Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 4392 - 4399
Received 16 February 2021; Accepted 08 March 2021.

Microgametogenesis Tolerant to Heat Stress in Some Maize Crosses

Medhat M. Elsahookie[1], Saddam H. Cheyed[2], A. A. Dawood[3]
[1]Department of Field Crop Sciences, College of Agriculture Engineering Sciences, University of Baghdad, Iraqi.
[3] Ministry of Agriculture, Office of Agriculture Research.
Email: [2]saddam.hakeem@coagri.uobaghdad.edu.iq

Abstract: A field experiment was conducted on the farm of Res. Sta. Coll. of Agric. Univ. of Baghdad during 2018-2020. The experiment involved 26 recently developed maize crosses plus an adopted check hybrid. Fall planting of maize in Iraq gives higher yield than spring planting, but it is confronted with high grain moisture at harvest (30-40%) through the rain season. The main goals of this experiment were; to have crosses of low grain moisture, high grain yields, and harvesting before rain season. Seeds were planted about 10 days earlier than recommended, and harvested at mid-October. There were heat tolerant, semi-tolerant and sensitive crosses. The top five heat tolerant crosses were;1-pio3×60L, 2-60 frc × J1, 3-17A×J2, 4-Pio3 × B74 and 5-4f6×B74. Other crosses were either semi-tolerant or heat sensitive. It was concluded that high heat temperatures (45-55̊c) has negative impact on many growth parameters, enzyme activity, DNA damage, and microsporogenesis and/or microgametogenesis. Maize genotypes still have excellent opportunity to breed for high temperature and other abiotic stresses. Gene transfer will not be helpful as evaluation and screening if genetically diversed genotypes. Such plants characteristics could be controlled by hundreds of not thousands of genes. Cycles of reselection on some individual unique plants inbred population will be helpful to have new genetic variations in maize germplasm.
Key words: Microsporognesis, ROS, genetic diversity, grain yield, moisture.

INTRODUCTION

Maize (*Zea mays* L.) ranks the third after wheat and rice, and this explains its significance in animal feed, and human diet in the world. This crop is a C-4 crop which give it an excellent chance for high productivity. There are many constraints to produce higher grain yield of this crop and many other crops. Among those constraints is abiotic stress. In Iraq, maize is being grown for years into two planting seasons, spring planting in mid-March, and fall planting in mid-July. Spring planting gives about 60%grain yield of fall planting and harvested between end of June and beginning of July so, there will be no grain moisture problem and rainy season that makes soil muddy against harvesting equipments. For the higher productivity of maize in fall planting, farmers prefer this planting season. However, harvesting the crop of this planting season has the problem of high grain moisture which causes the growth of molds, and the difficulty of harvesting, because rain season starts at time of harvest. The low grain yield of spring planting is due to high temperatures at time of pollination causing poor seed-set. In fall planting, seed-set is better, so, the productivity is higher, but still the problem of high grain moisture. Several investigators have studied the impact of high temperatures on micro- gametogenesis in maize. Tiwari and Yadav (2019) reported that high temperature of environment has negative effects on anthesis, silking, and grain filling. Rieu et al (2017) also stared the negative effect of high temperature on male gametes of maize. Lyakh et al (1991) mentioned that maize pollen viability was strongly decreasing at 38 ºC, and that the tassel of the maize plant could lose high number of viable pollens leaving silks without fertilization, although the tassel could contain 5-50 millions of pollen grains (Poehlman 1959). The negative effects of high temperature on pollen viability was attributed to reduced starch content, decreased enzymatic activity, and reduced pollen germination (Begcy et al 2019). However, the last authors also concluded that negative effects of high temperature will be the most through the development stage of microsporogenesis to microgametogenesis. On the other hand, silk dryness is another negative effect of high temperature on maize seed-set and yield (Naveed et al 2014). This experiment aimed screening some recently developed single crosses of maize to high temperatures at time of pollination, by early planting in July to avoid the problem of high grain moisture at harvest, causing molding and loss of high quantities of this crop in Iraq, besides the need to spend energy to dry grain before storage.

MATERIALS AND METHODS:

On the farm of Research Station, of the College of Agric., University of Baghdad, Abu-Gharib, an area of land was prepared for planting. Some recently developed inbreds of maize were planted in fall season of 2018 for seed increase. These inbreds were Pio3, 60L, 4f6, A4, A7, J1, 17A, B74, 73brdwc, Den4, 60frc, and J2. Some of these inbreds were developed according to some promising traits (Elsahookie et al 2018). In fall 2019, seeds of these inbreds were planted in enough populations to do crossing among some of them according to our knowledge on combining ability of those inbreds. At time of maturity, seeds were collected and kept in room temperature for planting in next season. In fall of 2020, all enough seeds of crosses were grown on 50×25 cm spacing. Weeds and corn borer control, irrigation and fertilization were applied as recommended (Elsahookie 1990). There were 26 single crosses grown plus a control (Zm 60 × Zm 21) which is a registered and adopted hybrid in central Iraq. The crosses parents and their symbols are shown in Table 1.

Table 1. Number and parents of maize crosses used.

No.	Crosses	No.	Crosses	No.	Crosses	No.	Crosses
1	Pio3 × 60L	8	A4 × 17A	15	17A× J1	22	73brdwe×J2
2	4f6 × 60L	9	A4 × B74	16	17A×60L	23	A7 × Den4
3	A7 × J1	10	A4 × Den4	17	17A×Den4	24	73brdwe×J1
4	A7 × 17A	11	A4 × J1	18	60frc×17A	25	Pio3×B74
5	A7 × B74	12	73brdwc×60L	19	60frc× J2	26	4f6×B74
6	A7×73brdwc	13	73brdwc×17A	20	60frc× J1	27	Zm60×Zm21
7	A4 × 60L	14	73brdwc×Den4	21	17A×J2		

Planting was in the first week of July, 2020 to give plants higher chance to suffer from heat at time of pollination. The design used was an RCBD of four replicates. The sampled plants measured for agronomic traits were tagged to be taken for grain yield. Plots were 4m long and 3m wide. Each plot contained 6 rows of 50 cm apart. At the end tasseling, agronomic

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 4392 - 4399
Received 16 February 2021; Accepted 08 March 2021.

measurements were taken as shown in the Tables. Leaf area was taken on five plants of each experimental unit. The formula used was:

Leaf area / plant = $\sum Lb^2 \times 0.65$.

Lb = is the length of leaf which is located below ear leaf (Elsahookie 1990).

Stem volume was measured as a cylinder. Chlorophyll index was taken by Spad at harvest maturity. Ears were left to dry for 5 weeks in room temperature. Ears were hand threshed, cleaned, and weighed with 15% grain moisture. Data were tabulated and statistically analyzed.

RESULTS AND DISCUSSION:

Field plant traits:

Data taken on plant height, leaf area, stalk volume, and chlorophyll index (Spad readings) are shown in Table 2. Crosses were significantly different (α5%) for the four measured traits. Taller plants of crosses did not necessarily give wider leaf area. This implies that shorter plants had wider and/or longer leaves to substitute for leaf area. Genotypes leaf area if not higher in photosynthesis efficiency, they will not show better growth or higher grain yields. Stalk volumes of crosses plants show significant

Table 2. Means of yield components and grain yield of maize crosses studied.

Cross number	Plant height (cm)	Plant leaf area (m^2)	Stalk volume (cm^3)	Spad index
1	163	0.621	288	29.5
2	183	0.522	369	44.5
3	164	0.621	290	25.8
4	154	0.677	260	32.8
5	164	0.711	317	32.7
6	137	0.551	232	39.2
7	140	0.590	238	36.7
8	142	0.668	312	37.8
9	150	0.704	290	49.2
10	171	0.677	128	32.0
11	185	0.649	327	37.02
12	163	0.490	206	31.5
13	145	0.537	267	36.8
14	172	0.572	278	37.2
15	137	0.545	171	30.5
16	121	0.517	161	37.5
17	154	0.658	226	45.7
18	124	0.589	240	39.0
19	169	0.571	215	43.7
20	188	0.594	304	36.5
21	143	0.599	266	49.5
22	147	0.541	249	32.8

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 4392 - 4399
Received 16 February 2021; Accepted 08 March 2021.

23	**186**	**0.706**	**300**	**23.7**
24	**156**	**0.468**	**167**	**26.0**
25	**152**	**0.586**	**246**	**27.3**
26	**185**	**0.460**	**405**	**42.2**
27	**144**	**0.563**	**290**	**34.0**
LSD 5%	**5**	**0.036**	**42**	**4.5**

differences in this trait. Chlorophyll index values taken on flag leaf or the penultimate leaf (if flag leaf was damaged) show significant differences. These four studied traits in general were related. However, it could be noted that a tall plant with lower leaf area such as cross number 2 of 183cm gave 0.522m^2, while cross number 15 of 137cm gave 0.545m^2 leaf area. If we look at plant leaf area and stalk volume, we may be notice a similar thing. The cross number 9 gave 0.704m^2 leaf area and 290 cm^3 stalk volume, while cross number 26 gave 0.460m^2 leaf area and high stalk volume (405cm^3). When stalk volumes were studied, we kept in mind that stalks with higher volume will probably have more photosynthesis to feed grain filling, but the case was not, although, we can say that plant with lower stalk volumes will give better harvest index. The same trend is found between stalk volume and Spad index. The cross number 15 had 171cm^3 stalk volume and 30.5 chlorophyll index, the opposite case is with the cross number 3 which had 290cm^3 stalk volume corresponding to 25.8 chlorophyll index. These results show why researchers need to study their genotypic populations for all traits under study for different genotypes have different relationships among their agronomic traits.
There were some deleted crosses, (not involved in Table 2) that was for those crosses were so sensitive to high temperature at time of pollination. Temperatures measured at time of pollination ranged between 45-55 ºC for a few hours a day, so, in this way, we had a good screening chance for all heat sensitive crosses. Those sensitive crosses gave only a few kernels per ear, and many of them without grains (Plate 1).

Plate1. Ears of sensitive semi-talent, and tolerant crosses of maize to high temperatures.

High temperatures through time of pollination cause pollen dryness and death, and also the silks will dry off and cannot be receptive to pollens (Naveed et al 2014). The most amazing point that these authors reported that: every one degree of temperature higher than 35ºC during pollination and grain filling decreases maize grain yield by 101 kg/ha every day. In

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 4392 - 4399
Received 16 February 2021; Accepted 08 March 2021.

case that pollen was active and has pollinated the silk, under temperatures 45ºC and up, it is still difficult to pollen tube to grow will. However, when temperatures measured at time of pollution (45-55ºC), that was in the shade, but plants were grown under direct sun, that will be more harmful to pollens, silks, and pollen tube growth. Many disruptions in metabolism and nutrient translocation will also take place, and have negative effects on pollens, silks activity, and grain filling. Palmer (2019) mentioned that starch, sucrose, fructose, enzymatic activities, and DNA were involved in fertilization failure under high temperatures. Janni et al (2020) emphasized on the need to study the mechanisms of cultivated plants to cope with abiotic stresses. Meanwhile, these mechanisms are not easily transformed by new technologies of gene transfer, for they should be in hundreds, if not thousands of genes. Kandel et al (2018) stated that plant heat tolerance is a quantitative complex trait, and selecting genotypes tolerant to heat is the best way in a breeding program. This explains that germplasm collection and screening is a premium step towards having heat tolerant elite hybrids of maize. El Sabagh et al (2020) stated that it is so necessary to breed for heat tolerance and other abiotic stresses due to climate change to boost crop production for increasing human population. This idea has been recommended also by some other investigators (Elsahookie 2013, Lorenz et al 2019 and Prasad et al 2017 and 2018).

Grain yield and components:

When maize genotypes grown under high population densities, it is seldom to find a genotype of more than one ear/plant as a mean for that genotype. These crosses were grown under 80 thousand plants/ha (50×25cm), and this is why all crosses plants gave only one ear per plant. Data of Table 3 show the results obtained on yield components and grain yield of crosses under study. Grain number per ear is one of principal component of grain yield in unit area along with grain weight. The crosses differed significantly in this trait and in the other three traits shown in the Table. If the cross gives ears with high number of grains coincided with heavy grains, this cross will produce higher grain yield in unit of area. Ear grain number was ranging from 215.8 in cross 4 to as high as 533.5 grains/ear of the cross 21. It can be noted that cross 4 gave lower grain yield (6.64Mg/ha), and cross 21 gave high grain yield (12.16 Mg/ha), although there was cross 1 which gave 14.20 Mg/ha but it was not significantly higher. The second component is the grain weight. Table 3 shows that the heavier grain weight was with cross 4 (0.385 g/grain) but its grain number per ear was low. The least grain weight was with cross 5 which gave only 0.205g/grain and did not give high grain yield (7.52 Mg/ha). Ear grain weight is the resultant of both components; ear grain number and grain weight. So, all what is needed just to multiply their outcome by planting population density to have grain yield/ha. Kernel size and weight have specific loci in maize (Hao et al 2019) while Allard (1960) stated that seed weight is the most inherited trait among other traits in plants. In case of crossing, kernel weight and number could be increased so well in some

Table 3. Means of yield components and grain yield of maize crosses studied.

Cross number	**Ear grain number**	**Grain weight (g)**	**Ear grain weight (g)**	**Grain yield (Mg/ha)**
1	**422.5**	**0.350**	**158.0**	**14.20**

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 4392 - 4399
Received 16 February 2021; Accepted 08 March 2021.

3	**479.2**	**0.279**	**133.6**	**10.70**
4	**215.8**	**0.385**	**82.8**	**6.64**
5	**459.5**	**0.205**	**94.0**	**7.52**
11	**336.5**	**0.312**	**105.0**	**8.40**
12	**470.8**	**0.237**	**111.2**	**8.90**
13	**304.0**	**0.280**	**85.0**	**6.80**
14	**336.5**	**0.255**	**86.0**	**6.87**
16	**368.5**	**0.221**	**81.2**	**6.50**
17	**318.0**	**0.275**	**87.2**	**6.98**
18	**497.0**	**0.217**	**107.8**	**8.62**
19	**434.8**	**0.336**	**146.0**	**9.18**
20	**497.2**	**0.312**	**156.0**	**12.48**
21	**533.5**	**0.285**	**152.0**	**12.16**
22	**308.0**	**0.320**	**98.5**	**7.88**
24	**369.0**	**0.330**	**122.0**	**9.76**
25	**482.2**	**0.320**	**154.2**	**12.34**
26	**461.2**	**0.335**	**154.5**	**12.36**
27	**377.0**	**0.320**	**123.8**	**9.90**
LSD 5%	**45.5**	**0.007**	**16.0**	**2.66**

elite crosses as found by Elsahookie et al (2020). The problem of climate change and its impact on crop productivity has been discussed by several investigators (Westgate et al 2003, Shiferaw et al 2001, Sehgal et al 2018, Takahashi and Shimozaki 2019 and Lorenzo et al 2019).

Ear grain yield was significantly higher in five crosses; cross number 1 (158.0g/ear), cross number 20 (156.0g/ear), cross number 26 (154.5g/ear), cross number 25 (154.2g/ear), and cross number 21 (152.0 g/ear). These five crosses produced higher grain yield as compared to all other crosses. They produced 14.20, 12.48, 12.36, 12.34, and 12.16 Mg/ha, respectively. Our breeding program for heat tolerant crosses should focus on the increase of inbred seeds involved in these elite crosses. The check hybrid Zm60 ×Zm21(given number 27 in Table 3) has ranked sixth in grain yield (Mg/ha) after these five crosses. These five crosses were the most prominent crosses in heat tolerance. Other crosses of less heat tolerance such as those holding the numbers 3, 24, and 27 are still promising, while the rest of crosses were semi-tolerant crosses, and those deleted from the table were heat sensitive.

There were too many ideas reported on mechanisms of heat tolerance, and what is happening under that extreme temperatures. Elsahookie (2013) and, Tiwari and Yadav (2019) reported the negative effects of high temperature due to ROS production, especially in the chlorophyll. Several disruptions in cell will take place which lead to cell death. Sehgal et al (2018) stated that even grain filling will be affected by high temperatures. This idea was confirmed by Tiwari and Yadav (2019). However, Takahashi and Shimozaki (2019) found that most of heat stress genes are involved in primary and secondary metabolism, translation, transcription, regulation, and many other signaling in the plants, including modifications of proteins and phosphorylation. Probably some could say that tassels produce pollens up to 50 million, should we expect all that number of pollens will dry? pollen density to pollinate a plant silk should be high enough in number to have good seed-set. Westgate et al (2003) estimated the number of active pollens to pollinate one maize plant silk should not be less than 3000 viable pollens.

Finally, this research aimed screening some recently developed maize crosses for heat tolerance. Planting about 10 days earlier than recommended have resulted harvesting at mid-October. Grain moisture at harvest raged between 18%-25%, while in conventional fall harvest in Iraq is in December, with grain moisture 30%-40%. Rain season in Iraq starts usually in November-December. Temperatures between 45-55ºC at pollination has dramatic negative effects on pollen viability. So, microgametogeneses has been negatively affected by that temperature. Some tassels of some crosses did not show any pollens, this could be attributed to negative heat effect on microsporogenesis too. Reactive oxygen species, starch, sugars, chlorophyll photosynthesis, DNA damage, and many other metabolisms could be disrupted by heat. We can conclude that some maize germplasm still have high ability to tolerate temperatures beyond 50 ºC at time of pollination. So, our program will proceed to propagate inbreds seeds involved in those heat tolerant crosses. Genetic diversity can be created by breeders from their own adapted inbreds, but it is recommended to cross with some other geographic regions germplasms to have wider genetic diversity for high temperature tolerance.

REFERENCES

[1]. Allard R 1960. Principles Plant Breeding, John Wiley and Sons, Inc., N.Y., USA, pp:485.

[2]. Begcy K, Nasenko T, Zhou L-Z, Fragner L, Weckweth W and Dresselhaus T 2019. Male sterility in maize after transient heat stress during the tetrad stage of pollen development. Plant Physiol.,181:683-700.

[3]. EL Sabagh A, Hussain A, Iikal M, Barutcular C, Islam M, … et al 2020. Maize adaptability to heat stress under changing climate. In Plant Stress Physiology, IntechOpen, DOI: http://dx.doi.org./10.5772/intechopen.92396.

[4]. Elsahookie MM 1990. Maize Production and Breeding. Ministry of Higher Education Press (in Arabic) Baghdad, Iraqi pp:400.

[5]. Elsahookie MM 2013. Breeding Crops for Abiotic Stress-A Molecular Approach and Epigenetics (in Arabic). College of Agriculture, University of Baghdad Publications pp:244.

[6]. Elsahookie MM, Al- Khafaji MJ and Dawood AA 2018. A promising approach to develop maize inbreds and test their performance. Iraqi J. Agriculture Science 49(5):748-754.

[7]. Elsahookie MM, Cheyed SH and Dawood AA 2020. Improving kernel weight and number in some maize subspecies crosses. Sys. Rev. Pharm 11(9):803-807

[8]. Hao D, Xue L, Zhang Z, Chang Y, G. Chen, Zhou G, Lee P, Yang Z and Xu C 2019. Combined linkage and association mapping reveal candidate loci for kernel size and weight in maize. Breeding Science Preview. doi: 10.1270(1) jabba.18185.

[9]. Janni M, Gulli M, Maestri E, Marmiroli M, Valliyodan B, Nguyen H and Marmiroli N 2020. Molecular and genetic base of heat stress responses resilience and productivity. J. Exptl. Bot,71(3):3780-3802.

[10]. Kandel M, Ghimire S and Shrestha J 2018. Mechanisms of heat stress tolerance in maize. Azarian J. Agric. 5(1): 20-27.

[11]. Lorenz R, Stalhandske Z and Fischer E 2019. Detection of climate change signal in extreme heat, heat stress, and cold in Europe from observations. Geophysical Res. Letters, 46:8363-8374.

[12]. Lyakh V, Kravechenko A, Soroka A and Dryuchina E 1991. Effects of high temperatures on mature pollen grains in wild and cultivated maize accessions. Eyphytica, 55: 203-207

[13]. Naveed S, Aslam M, Magbool M, Bano S, Zaman Q and Ahmed R 2014. Physiology of high temperatures stress tolerance ot reproductive stages in maize. The J. Animal of Plant Sci., 24(4): 1141-1146.

[14]. Palmer L 2019. The heat is on the maize pollen development after a heat wave. In: Plant Physiology News and Views. https://plantae.org/the-heat-is-on-maize-pollen-development-aft.

[15]. Poehlman J 1959. Breeding Field Crops. Holt, Rinehart, and Winston, Inc., N.Y, USA, pp:427.

[16]. Prasad P, Bheemanahalli R and Jogadish S 2017. Field crops and the fear of heat stress-opportunities, challenges, and future directions. Field Crop Res. 200:114-121.

[17]. Prasad R, Gunn S, Rotz C, Kersten H, Roth G, Buda A and Stoner A 2018. Projected climate and agronomic implications for corn production in the Northern United States. PLOS One, 13:e 0198623.

[18]. Rieu I, Tell D and Giron N 2017. Pollen development at high temperatures: From acclimation to collapse. Plant Physiol., 173:1967-1976.

[19]. Sehgal A, Sita K, Siddiqui K, Kumar R, Bhogireddy S, Varshney R, et al 2018. Drought and/or heat stress effects on seed filling in food crops. Impact on functional biochemistry, seed yields, and nutritional quality. Frontiers in plant Sci., 9:1705. DOI: 10.3389/ fold. 2018.01705.

[20]. Shiferaw B, Prasanna B, Hellin J and Danziger M 2011. Crops that feed the world: Past successes and future challenges to the role played by maize in global food security. Food Security 3:307-311.

[21]. Takahashi F and Shimozaki K 2019. Long distance signaling in plant stress response. Current Opinion in Plant Biology 47:106-111.

[22]. Tiwari Y and Yadav S 2019. High temperature stress in maize (*Zea mays* L.): Physiology and Molecular Mechanisms. J. Plant Biol., 62: 93-102.

[23]. Westgate M, Lizaso J and Batchelor W 2003. Quantitative relationship between pollen shed density and grain yield in maize. Crop Science 43(3): 934-942.