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GENETIC VARIABILITY FOR GAMETOPHYTIC HEAT TOLERANCE IN MAIZE INBRED LINES

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SUMMARY

The pollen grains of 10 homozygous inbred lines were subjected to heat stress (36°C) for 2 and 4 hours duration in the laboratory and cultured on agar pollen germination medium. High temperature treatment completely inhibited pollen germination and tube growth in seven inbred lines and only moderately in 3 lines - BTM 14, BTM 15 and BTM 1 suggesting the genetic variability for gametophytic heat tolerance among selected inbred lines. Heat treated pollen grains were also used to self-fertilize the field grown plants of ten inbred lines. High temperature stress significantly reduced the seed set in all the inbred lines. However two inbred lines BTM 14 and BTM 15 produced relatively more number of seeds per cob suggesting their tolerance to heat stress. The lines which showed gametophytic tolerance to heat stress under *in vitro* conditions produced more number of seeds per cob after heat treatment to pollen grains.

Key words: Heat stress, tolerance, gametophyte, pollen germination, inbreds

Key findings: In vitro pollen screening for high temperature tolerance has a potential for rapid and inexpensive screening of a large set of genotypes for abiotic stress tolerance in plant breeding.

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INTRODUCTION

Abiotic stresses such as drought, heat, cold and salinity are major factors responsible for reduced global agriculture productivity. One major abiotic stresses, heat or high temperature stress, has an independent mode of action on all vegetative and reproductive stages of plant growth (Craita and Tom Gerats, 2013). Reproductive stage in particular has been considered as extremely sensitive to heat stress, often a limiting trait in crop productivity (Barnabas *et al.,* 2008). 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). Given the importance of fertilization and seed set in productivity, a number of attempts have been made to understand the thermo sensitive stage of reproductive phase. Depending upon the timing, duration and severity, heat stress can limit fertilization at all the stages of gametophyte development, pollen germination and tube growth or female tissues required for fertilization success (Snider *et al.,* 2013). The male gametophyte is particularly vulnerable to high temperature at all the stages of development compared to female

gametophyte (Hedhly, 2011). Male sterility and the impairment of pollen development have been the main factors for reduced seed set and productivity under high temperature stress (Sakata and Higastitani 2008; Burke and Chen, 2015).

Pollen viability and seed-set as measures of heat stress tolerance has been proposed (Craita and Tom Gerats, 2013). The inhibitory effect of stress factor on *in vitro* and *in vivo* pollen performance and fertilization success has been reported for various stresses pathotoxin (Laughnan and Gabay, 1973; Ratna babu and Ravikumar 2010); metal ion (Searcy and Mulcahy, 1990); drought (Ravikumar and Patil 2004); herbicide (Sari-Gorla *et al.,* 1989); salt (Reynolds *et al*., 2005); and temperature (Eva Dominguez *et al.,* 2005). Based on the performance of pollen grains in seed set under heat stress, temperature sensitive and tolerant genotypes were identified (Zinn *et al.,* 2010). Kakani (2002) also showed genotypic differences in groundnut for temperature tolerance based on pollen characters.

Maize with an area of 184.23 million ha, production of 1016.43 million tons and productivity of 5.56 tons/ha is the third most important crop in the world after rice and wheat. India has an area of 9.50 million ha with production of 23.29 million tons, and productivity of 2.45 tons/ha (FAO stat, 2013). It has been speculated that global climate change particularly temperature is the most detrimental factor for crop production (Cumhur *et al*., 2008). Thomson (1975) showed that a 6°C increase in temperature during the grain filling period resulted in a 10% yield loss in the U.S. Corn Belt. At temperatures above 38°C, poor seed set in maize has been attributed to both direct effect of high temperature (Carberry *et al*., 1989) and pollen desiccation (Schoper *et al*., 1986; Lonnquist and Jugenheimer 1943). Tolerance to high temperature is a multi-genic character 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 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 this study, an attempt has been made to study the relationship between *in vitro* and *in vivo* performance of the heat stressed pollen grains in terms of germination, tube growth and ability to fertilize to produce grains in ten inbred lines of maize.

MATERIALS AND METHODS

Plant material

Ten homozygous inbred lines BTM 1, BTM 2, BTM 4, BTM 5, BTM 6, BTM 7, BTM 9, BTM 11, BTM 14 and BTM 15 were chosen for the present study. The inbred lines were developed at this station essentially selected for heat tolerance. The inbreds were developed from drought tolerant populations and lines available at our station by selfing the selected plants for 6 generations. During the process of development of inbred lines, the selected plants were grown during summer season and the plants were subjected to heat stress at flowering. Further, these lines were tested for heat tolerance under field conditions during summer at ARS, Gulburga (Karnataka), which is a known station for testing heat tolerance. The inbred lines showed high variability for heat tolerance from high susceptibility to heat tolerance under field conditions making them ideal for gametophtyic heat tolerance studies.

Pollen culture and in vitro bioassay for heat tolerance of pollen

Pollen germination was conducted on cavity slides on agar pollen germination medium with bactoagar 0.72%, sucrose 18%, calcium nitrate 0.036% and boric acid 0.012%. This medium was modified from a basic medium proposed by Pfahler and Linsken (1972), which gave only 18-20% pollen germination in the inbred lines selected for this study (data not shown). The modified medium was melted in microwave oven and 200µl was poured in to each cavity of

cavity slides and allowed 10 minutes to solidify. All the 10 inbred lines were grown in the field in a single row of 10 plants during post rainy season under irrigation. The pollen grains at flowering were freshly collected in the morning (9:00 A.M.), brought to the laboratory and immediately transferred to 0.5 ml Eppendorf tubes and sealed with parafilm. In general the pollen grains on 2-4 days after anthesis were used for the study. Equal volume of pollen grains from each genotype was filled into four tubes and the following four treatments were given. One tube incubated at room temperature for two hours and another tube for four hours. The third and fourth tubes were incubated at 36°C in a Thermal cycler for two and four hours respectively. After incubation, the pollen grains were uniformly sprinkled over the surface of pollen germination medium in cavity slides. The inoculated cavity slides were incubated in humid chamber (petri plates with moist filter paper above and below) at room temperature (22-24°C) for one hour. After one hour the slides were observed under microscope (100X) and pollen germination was determined on the screen of computer using Image focus version 3.0 software (Novex Holland) which could record points, length etc. The projections on computer screen frame were used to count the pollen grains. The number of pollen grains germinated (tube length more than half the diameter of pollen grains) and total number of pollen grains were counted in each frame and expressed as % pollen germination.

For each genotype, four cavities were used and from each cavity eight fields were randomly chosen for recording observations. In each field five germinated pollen grains were selected and the tube length was measured on the screen under 10X magnification and recorded in micro meters (Image focus version 3.0).

In vivo pollination with heat stressed pollen grains

All the ten inbred lines were grown in the field in a single row of 2.5 m during post rainy season of 2013-14 under irrigated conditions. The maximum temperature prevailed at reproductive stage was 28°C during the day. At reproductive stage the tassel and the main cob in the plant were covered with paper bags to avoid open pollination on the second day after anthesis of the tassel, the pollen grains from individual plants were collected separately and the following 3 treatments were given to pollen grains.

1. Pollen grains incubated at 36°C for 2 hours
2. Pollen grains incubated at 36°C for 4 hours
3. Pollen grains incubated at room temperature (22°C) for 4 hours as control

Equal quantity by volume of the treated pollen grains was used to self-pollinate the plants. The pollen grains from the same plant was used for self-pollination after treatment. Two plants were used for each treatment in each inbred line. The selfed cobs were harvested and the number of grains formed in each cob and treatment was determined for each inbred line.

RESULTS

Effect of heat stress on pollen germination and tube growth

In the absence of temperature treatment, the agar based pollen germination medium gave high frequency of pollen germination and tube growth in maize inbred lines. The incubation of pollen grains for 2 hours at room temperature did not affect the pollen grain germination and tube growth significantly compared to fresh pollen grains. The pollen germination and tube growth of the inbred lines after 2 hours of incubation at room temperature ranged from 53.73 to 85.10% and 951.33 μm to 1361.7μm respectively. The four hour incubation at room temperature reduced the germination and tube growth. The germination ranged from 32.03 to 68.05% and tube length from 804.0 to 1075.5 μm after 4 hours incubation at room temperature (Table 1 and 2). The reduction in germination and tube growth was found in all the inbred lines.

The temperature treatment to pollen grains decreased the pollen germination and tube growth in all the inbred lines (Table 2). The major effect was on pollen germination.

Table 1. Effect of high temperature treatment to pollen grains on pollen germination of maize inbred lines.

No.	Inbred line	% Germination			
		RT-2hrs	36°C-2hr	RT-4hr	36°C-4hrs
1	BTM 1	69.68±4.55	32.32±0.50 (53.61)	53.26±2.09	26.56±0.52 (50.13)
2	BTM 2	80.00±4.50	25.49±0.47 (68.13)	58.51±5.03	00.00 (100.00)
3	BTM 4	85.10±5.09	00.00 (00.00)	68.05±5.03	00.00 (100.00)
4	BTM 5	61.50±7.91	21.39±4.70 (65.21)	59.30±5.51	00.00 (100.00)
5	BTM 6	66.00±7.10	22.00±3.06 (66.67)	52.94±6.83	00.00 (100.00)
6	BTM 7	54.95±7.00	27.35±4.66 (50.22)	52.38±6.55	00.00 (100.00)
7	BTM 9	53.73±3.91	00.00 (00.00)	51.26±0.55	00.00 (100.00)
8	BTM 11	79.57±5.81	00.00 (00.00)	32.03±2.26	00.00 (100.00)
9	BTM 14	74.20±4.33	39.25±2.83 (47.10)	62.03±2.94	12.70±3.51 (79.52)
10	BTM 15	56.89±5.84	29.59±2.11 (47.98)	52.29±3.34	28.36±0.77 (45.76)

Values in parenthesis are respective per cent of control (incubation at room temperature)

Table 2. Effect of high temperature treatment to pollen grains on pollen tube length of maize inbred lines.

Inbred line	Tube length (μm)			
	RT-2hrs	36°C-2hr	RT-4hr	36°C-4hrs
BTM 1	1213.70±74.01	880.75±66.91 (27.43)	1043.4±49.50	3.00 (negligible) (99.71)
BTM 2	1075.20±53.94	586.67±65.45 (45.43)	984.57±110.62	0 (00.00)
BTM 4	1029.70±89.36	000.00 (00.00)	954.1±87.34	0 (00.00)
BTM 5	1132.80±148.75	676.50±67.25 (40.28)	948.84±122.17	0 (00.00)
BTM 6	1122.30±110.14	724.5±65.52 (35.44)	804.00±70.79	0 (00.00)
BTM 7	951.33±122.24	751.08±54.41 (21.04)	921.25±115.75	0 (00.00)
BTM 9	1361.70±212.23	000.00 (00.00)	1070.2±161.89	0 (00.00)
BTM 11	1112.30±40.06	000.00 (00.00)	1075.5±79.50	0 (00.00)
BTM 14	1161.20±101.83	920.20±88.71 (20.75)	947.53±93.13	712.22±98.00 (24.82)
BTM 15	1066.30±70.25	898.25±102.22 (15.76)	984.94±98.75	797.12±48.87 (18.98)

Values in parenthesis are respective per cent of control (incubation at room temperature)

The heat stress (36°C) for two hours has completely inhibited the pollen germination in three inbred lines BTM 4, BTM 9 and BTM 11. In the remaining inbred lines the germination ranged from 21.39 to 39.25%. Similarly the four hour heat stress (36°C) completely inhibited the pollen germination in seven of the 10 inbred lines. Three inbred lines (BTM 15, BTM 14 and BTM 1) recorded moderate level of germination ranging from 12.70 to 28.36%. These 3 inbred lines recorded high pollen germination both after two and four hours of heat stress treatment indicating their tolerance to high temperature compared to other inbred lines. The heat treatment also reduced the tube growth among the germinated pollen grains (Figure 1 and Table 2). The increase in duration of treatment further reduced the tube growth. Among the three inbred lines, which showed pollen germination after heat stress, only two inbred lines produced good tube growth. The results of pollen germination and tube growth suggested that maize inbred lines produced pollen grains that differ in their sensitivity to heat treatment. For example, inbred lines BTM 14 and BTM 15 showed higher germination and tube growth even after four hour heat treatment to pollen grains. On the other hand the pollen grains of BTM 4, BTM 9 and BTM 11 completely lost their viability in 2 hours heat treatment suggesting their sensitivity to heat stress.

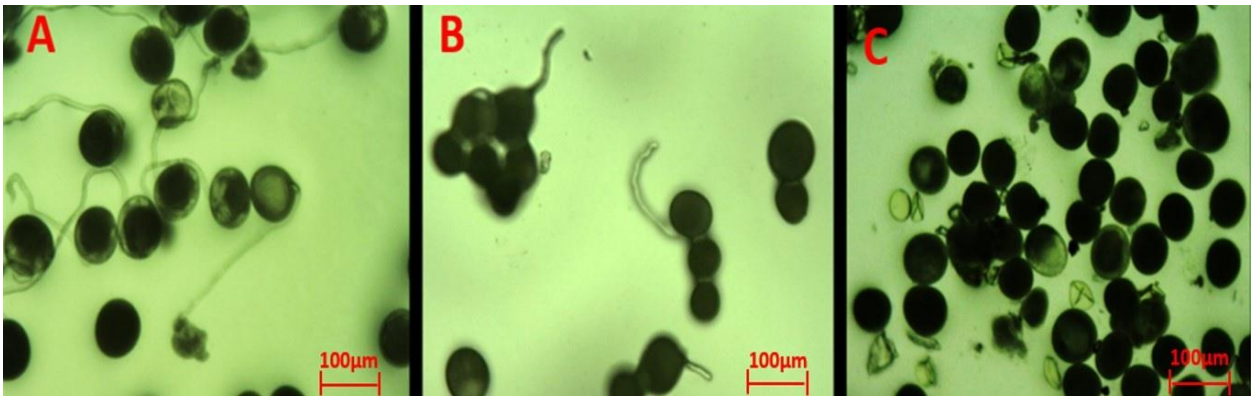

Figure 1. Effect of high temperature on pollen germination and tube growth of maize inbreds
A: Control; B: 36°C (2 hours); C: 36°C (4 hours)

The pollen grain incubated at room temperature for four hours did not affect the capacity of the pollen grains to fertilize and produce seeds. The number of grains per cob ranged from 238 to 530 (Table 3). However, high temperature stress to pollen grains reduced the viability of pollen grains and fertilization success. The number of grains per cob ranged from 8 in BTM 1 to 101 in BTM 14, when pollinated with pollen grains stressed for two hours at 36°C. The increased duration of heat treatment (4 hours) further reduced the capability of pollen grains to fertilize and produce grains. The number of grains per cob ranged from 0 to 25.5 in pollination using pollen grains incubated for four hours at 36°C. The grains/cob in 3 inbred lines (BTM 2, BTM 5 and BTM 9) was nil in this treatment and five inbred lines (BTM 1, BTM 4, BTM 6, BTM 7, BTM 11) showed less than 6 grains per cob suggesting that susceptibility of these inbred lines to heat stress. Two inbreds BTM 14 and BTM 15 which recorded better *in vitro* pollen germination and tube growth under stress also produced relatively more number of grains per cob - 17.5 and 25.5 respectively, suggesting their tolerance to heat stress at gametophyte stage. The results indicate that heat stress reduced the *in vivo* pollination ability of pollen grains in all the inbred lines. However, the inbred lines differ in their susceptibility to heat stress at gametophyte stage.

The heat stress has also reduced the capacity of the pollen grains to fertilize and produce seeds under *in vivo* fertilization.

Table 3. Mean number of grains per cob in maize inbred lines self-pollinated with heat stressed pollen grains.

No.	Inbred line	36°C (2 hours)	36°C (4 hours)	RT (4 hours)
		Mean	Mean	Mean
1	BTM 1	8	0.5	530
2	BTM 2	27	0.0	239
3	BTM 4	51	1.5	434
4	BTM 5	33	0.0	238
5	BTM 6	19	2.5	256
6	BTM 7	52	0.5	365
7	BTM 9	60	0.0	280
8	BTM 11	72	6.0	294
9	BTM 14	101	17.5	320
10	BTM 15	75	25.5	345

RT (4hr): Pollen grains incubated at room temperature for 4 hours; 36°C (2hr): Pollen grains incubated at 36°C for 2 hours; 36°C (4hr): Pollen grains incubated at 36°C for 4 hours

The number of seeds per cob ranged from 8 to 101 and 0.0 to 25.5 when 2 and 4 hours heat stressed pollen grains were used for pollination compared to 238 to 530 seeds/cob in non-stressed pollen grains (Table 3). The inbred lines differed in their response to heat treatment of pollen grains in terms of number of seeds produced per cob. Three inbred lines did not produce any seeds and 5 inbreds produced less than 6 seeds per cob when 4 hours heat stressed pollen grains were used for pollination. Two inbred lines BTM 14 and BTM 15 produced an average of 17.5 and 25.5 seeds/cob in the same stress treatment.

DISCUSSION

The most comprehensive research on gametophyte selection and association of gametophyte performance to resulting sporophyte traits was conducted in maize (Ottaviano and Malcahy 1986; Ottaviano *et al.,* 1991). Later, the components of pollen performance; *in vitro*/*in vivo* pollen germination and tube growth in the presence of biotic and abiotic stress factors were found to be linked to the plant genotypes producing pollen grains (Ravikumar and Chikkodi, 1998; Herrero and Hormaza, 1996; Zinn, *et al.,* 2010). The effect of temperature stress on pollen grains is well documented in many plant species (Zinn *et al.,* 2010, Young *et al.,* 2004). Heat stress applied during pollen release decreased seed number and the severity of seed set reduction was genotype dependent. Fertilization was unsuccessful when pollen subjected to heat stress prior to application to the spikelet, in maize and tomato (Zinn *et al.,* 2010). In this experiment also, the high temperature treatment (heat stress) clearly affected the pollen viability and vigor as indicated by the reduced germination and tube growth under *in vitro* conditions in pollen grains treated with 36°C for 2 and 4 hours. The inhibitory effect of heat stress was not uniform across inbred lines. The inbreds BTM 1, BTM 14 and BTM 15 consistently produced higher germination and tube growth both at 2 and 4 hour duration of heat stress, suggesting tolerance to heat stress compared to other inbred lines.

However, among 3 tolerant inbreds only two BTM 14 and BTM 15 produced normal pollen tube growth after 4 hours of heat stress while the tube growth was completely inhibited in another tolerant line BTM 1. Pollen heat tolerance of inbred lines is intriguing as these inbred lines were derived from the genetic pools developed under high temperature.

The value of pollen determination of heat tolerance of an inbred line is dependent on the extent to which *in vivo* performance of pollen grains in terms of pollination and fertilization in field under heat stress. In our study, there was a good correspondence between

in vitro performance of heat stressed pollen grains and their capacity to fertilize under *in vivo* conditions. The two inbred lines BTM 14 and BTM 15, which showed high level of tolerance under *in vitro* conditions recorded the highest grain number / cob when heat stressed pollen grains were used for pollination and fertilization. High temperature stress during reproductive stage results in reduction of seed yield in canola (Nuttal *et al.,* 1992); tomato (Sato *et al.,* 2002); wheat (Saini *et al.,* 1983) and corn (Carlson, 1990). The reduced gametophyte fertility or pollen viability or abnormal gametophyte development due to high temperature stress has been observed in many crop plants (Polowick and Sawhney 1988; Sato *et al.,* 2002; Young *et al.,* 2004). The proteomic study of pollen indicated a considerable overlap in heat shock proteins (HSPs) in gametophyte and sporophyte heat tolerant and susceptible rice genotypes speculating the accumulation of HSP conferring heat tolerance in rice (Zinn et *al.,* 2010). Further, Pollen is the only organ that has insufficient induction of HSPs in response to heat stress and has been reported to be sensitive to elevated temperatures throughout its prior to development and and also after dehiscence (Burke and Chen 2015).

Although, in this study the sporophyte performance of the inbred lines under heat stress is not characterized, the pollen performance both *in vivo* and *in vitro* indicated that the potentiality of using pollen bioassay as a reliable technique to screen the maize inbred lines for heat tolerance. It is important to test the heat tolerance of these inbred lines under field conditions to unequivocally demonstrate the relationship between gametophyte and sporophyte tolerance which will be carried out in the ensuing summer season. The technique is also helpful in pollen selection for heat tolerance in a population of pollen grains (Eva Domınguez *et al.,* 2005). Heat stressing pollen grains and pollinating the plants might allow to select genetically tolerant pollen grains for successful fertilization. The subsequent progeny might be more tolerant to heat stress. The inheritance of traits selected through gametophytic tolerance has been demonstrated in chickpea against *Fusarium* wilt and cold tolerance (Ravikumar *et al.,* 2007; Clarke *et al.,* 2004; Kron and Husband 2006). It is important to demonstrate the effect of gametophytic tolerance for heat stress on the progeny performance at high temperature stress. Hybridization has been made between tolerant and susceptible inbred lines and the gametophyte of the hybrid plants will be subjected to heat stress to study the effect on F_2 generation.

REFERENCES

Barnabas BK, Jager, Feher A (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ. 31*:11–38.

Burke JJ, Chen J (2015). Enhancement of Reproductive heat tolerance in plants. *PLOS ONE.* 10(4): e0122933. doi:10.1371/journal.pone.0122933

Carberry PS, Muchow RC, Mccown (1989). Testing the CERES-Maize simulation model in semi-arid tropical environment. *Field Crops Res.* 20: 297-315.

Carlson RE (1990). Heat stress, plant-available soil moisture, and corn yields in Iowa: A short and long term view. *Journal of Production Agriculture.* 3: 293-297.

Chennakrishnan P, Raja K (2012). Maize production in India: fighting hunger and malnutrition. US Department of Agriculture, *Maize Monthly Report* 8: 2011-12.

Clarke HJ, Khan TN Siddique KHM (2004). Pollen selection for chilling tolerance at hybridization leads to improved chickpea cultivars. *Euphytica* 139: 65–74.

Craita E, Bita, Tom Gerats (2013). Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress tolerant crops. *Frontiers in Plant Science (Crop Science and Horticulture).* 4 (273): 1-18.

Cumhur A, Malcolm SC (2008). The effects of global climate change on agriculture. *J. Agric. & Environ. Sci.,* 3 (5): 672-676.

Dominguez E, Cuartero J, Fernandez-Munoz R (2005). Breeding tomato for pollen tolerance to low temperatures by gametophytic selection. *Euphytica* 142: 253–263.

Food and agriculture organization of the United Nations (2013). FAO Stat. Databases, www.fao.org.

Frank GE, Pressman R, Ophir L, Althan RS, Freedman M (2009). Transcriptional profiling of maturing tomato (*Solanum lycopersicum* L.) microspores reveals the

involvement to heat shock proteins, ROS scavengers, hormones, and sugars in the heat stress response. *J. Exp. Bot.* 60: 3891-3908.

Hedhly A (2011). Sensitivity of flowering plant gametophytes to temperature fluctuations. *Environ. Exp. Bot.* 74: 9-16.

Herrero M, Hormaza JI (1996). Pistil strategies controlling pollen tube growth. *Sex Plant Reprod.* 9: 343-347.

Kakani VG, Prasad PVV, Craufurd PQ, Wheeler TR (2002). Response of *in vitro* pollen germination and pollen tube growth of groundnut (*Arachis hypogaea* L.) genotypes to temperature. *Plant Cell Environ* 25: 1651-1661.

Kelly E, Zinn MTO, Jeffrey FH (2010). Temperature stress and plant sexual reproduction: uncovering the weakest links. *J. Exp Botany* 61 (7): 1959-1968.

Kron P, Husband BC (2006). The effects of pollen diversity on plant reproduction: insights from apple. *Sex Plant Reprod*. 19: 125–131.

Laughnan JR, Gabay SJ (1973). Reaction of germinating maize pollen *Helminthosporium* maydis pathotoxins. *Crop Sci.* 13: 681-684.

Lonnquist JH, Jugenheimer RW (1943). Factors affecting the success of pollination in corn. *J. of Am. Soc. Agron*. 35: 923-933.

Nuttal WF, Moulin AP, Townley Smith LJ (1992). Yield response of canola to nitrogen, phosphorus, precipitation, and temperature. *Agronomy Journal* 84: 765-768.

Ottaviano E, Mulcahy DL (1986). Gametophytic selection as a factor of crop plant evolution. *In:* C Barigozzi (ed), *The origin and domestication of cultivated plants*, Elsevier, New York. pp 101-120.

Ottaviano E, Pe ME, Binelli G (1991). Genetic manipulation of male gametophytic generation in higher plants. In *Subcellular biochemistry: Plant genetic engineering* (eds. B.B. Biswas and J.R. Harris), pp. 107-142.

Pfahler P, Linskens H (1972). *In vitro* germination and pollen tube growth of maize (*Zea mays* L.) pollen. *Theor. Appl. Genet.* 42 (3): 136-140.

Polowick PL, Sawhney VK (1988). High temperature induced male and female sterility in canola (*Brassica napus* L.). *Annals of Botany*. 62: 83-86.

Ratna Babu D, Ravikumar RL (2010). Genetic evidence for resistance to Fusarium wilt of pollen grains in chickpea (*Cicer arietinum* L.) *Curr. Sci.* 96: 6-25.

Ravikumar RL, Chikkodi SB (1998). Association between sporophytic reaction to *Alternaria helianthi* and gametophytic tolerance to pathogen culture filtrate in sunflower (*Helianthus annuus* L.) *Euphytica* 103: 173-180, 1998.

Ravikumar RL, Patil BS, Soregaon CD, Hegde SG (2007). Genetic evidence for gametophytic selection of wilt resistant alleles in chickpea. *Theor. Appl. Genet.* 114: 619-625.

Ravikumar RL, Patil BS (2004). Effect of gamete selection on segregation of wilt susceptibility-linked DNA marker in chickpea. *Curr Sci.* 86: 642-643.

Reynolds MP, Mujeeb-Kazi A, Sawkins M (2005). Prospects for utilising plant-adaptive mechanisms to improve wheat and other crops in drought-and salinity-prone environments. *Annals of Appl. Biol*. 146 (2): 239-259.

Saha S, Hossain M, Rahman M, Kuo C, Abdullah S (2010). Effect of high temperature stress on the performance of twelve sweet pepper genotypes. *Bangladesh J. Agric. Res. 35*: 525-534.

Saini, HS Sedgley M, Aspinall D (1983). Effect of heat stress during oral development on pollen tube growth and ovary anatomy in wheat (*Triticum aestivum* L.). *Australian Journal of Plant Physiology*. 10: 137-144.

Sakata T, Higashitani A (2008). Male sterility accompanied with abnormal anther development in plants genes and environmental stresses with special reference to high temperature injury. *Int. J. Plant Dev. Biol*. 2: 42-51.

Sari-Gorla ME, Ottaviano E, Frascaroli, Landi P (1989). Herbicide tolerant corn by pollen selection. *Sex Plant Reprod.* 2: 65-69.

Sato S, Pet MM, Thomas JF (2002). Determining critical pre- and post-an thesis periods and physiological processes in *Lycopersicon esculentum* Mill. exposed to moderately elevated temperatures. *Journal of Experimental Botany*. 53: 1187-1195.

Schoper JB, Lambert RJ, Vasilas BL (1986). Maize pollen viability and ear receptivity under water and high temperature stress. *Crop Sci.* 26: 1029-1033.

Searcy KB, Mulcahy DL (1990). Comparison of the response to aluminium toxicity in gametophyte and sporophyte of four tomato (*Lycopersicon esculentum* Mill.) cultivars. *Theor Appl. Genet.* 80: 289-295.

Snider JL, Oosterhuis DM, Collins GD, Pilon C, Fitzsimons TR (2013). Field-acclimated

Gossypium hirsutum cultivars exhibit genotypic and seasonal differences in photosystem II thermostability. *J. Plant Physiol.* 170(5): 489-96.

Thomson LM (1975). Weather variability, climatic change, and grain production. *Sci*. 188: 535-541.

Young LW, Wilen RW, Bonham Smith PC (2004). High temperature stress of *Brassica napus* during flowering reduces micro and mega gametophyte fertility, induces fruit abortion, and disrupts seed production. *J. Exp. Bot.* 55: 485-495.

Zinn KE, Tunc-Ozdemir M, Harper JF (2010). Temperature stress and plant sexual reproduction: uncovering the weakest links. *J. Exp. Bot.* 61: 1959-1968.