relative length of the vectors represented the relative amount of variability of each variable denoted. Collectively in PC1 and PC2, pollen grain size, pollen germination %age, pollen viability %age and pollen moisture contents were well represented in the plot but cell membrane thermostability, leaf temperature and pollen tube length showed minimum differences (Figure-1). The traits viz., pollen production, pollen tube length and pollen viability %age were the most discriminating because of longer vector length (Figure-1) and used as basis for ranking of maize accessions under heat stress (Table-3). Result on the basis of PCA, plot score and biplot analysis showed high variability for cell membrane thermostability, leaf temperature and pollen related traits in the accessions; B-321, EV-323, POP-209, B-308, B-316, F-127, B-236, EV-340, F-143, and SAWAN-3 under prevalence of high temperature stress. These genotypes were farther away from the origin with reference to the discriminating traits in biplot graph (Figure-1).

Table-1: Mean square comparison for eight parameters in 70 maize genotypes under high temperature stress.

SOV	df	LT (°C)	CMT	PV %	PG %	PP (mg)	PGS(µm)	PTL (µm)	PMC(%)
Replication	2	67.4298	10.027	4264.13	224.286	15805.1	2.79405	1218.74	506.071
Genotypes	69	6.5338**	583.745**	979.62**	688.437**	1416.5**	2.20650**	929.56**	30.331**
Error	138	0.8409	1.508	2.18	0.460	36.9	0.35371	30.15	1.612
Total	209								

Table-2: Principal Components (PC's) for eight characters in 70 maize genotypes under high temperature stress.

Variables	Eigenvalue	Proportion	Cumulative	PP (mg)	PV %	PG %	PTL (µm)	PMC	PGS (µm)	LT (°C)	CMT
PC1	2.768	0.346	0.346	0.375	0.519	0.501	0.430	-0.374	-0.374	-0.004	-0.070
PC2	1.298	0.162	0.508	0.386	-0.101	-0.233	-0.060	-0.173	0.451	-0.608	-0.424
PC3	1.168	0.146	0.654	-0.26	0.036	0.148	-0.170	-0.257	0.504	0.575	-0.481

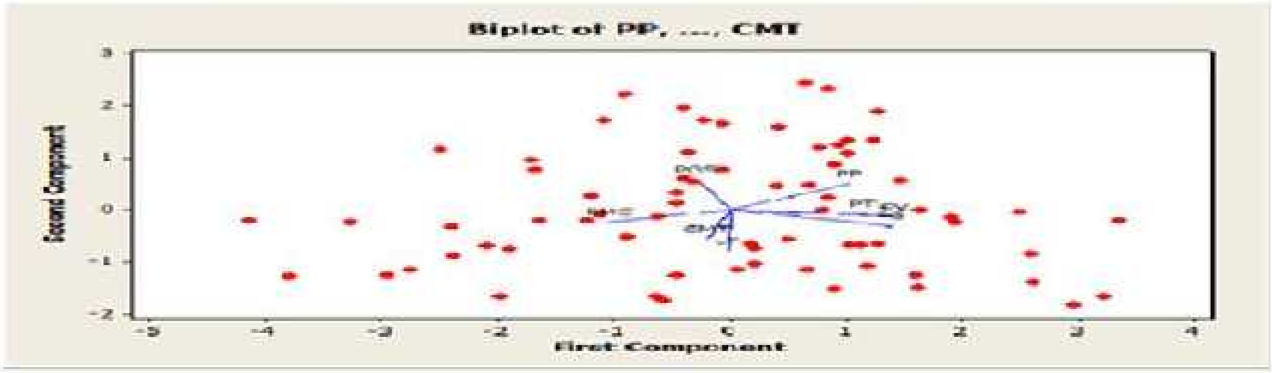

Figure-1: Biplot graphical presentation of eight traits in 70 maize genotypes against high temperature stress

DISCUSSION

High temperature increases kinetic energy of molecules across membranes which results in loosening of membranes either because of increase in unsaturated fatty acids or due to protein denaturation (Savchenko *et al*., 2002). Cell membrane thermostability was reduced because of change in secondary and tertiary structure due to higher electrolyte losses (Wahid *et al*., 2007). Steven *et al.* (2002) reported that photosynthetic apparatus of maize is very much sensitive to high temperature stress. Photosynthesis efficiency reduces beyond 38ºC and severe inhibition occurs due to rapid increase in temperature. Results indicated that maximum leaf temperature was lower than 38ºC of POP-209 (35.9ºC), which clarifies that photosynthetic activity was not inhibited in these accessions under heat stress. Transpiration rate increases with increase in leaf temperature. Ribulose bisphosphate carboxylase activation decreases at 32.5ºC with almost complete inactivation at 45ºC, which results in reduced plant growth. Photosynthetic activity and transpiration rate respond variably in maize accessions as indicated by significant differences in their leaf temperature which ultimately provides an opportunity to select least affected accessions by high temperature. Stomatal conductance reduces either by tremendously lower or higher leaf temperatures which were approximately 5ºC and 45ºC, respectively and highest at the temperature range of 20ºC to 30ºC (Baldocchi, 2005). Under drought conditions these accessions were not feasible to grow as efficiently water user.

It is well known that high temperature reduces cell membrane thermostability (CMT). The accessions showing high CMT are always considered as tolerant to high temperature stress. CMT is used as indirect measure of heat stress tolerance in wheat, cotton, soybean, tomato, potato, sorghum, barley and cowpea (Wahid *et al*., 2007). Genotypes EV-343 and MT-1 showed the highest CMT values and were considered as heat stress tolerant. Higher value of CMT indicates lower value of cell membrane injury, and higher ability to prevent the membranous protein denaturation, inhibition of electrolyte leakage, and higher tolerance to prevailing heat stress (Wahid *et al*., 2007). Genotypes F-113 and EV-310 have very low CMT values which are indication of susceptibility. Ibrahim and Quick (2001) studied membrane thermal stability as indicator of heat stress induced damage in plants. Membrane thermal stability measures the electrolyte leakage from leaf tissue after exposure to heat stress.

Accessions with highest pollen viability (B-321, B-316, POP-209, and B-308) may result in higher pollination success consequently bear higher output in the form of better seed setting under heat stress prevalence at reproductive stages of maize. Pollen formation, pollen shedding and viability are the most sensitive stages to heat stress in cereals (Stone, 2001). According to Johnson (2000), the heat stress affected the pollen viability and germination which resulted in poor seed set and ultimately poor grain yield and quality. Matsui *et al* (2001) reported that pollen sterility and poor pollen production were the causes of lesser number of viable pollens on stigma. It is well explained now that high temperature at anthesis reduced pollen viability actively whereas low temperature and high humidity favors pollen viability by preserving functional life of pollen. Therefore, the accessions with higher pollen viability are considered as heat tolerant relative to the accessions with lower pollen viability.

Pollen viability, germination and tube growth rate are much prone to heat stress and adversely affected by long time high temperature or short time extreme temperatures (Prasad *et al*., 2000). In maize, the pollen germination was affected by long time exposure to high temperature (Kakani *et al.*, 2002). Aylor (2003) reported sensitivity of ear in maize to high temperature and ultimately poor kernel growth. Adventitious pollen production and proper quantification reduces under high temperature (Lizaso *et al.*, 2003). Pollen production, silk development, pollen germination and pollen tube elongation rate are more sensitive to heat stress (Cross *et al.*, 2003). Several physiological and biochemical changes take place during pollen germination which initiated from hydration leading to transmission of generative nuclei to ovule through pollen tube. In view of the studies in different crops under heat stress, it is obvious that genotypes showing more success in pollen germination on stigma result in embryo development and subsequently confers higher grain yield.

Cárcova and Otegui (2001) treated the ears in maize with a temperature 4.5°C higher than normal which resulted in reduced number of kernels per ear. Pollen grains of maize have much variability in its shape and size ranging from round to oval which matters in defining the normal functioning of pollens. Due to exposure to high temperature normal pollen grain size is disturbed (Cárcova and Otegui, 2001). Sakata *et al.* (2000) reported damage in signal transfer mechanism due to high temperature following inhibition of pollen tube growth with almost 21% reduction in seed setting. All the reproductive processes starting from microsporogenesis, megasporogensis, anthesis, pollen tube growth, pollination, fertilization and embryo development are extremely susceptible to heat stress. Miscarriage of any of these processes reduces fertilization or raised early embryo abortion, leading to lesser number of grains (Crafts-Brandner and Salvucci, 2000).

In maize the reduced moisture contents in pollens readily reduce the viability therefore they are known as desiccation intolerant. Pollen viability reduced by 20% in first hour and after two hours pollens become totally non-viable in hot and dry field conditions (Luna *et al.*, 2001). Moisture content in maize pollen is directly associated with viability and aerodynamics of airborne pollen. Therefore, the quantification of dynamics of desiccation of pollen is much important to evaluate the crossing and dispersal potential of pollens. So pollen viability decreases gradually with pollen desiccation (Aylor, 2003).

Conclusions: Performance of maize accessions for high temperature responsive indicators was evaluated by PCA and biplot graphical displays. According to findings of this study, POP-209, B-308, B-316, F-127, EV-323, and B-321 showed higher variability based on all the studied traits. These genotypes were found to be more heat tolerant and exploitation of these accessions will bear positive outcomes in further breeding programs.

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