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Breeding for Heat Stress Tolerance of Maize in Pakistan

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Abstract: Each plant species rather more specifically each genotype, has an optimum range of temperature for growth and development. When temperature rises beyond this critical limit, it generates temperature stress which affects its performance adversely. Considerable variation exists among genotypes regarding heat stress providing opportunities for breeders to improve crops through genetic means. Keeping in view the importance of global warming as a potential threat to maize production in the province of Punjab, Pakistan; studies have been initiated to develop thermo tolerant inbred lines by providing high temperature at the time of anthesis and subsequent developmental stages of grain formation. The studies were focused on screening of heat tolerant lines for the production of maize hybrids that are adaptable in agro-climatic conditions of Pakistan especially in spring season when temperature fluctuates between 40-45ºC and sometime reaches up to 48ºC. The inbred lines along with local hybrids were grown in field as well as under tunnel conditions keeping multinational hybrids as standard. The results revealed that anthesis and grain filling stages were more sensitive. A number of lines set seed showing tolerance against high temperature, which were increased and used in hybrid production. The local hybrids YH-1898 and YH-1921 showed reasonable tolerance against high temperature with 40-50% seed setting as compared to commercial hybrids (DK-6525, DK-6142 and NK-8441) with 20 - 25% seed setting. More recently the screening studies have been further substantiated with glasshouse conditions.

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1. Introduction

In Pakistan maize is third important cereal crop after wheat and rice. Maize is being grown on an area of 1.117 million hectares with annual production of 4. 527 million tons and average grain yield of 4053 kg ha^{-1} (Anonymous, 2014). In Pakistan maize is grown during two seasons i.e. kharif and spring. The adoption of spring maize has significantly increased since the active involvement of multinational companies in seed business. Today the spring maize acreage is 12-15% of total maize area and almost 30-35% of total annual production. A significant increase in maize area during spring has been observed which is mainly due to very good yield levels (7-8 ton ha^{-1}). Spring maize is one of the success stories in Pakistan agriculture. Maize plants are prone to rapid and constant changes due to global warming (Porter, 2005; Wahid et al., 2007). However, high temperature (45 – 48ºC) at reproductive stage is the most alarming factor in Pakistan as well as in the world that standardizes the crop growth and eventual the yields (Al khatib and Paulsen, 1999, Ulukan, 2009; Rahman et al., 2013). As the temperature increased beyond optimum temperature which significantly decreases the plant growth as well as yield (Campos et al., 2004).

Higher temperature at reproductive stage decreased the seed setting percentage and kernel development that ultimately has negative effect on grain yield and quality as well (Wilhelm et al., 1999; Commuri and Jones, 1999; Talwar et al., 1999; Cicchino et al., 2010; Rehmani et al., 2014; Sattar et al., 2015). As higher temperature with low humidity withers the open silk and pollen grains and condensed the pollen germination (Sinsawat, 2004). It also negatively affects many physiological aspects of the plant like variation in protein metabolism, decreasing the process of photosynthesis (Dubey, 2005; Kim et al., 2007; Ristic et al., 2009), initiation state of Rubisco declines at 32.5°C (Crafts-Brander and Salvucci, 2002) which almost completely stopped at 45°C and plant dies at 54°C (Smith, 1996 and Steven et al., 2002). The effects of heat stress can be reduced through optimum agronomic practices like irrigation management can minimize the moisture stress but it cannot eliminate all of the destructive impact of high temperature (Chen et al., 2010). Hence, the development of heat tolerance in maize inbred lines through breeding can maintain the optimum plant growth and productivity under heat stress (Wang et al., 2003).

Variation in maize landrace genetics is naturally and is helpful in coming breeding advancement (Reif et al., 2004); it is a crucial requirement to permit constant development in agricultural productivity (Smith, 2007). Genetic resources of the plants are the essential constituent of all agricultural systems, thus maintenance, evaluation and improvement of germplasm is an indispensable (Ortiz et al., 2008). For many years, prevailing landraces were the sources for the development of new open pollinated varieties, which in turn are frequently used to develop promising hybrids (Taba et al., 2005). The meteorological data showed that highest maximum temperature in Central Punjab region of Pakistan reaches up to 48°C during the month of May when maize crop is in the reproductive phase. Keeping in view the current and expected future environmental changes, studies were initiated at Maize and Millets Research Institute (MMRI), Yusafwala - Sahiwal, Punjab (Pakistan) during 2004 to develop thermo-tolerant maize inbred lines by providing high temperature at the time of flowering and subsequent developmental stages and selecting those which set seed under heat stress. These lines are being utilized for production of heat tolerant maize hybrids which have the potential to perform better under severe agro-climatic conditions of Pakistan especially during spring season. Maize crop cannot be sown earlier in month of January, due to lower temperature which is unfavorable for germination and growth and late sowing is affected by high temperature stress at reproductive stage. Therefore, heat stress tolerance is a top priority breeding objective for the maize researchers in Pakistan.

2. Materials and Methods

The research work regarding screening of maize inbred lines developed at MMRI, Yusafwala-Sahiwal, Punjab, Pakistan against high temperature stress was started in spring season, 2004 initially under field (uncontrolled) conditions. The procedure was improved by coupling it with tunnel (semi-controlled) conditions in spring season, 2010. More recently, the procedure has been further substantiated by utilizing glasshouse facilities (controlled conditions) during spring season, 2011.

2.1 Screening under Field Conditions

Maize planting in spring season in Pakistan starts from last week of January to mid-February. Normal flowering occurs in April when the temperature ranges 35-40°C. May and June are severe months and temperature fluctuates around 45-48°C. For screening against high temperature stress; 92, 116, 101, 45, 60, 47 and 37 maize inbred lines were planted during spring season in the last week of March 2004, 2005, 2006, 2007, 2008, 2009 and 2010 respectively to give high temperature (45-48°C) exposure at flowering in May-June.

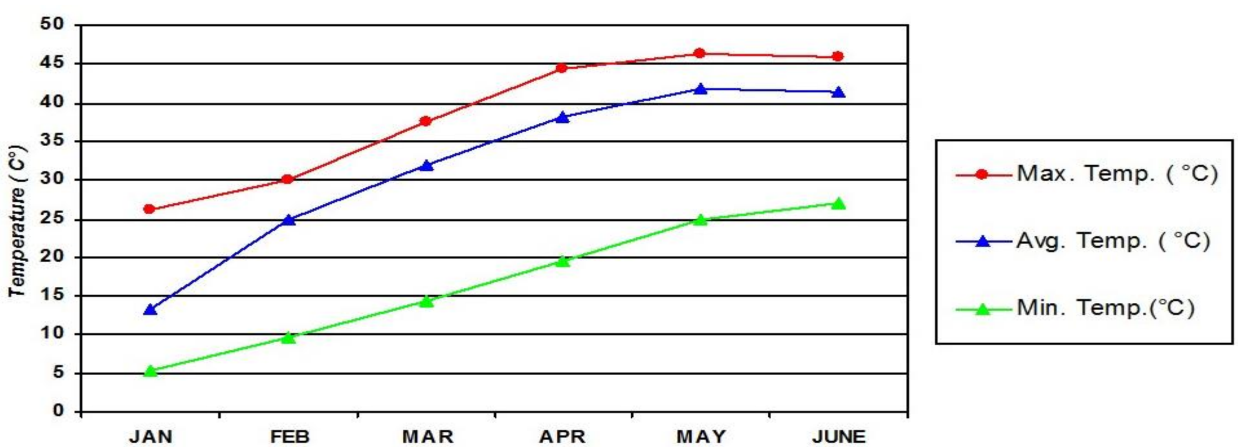

Fig. 1: Monthly averages of daily Maximum, Minimum and mean temperatures during spring season at Maize & Millets Research Institute Yusafwala Sahiwal during 2004-11.

Table1: Maximum temperature range at different growth stages.

Crop Growth Stage	Outside Tunnel	Inside Tunnel	Delta Temperature
Flowering (24th April to 28th April)	42 - 43 °C	45 - 47°C	3-4°C
Pollination (29th April to 4th May)	42 - 45 °C	45 - 47°C	2-3°C
Grain formation	45 - 47°C	47 - 51°C	2-4°C

Inbred lines were planted ear to row and all plants having silks emergence and pollen production in high temperature were self pollinated. Single plant selections were made on the basis of seed setting percentage. Date of pollination and daily maximum temperature were recorded. More seed setting percentage confirmed the pollen viability/silks receptivity at high temperature. Selected inbred lines/plants were sown during following spring season for next cycle of screening. The elite lines so screened were utilized for development of hybrids having fair possibility of tolerance against heat stress.

2.2 Screening under tunnel and field conditions

Twenty one (21) locally developed inbred lines along with eight hybrids (including three multinational standards) were sown on 22nd February 2010 both under tunnel as well as in the open field. The temperature at sowing was 25ºC. The plot size was kept as 5 m x 0.75 m. The standard production/protection practices were followed. Data on temperature both outside and inside the tunnel at different crop stages (Table 1), agronomic traits and number of viable seeds on five (5) self-pollinated cobs were recorded (Table 3A, 3b).

3.3 Screening in the glasshouse and field conditions

All the maize inbred lines and hybrids (29) planted in tunnel during spring 2010 along with seven new derivatives (total 36) were planted both in glasshouse as well as in the open field on February12, 2011. The temperature at sowing was 20ºC in glasshouse. The plot size was kept as 5m x 0.75m. The standard production/protection practices were followed. Data on daily maximum temperature, both outside and inside the glasshouse, agronomic traits and number of viable seeds on five (5) self pollinated cobs were recorded. High temperature shock of 50ºC (±1) for 4-hour duration, during day time, was imposed for three (3) weeks at flowering and grain formation stage.

3. Results and discussion

3.1 Screening under Field Conditions

The studies revealed that most of the inbred lines showed variable response against high temperature. The tested lines were selected on the basis of seed setting. The lines with more than 25% seed setting were selected. The year-wise detail is given in Table 2. The selected lines have been utilized for development of hybrids expected to be heat tolerant. Although screening in the field is not very accurate due to fluctuations in the temperature during day and night that may favor seed setting, it provides basic information regarding behavior of genotypes under thermal-stress. The combinations constituted on the basis of such screening expressed heat tolerance in comparison to commercial hybrids as shown in the following studies.

3.2 Screening under tunnel and field conditions

The data on seed setting (Tables 3, 4) showed that high temperature **(**47 – 51ºC) inside the tunnel during and after pollination severely affected seed setting both of inbred lines and hybrids. Most of the inbred lines showed variable tolerance against high temperature (Table 3). Twelve (12) lines out of twenty one (21) viz. Y-12, Y-22, Y-5, Y-35, Y-29, Y-3, Y-27, Y-36, Y-37, Y-2, Y-38 and Y-50 did not produce any seed. The others varied with range of one (1) grain (Y-24 and Y-19) to forty-five (45) grains (Y-26).

Table 2. Number of inbred lines studied under field condition (2004-2010)

Season/Year	Lines Tested	Lines Selected (Seed Setting >25%)
Spring-2004	92	25
Spring-2004	116	40
Spring-2006	101	40
Spring-2007	45	10
Spring-2008	60	5
Spring-2009	47	5
Spring-2010	37	2

As regards hybrids, local hybrids developed at MMRI viz. YH-1898 and YH-1921, FH-810 and FH-793 by utilizing heat tolerant inbred lines depicted considerable tolerance against heat stress with 30-50% seed setting as compared to commercial hybrids used as checks viz. DK-6525, DK-6142 and NK-8441with 20-25% seed setting. In open field, inbred lines showed more seed setting as compared to tunnel planting while local hybrids (YH-1898, YH-1921, FH-963, FH-793 and FH-810) exhibited 100% seed setting as compared to multinational hybrids (DK-6525, DK-6142 and NK-8441) with 50-60% seed setting. As shown in table 1, there was a difference of 2-4ºC in the ambient temperature inside and outside the tunnel that may be attributed to respective differences in the seed setting. A single degree change in ambient temperature may lead to a series of changes in the plant behavior (IPCC, 2007; Wahid et al., 2007). However, it is worth mentioning that the chances of escape are minimized due to excessive thermal exposure inside the tunnel.

3.3 Screening in the glasshouse and field conditions

The data on seed setting (Tables 5, 6) showed that high temperature **(**50^{0}C ±1) during day time at flowering and grain formation stages inside the glasshouse influenced seed setting of inbred lines and hybrids. Most of the inbred lines showed differential tolerance against high temperature. Six (6) lines out of twenty-one (21) viz. Y-29, Y-2, Y-37, Y-38, Y-19 and Y-35 did not set any seed. The others ranged from 2 grains (Y-3) to 45 grains (Y-26).Among new derivatives, four (4) out of seven (7) did not develop any seed while derivative DR-35 produced highest number of grains (100).

Table-3: Agronomic traits and seed setting of inbred lines under tunnel (Spring-2010).

Entry	Days to 50% tassel	Days to 50% silk	Plant Height (cm)	Cob Height (cm)	No. of Viable Seeds (5-cobs)
Y-26	65	68	125	60	45
Y-9	67	70	115	47	20
Y-14	65	69	125	64	15
Y-13	65	68	155	75	15
Y-25	65	68	110	55	10
Y-11	66	69	125	57	10
Y-32	64	67	125	62	3
Y-19	67	69	115	55	1
Y-24	66	69	120	65	1
Y-12	69	72	155	140	-
Y-22	69	71	147	71	-
Y-5	67	69	120	60	-
Y-35	70	71	130	65	-
Y-29	70	71	110	50	-
Y-3	70	71	106	52	-
Y-27	68	71	120	55	-
Y-36	67	69	145	71	-
Y-37	67	69	126	65	-
Y-2	69	70	155	79	-
Y-38	68	70	155	65	-
Y-50	67	70	139	67	-

Table-4: Agronomic traits and seed setting of hybrids under tunnel (Spring-2010).

Entry	Days to 50% tasseling	Days to 50% silking	Plant Height (cm)	Cob Height (cm)	No. of Viable Seeds (5-cobs)
YH-1898	66	67	180	90	50
YH-1921	67	68	178	90	40
FH-793	64	67	195	100	30
FH-810	68	71	190	97	30
NK-8441	68	70	201	100	25
FH-963	63	66	189	89	20
DK-6525	64	67	187	90	20
DK-6142	64	68	20	98	20

Table-5: Agronomic traits and seed setting of inbred lines in glasshouse (Spring-2011).

Entry	Days to 50% tassel	Days to 50% silk	Plant Height (cm)	Cob Height (cm)	No. of Viable Seeds (5-cobs)
Y-26	76	89	133	70	45
Y-9	78	82	128	63	22
Y-11	73	76	138	70	21
Y-14	76	79	143	72	20
Y-13	70	73	160	81	19
Y-25	68	69	125	64	17
Y-32	74	76	140	70	12
Y-24	78	81	128	65	10
Y-27	78	81	143	73	7
Y-36	67	70	155	75	7
Y-22	72	75	154	76	7
Y-5	68	71	136	70	6
Y-50	73	75	150	78	3
Y-12	70	72	150	77	3
Y-3	68	71	118	58	2
Y-29	73	76	119	58	-
Y-2	73	75	163	80	-
Y-37	75	78	141	71	-
Y-38	73	76	160	80	-
Y-19	70	73	123	65	-
Y-35	72	75	139	71	-
DR35	73	76	127	65	100
DR2	68	70	128	65	15
DR135	77	80	135	63	10
DR1	73	75	117	55	-
DR3	70	73	120	59	-
DR5	68	70	121	60	-
DR6	68	71	130	61	-

Table-6: Agronomic traits and seed setting %age of hybrids in glasshouse (Spring-2011).

Entry	Days to 50% tasseling	Days to 50% silking	Plant Height (cm)	Cob Height (cm)	No. of Viable Seeds (5-cobs)
YH-1898	69	72	189	93	50
YH-1921	71	74	190	95	40
FH-793	67	71	203	106	40
FH-810	73	77	200	106	35
NK-8441	73	76	209	104	35
FH-963	66	70	190	97	30
DK-6142	67	69	205	100	25
DK-6525	68	71	197	95	25

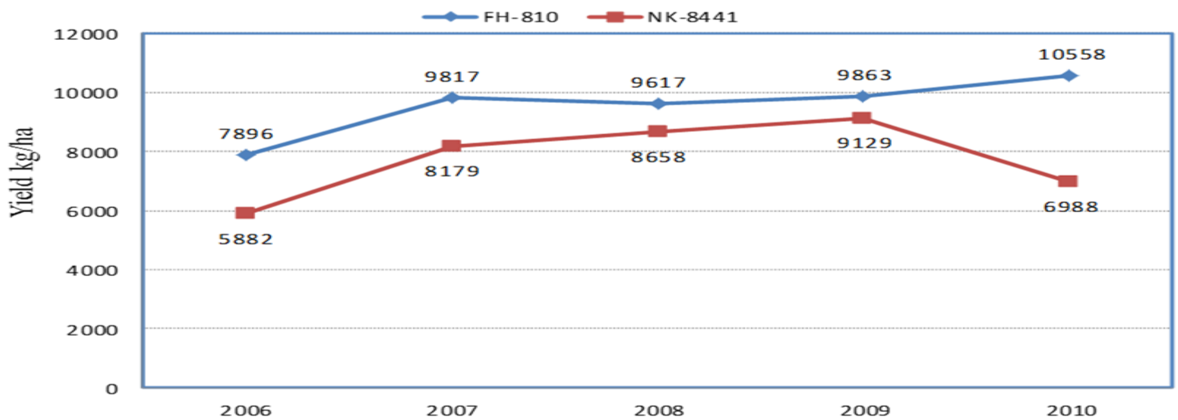

Fig. 2: Performance of local and commercial maize hybrids under heat stress

As regards hybrids, local hybrids (YH-1898, YH-1921, FH-793 and FH-810) showed considerable thermo-tolerance having 35-50% seed setting as compared to commercial hybrids (DK-6525, DK-6142 and NK-8441) included as checks with 25-35% seed setting. The same lines and hybrids which were sown in open field on same date, performed better in terms of seed setting. It was interesting to note (Tables 3 and 5) that although the response of inbred lines towards heat stress was almost similar in the tunnel and glasshouse, tunnel conditions were more severe than glasshouse regarding seed setting. Hussain et al. (2010) have reported changes in growth and yield of maize grown in the glasshouse and suggested that maize is greatly responsive to glasshouse conditions being an excellent system for the screening of suitable germplasm against thermal stress.

4. Conclusion

Heat stress may affect any plant species at any stage. Pollination and grain formation phases are highly sensitive in maize. Screening under field conditions provides basic but useful information if the sowing be done at appropriate time to expose the breeding material to the harsh climatic conditions at flowering stage. It may provide temperature stress for screening of genotypes. However, under tunnel conditions the temperature is more conducive to screen out the susceptible germplasm as higher temperature under tunnel minimizes the chances of any escape which may occur under field conditions. Nevertheless, for getting more credible results the glasshouse provides an ideal system for screening under desired set of environmental factors including heat stress.

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