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Evaluation of Parental Lines for Heat Tolerance and Development of F_2 Population to Study the Effect of Pollen Selection for Heat Tolerance

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Abstract

In the present study four previously identified- tolerant and susceptible inbred lines (BTM1, BTM4, BTM6, and BTM15) were screened for high temperature tolerance by TIR technique in the laboratory at seedling stage and at plant level in field condition by growing under high temperature condition at ARS gulburga. At seedling stage in TIR, BTM6 and BTM15 showed highest survival percentage, with lowest reduction in root, shoot and dry weight. In field condition also BTM6 showed the highest seed yield (24 g). Based on both TIR and field evaluation of inbred lines BTM1 and BTM4 were found to be susceptible whereas, BTM6 and BTM15 were tolerant to high temperature stress. One hundred thirty SSR primers were used to characterize the four inbred lines. Among 130 markers, twenty were polymorphic between BTM4 and BTM6, fifteen markers were polymorphic between BTM4 and BTM15 and only 12 primers were polymorphic between BTM1 and BTM15. Three crosses were made between susceptible and resistant inbred lines (BTM1×BTM15, BTM4×BTM15 and BTM4×BTM6) to produce 3 hybrids. All the thirty F_1 plants of each hybrid were tested for purity using 2 SSR markers and confirmed that all the plants were true hybrids. The F_1 plants were selfed with gamete selection (exposing the pollen grains at 36 ^{0}C for 3 hours before selfing) and without gamete selection (control) to produce segregating F_2 population and to study the effect of gamete selection for heat tolerance. There was a significant reduction in the seed set in the F_1 plants selfed after gamete selection.

Keywords : Temperature induce response (TIR), polymorphic, gamete selection, F_2 population

Maize (*Zea mays* L.*)* is one of the oldest cultivated crops. It provides around 30% calories together with rice and wheat to 94 developing countries of 4.5 billion people. Worldwide maize is cultivated in an area of 184.80 mha with production of 1037.79 mt and productivity of 5615.7 kg / ha. In India maize is cultivated in an area of 9.25 mha with production of 23.67 mt and productivity of 2556.7 kg / ha (FAOSTAT, 2014). The productivity of maize is severely affected by drought and heat stress worldwide (Deryng *et al.,* 2014). High temperature stress has an independent mode of action on all vegetative and reproductive stages of plant growth (Craita and Tom Gerats, 2013). In maize each 1°C increase in temperature above optimum (25 °C) results in a reduction of 3 to 4 pre cent in grain yield (Shaw, 1983). Transitory or constant high temperature can cause an array of morpho-anatomical, physiological and biochemical changes in maize crop. High temperature (>30 °C) greatly affects the germination percentage and seedling growth of maize (Weaich *et al*., 1996). The inability of germination of maize seeds at high temperature indicates the sensitivity of embryo to high temperature while, poor seedling growth is a manifestation of poor reserve mobilization. The major impact of high temperature at vegetative stage of crop growth has been reported in the inhibition of the elongation of first internode and overall shoot growth of maize (Weaich *et al*., 1996).

Apart from this, maize reproductive stage is most sensitive for high temperature. High sensitivity to heat stress during flowering and significant reduction in seed set have been demonstrated in many cereals and legumes (Frank *et al.,* 2009; Saha *et al*., 2010). Pollen viability and seed-set as measures of heat stress tolerance has been proposed (Craita and Tom Gerats, 2013). Based on pollen grain performance heat tolerant and susceptible genotypes were identified (Zinn *et al.,* 2010).

Heat tolerance is a multi-genic trait and a wide variety of screenable traits are available. However, screening for heat tolerance under field conditions presents a challenge due to interactions of many other factors. Alternatively, pollen grains can be independently subjected to stress and subsequently tested for their performance in germination, viability, fertilization success and seed set to determine the tolerance of the inbred line / genotype producing pollen grains. A strong correlation exists between pollen and the sporophyte tolerance to various stresses (Herrero and Hormaza, 1996; Ravikumar and Patil, 2004).

In present study an attempt has been made to validate the resistance identified through TIR screening and actual field condition of inbred lines for heat tolerance and segregating populations were developed with and without gamete selection to assess the impact of pollen selection for heat tolerance.

Material and Methods

Plant material : In the present study four homozygous inbred lines (BTM1, BTM4, BTM6 and BTM15) developed specifically for temperature tolerance (Singh *et al.,* 2016) and one check MAI105 were chosen for confirmation of tolerance using TIR (Temperature induce response) technique and actual field evaluation at high temperature conditions. Further, these lines were also used for production of hybrids to study gamete selection for heat tolerance.

Evaluation of parents for heat tolerance : The Inbred line MAI105 was selected to standardize TIR (Temperature induce response) technique. A range of temperatures were tried (data not given) and the temperature 44 °C for 2h at RH 70 per cent was considered as optimum temperature for screening of the inbred lines for this study. The seeds were soaked in water for 24h and then transferred to petriplates (10.5"×10.5") containing moist blotting paper at bottom and allowed for germination for 48 h at room temperature. Germinated seedling with root length of 1.0–1.5 cm was selected for the study. Four petriplates were with 20 uniforms grown seedlings were chosen for heat treatments. Out of four petriplates two were selected as control and incubated at room temperature for germination and growth. Another two were exposed to 44 °C for 2h, at RH 70 per cent. After incubation seedlings were kept for three days at room temperature for recovery. After three days of recovery the seeds / seedlings were transferred to tray containing only sand in green house for establishment and allowed to grow for 6 days. After 6 days, the observations of seedlings the per cent establishment, root length, shoots length and dry weight were recorded for all the inbred lines and compared with their respective control.

The four inbred lines (BTM1, BTM4, BTM6 and BTM15) were also grown at gulburga (Karnataka) under high temperature conditions in summer (2015) with two replication along with other inbred lines. Five plants per replication were selected to record observation on RWC (Relative Water Content), SPAD (The Soil Plant Analysis Development) and seed yield per plant. Gulburga is identified as an ideal centre for high temperature tolerance screening in plants particularly maize and wheat.

Molecular characterization of parents : A total genomic DNA was extracted by CTAB (CetylTrimethyl Ammonium Bromide) method (Doyle and Doyle, 1987). The plants were grown in cups and from seedlings DNA was extracted.

One hundred thirty SSR primers were used to characterize the inbred lines. The PCR amplification were performed in 15µl reactions volume using thermal cycler (Mastercycler gradient, Eppendorf, Hamburg, Germany). The reaction mixture consist of 1.0 µl (50ng / µl) of template DNA, 1.5 µl (10X) taq buffer, 0.5 µl (2mM) dNTP mix, 1 µl (10mM) primer each and 0.2 µl (3U / µl) taq polymerase. Amplification was carried out in a thermal cycler programmed for an initial denaturation step at 95 ºC for 5 min followed by 40 cycles of 94 ºC for 1 min, (47-58 for 45 sec) 72 ºC for 90 sec and a final extension cycle of 72 ºC for 8 min. The PCR products from each tube along with 5 µl of loading dye were separated electrophoretically using 3.0 per cent agarose gel and 0.05µg / mL ethidium bromide. The amplification products were examined under UV light and photographed using Alpha digidoc 1000 gel documentation system (Alphainnotech corporation, USA).

Hybrid production and hybrid confirmation by molecular marker : Two heat susceptible inbred lines (BTM1 and BTM4) were crossed two tolerant lines (BTM6 and BTM15) producing three hybrids (BTM1×BTM15, BTM4×BTM15 and BTM4× BTM6). The hybrid seeds of three crosses were used to grow the F_1 plants during summer-2016 at GKVK.

Thirty F_1 plants from each cross along with parents were raised in field during the summer-2016 under irrigated condition. The DNA of all F_1 plants was extracted from15 days old seedlings by CTAB method (Doyle and Doyle, 1987). The true F_1 hybrids were identified using two polymorphic SSR primers and the F_1 plants were used for gametophytic selection for heat tolerance.

Gamete selection for heat tolerance in F_1 generation : The freshly dehisced pollen grains from individual F_1 plants were collected separately and the following treatments were given before selfing.

1. *Gamete selection*: Expose the pollen grains to 36 °C for 3 hours by incubating in PCR.
2. *No gamete selection (control)*: Pollen grains were incubated at room temperature (22 °C) for 3 hours.

For selfing equal quantity by volume of the treated and control pollen grains were used to self-pollinate the plants. The pollen grains from the same plant were used for self-pollination with and without gamete selection, respectively. Twelve plants were used for treatment (36 ^{0}C for 3 hours) and 10 plants were used as control (at room temperature 3 hours) in each cross. The selfed cobs were harvested and all the important cob parameters (cob length, cob diameter, number seeds per row, number of rows per cob, number of seeds per cob and seed weight per cob) were recorded to compare the effect of gamete selection on seed set.

Results and Discussion

Selection of parents for heat tolerance

Heat stress is one of the adverse climate conditions, which affects the crop production and its quality significantly. Hence, breeding for high temperature tolerance may be an effective tool to overcome current and future environmental effects produced by global climatic changes. Significant differences were observed among 4 inbred lines to heat tolerance at seedling stage in TIR testing. The observed survival percentage of BTM1, BTM4, BTM6 and BTM15 were 10, 31.2, 96.7 and 92.5 percent, respectively. Similarly, the percent reduction in shoot length was highest in BTM1 (72.05 %), BTM4 (56.43 %) and it was least BTM6 (26.96 %) and BTM15 (44.98 %). The same trend was observed for root length and dry weight also (Table I). The TIR technique is one of the important techniques for screening the genotypes for heat tolerance in different crops *viz.* pea, rice, groundnut and ragi (Srikanthbabu, 2002,

Table I

Response of inbred lines to heat stress in TIR and field conditions

	TIR data				Field data		
Inbred lines	Survival (%)	% reduction in root length	% reduction in shoot length	% reduction in dry weight	SPAD	RWC	Seed Weight (g)
BTM1	10	78.86	72.05	71.67	37.1	37.7	4
BTM4	31.2	68.41	56.43	67.21	38.0	41.1	0
BTM6	96.7	52.61	26.96	41.79	40.8	50.2	24
BTM15	92.5	55.07	44.98	47.78	39.2	50.4	0
CD	2.021	3.329	1.686	10.048			
SEm±	1.377	1.090	0.552	3.289			
CV (%)	1.69	4.24	2.72	14.34			

Sudhakar *et al.,* 2012; Selvaraj *et al.,* 2011 and Venkateshbabu *et al.,* 2013).

Screening of inbred lines for heat tolerance based on physiological parameters *viz*. RWC (Relative water content) and chlorophyll content indicated two inbred lines BTM6 and BTM15 were heat tolerant compared to BTM1 and BTM4 which show highest relative water content and chlorophyll content. However, only BTM6 produced significantly higher yield compared to other inbred lines (Table I). It is interesting to note that the inbred lines which were considered as heat tolerance through pollen screening (Singh *et al*., 2016) showed tolerance at sporophytic level in TIR and field screening. There is a correspondence between gametophyte and sporophyte tolerance to heat in maize (Laughnan and Gabay, 1973; Ratnababu and Ravikumar, 2010; Searcy and Mulcahy, 1990; Ravikumar and Patil, 2004; Sari-Gorla *et al.,* 1989; Reynolds *et al*., 2005 and Zinn *et al.,* 2010).

Under field conditions, high temperature stress is frequently associated with reduced water availability (Simoes-Araujo *et al.,* 2003). Cultivars with more RWC are a better indication of heat tolerance as observed in wheat and other plants (Kirkham *et al.,* 1980; Salim *et al.,* 1969). When temperature increases, chlorophyllase activity also increases and chlorophyllase degrade the chlorophyll, heat tolerant plants have low chlorophyllase activity. (Todorov *et al.,* 2003; Sharkey and Zhang, 2010).

Molecular characterization of inbred lines

Among one hundred thirty SSR markers, twenty were polymorphic between BTM4 and BTM6, 15 were polymorphic between BTM4 and BTM15 whereas, only twelve markers were polymorphic between BTM1 and BTM15 (Fig.1). The per cent

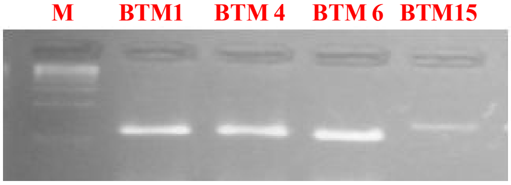

Fig.1: Amplification of SSR marker bnig-1118 in maize inbred

polymorphism observed was highest between BTM4 and BTM6 (15.38 %). It is clear from both phenotypic and molecular data that BTM4 and BTM6 are two highly contrast parents for heat tolerance. BTM4 is susceptible and BTM6 high heat tolerant parent. We have used these diverse lines to produce segregating population to evaluate the effect of gamete selection. The study is in progress.

Gamete selection for heat tolerance in F_1 generation

In the present study three crosses (BTM4 × BTM6, BTM4×BTM15, and BTM1×BTM15) were made and the true F_1 plants were identified using molecular markers (Fig.2). Molecular markers have

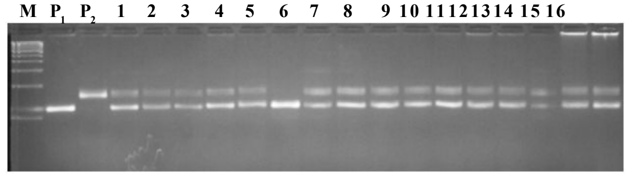

Fig. 2: Gel picture depicting Polymorphic SSR marker (p-umc-1914) pattern in parent F_1 of maize. P_1-BTM4, P_2-BTM6

been extensively used to identify true hybrids and genetic purity in commercial hybrid (Wu *et al.,* 2006). The SSR p-umc1914 and p-umc1472 were used to identify F_1 obtained from cross BTM4×BTM6. Similarly, p-umc1534 and p-umc1745 markers for cross BTM4×BTM15 and markers UMC1632 and UMC2180 were used to identify F_1 plants obtained from cross BTM1×BTM15.The SSR marker is currently the preferred molecular marker for purity identification in some crops (Smith and Register, 1998; Yashitola *et al.,* 2002) due to its highly desirable properties (high efficiency and simplicity).

The change in environment conditions like high temperature will have a very significant effect on pollen grains and it reduces the fertilization rate and reduces the seed set (Zinn *et al.,* 2010, Young *et al.,* 2004). In the present study an attempt has been made to observe the effect of high temperature stress on pollen grains, and their performance in fertilization. In all the three hybridization using heat selected pollen grains, significant reduction in number of seeds per cob, number of rows per cob, seed weight per cob (Table II and III) was observed. The reduction was more or less uniform in all the three hybrids. The pretreatment of pollen grains to stress under *in vitro* conditions affect the potentiality of the pollen grains to grow and

TABLE II

Effect of temperature treatment to pollen grains on different productive traits in three hybrids

Hybrid	Cob length (cm)			Cob diameter (cm)			Number of seeds per row of cob		
	Control	Treated	% reduction	Control	Treated	% reduction	Control	Treated	% reduction
BTM4×BTM6	15.95	14.68	7.96	3.32	2.82	15.12	26	9.09	65.04
BTM4×BTM15	14.85	12.71	14.4	3.09	2.56	17.15	21.2	5.58	73.68
BTM1×BTM15	13.5	11.54	14.52	3.09	2.60	15.86	22.6	5.08	77.50

TABLE III

Effect of temperature treatment to pollen grains on different productive traits in three hybrids

Hybrid	Cob length (cm)			Cob diameter (cm)			Number of seeds per row of cob		
	Control	Treated	% reduction	Control	Treated	% reduction	Control	Treated	% reduction
BTM4×BTM6	14.2	5.0	64.79	365.4	51.45	85.92	102.88	18.10	82.41
BTM4×BTM15	14.2	4.6	67.67	298.4	23.33	92.18	76.56	8.08	89.45
BTM1×BTM15	14.67	4.6	68.64	334.4	21.67	93.52	82.84	7.23	91.27

fertilize on the stigmatic surface. But does the treatment selectively affect the pollen genotype with susceptibility is a question that need to be answered. The present study is in progress and aims to answer these questions.

It can be conclud'ed that the high temperature stress had effect on pollen grains and their performance in fertilization and seed set. Among the three hybrids, the hybrid BTM4×BTM6 showed more tolerance to heat stress compared to other hybrids. The hybrid BTM4×BTM6 was chosen to study the effect of gamete selection for heat tolerance.

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