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RESEARCH ARTICLE

Consequence of cyclic pollen selection for heat tolerance on the performance of different generations in maize (*Zea mays* L.)

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Abstract. The reproductive stage in many crops, including maize, is very sensitive to heat stress and the genetic overlap between gametophytic and sporophytic phase gives an opportunity to select superior stress tolerant genotype at gametophytic stage. An attempt was made to evaluate the response of cyclic pollen selection in the F_1 and F_2 generations on the performance of F_3 generation progenies for seed yield and yield contributing traits under natural heat stress conditions. In this direction three groups of F_3 progenies, namely (i) pollen selection in F_1 and F_2 generations (GG), (ii) pollen selection only in F_2 generation (CG), (iii) no pollen selection in F_1 and F_2 generations (CC) were screened for heat stress at Agricultural Research Station (ARS), Bheemarayanagudi. The GG progenies recorded significantly higher chlorophyll content, more number of pollen grains per anther and less pollen sterility compared to CG and CC group of progenies under heat stress. Further, the F_4 progenies obtained through cyclic pollen selection (in F_1, F_2 and F_3) were also tested for heat stress tolerance at seedling stage. The significant improvement for heat stress tolerance was recorded in F_4 progenies derived through cyclic pollen selection as compared to control (no pollen selection for heat tolerance in any generation) F_4 progenies. The results indicated that cyclic pollen selection in F_1, F_2 and F_3 generations improved the heat stress tolerance of the progenies in the succeeding generations. To provide genetic evidence for the effect of pollen selection for heat tolerance, the control F_2 (C) and selected F_2 (G) populations were compared for the segregation of SSR markers. The selected F_2 (G) population showed significant deviation from normal Mendelian ratio of 1:2:1 and showed skewness towards the alleles selected from male parent. The results provide strong evidence for an increase in the frequency of parental alleles in the progenies that impart heat stress tolerance.

Keywords. heat stress; maize; cyclic pollen selection; genetic evidence; allele frequency.

Introduction

Maize is the most important cereal crop next to rice and wheat playing a significant role in food security in India. Maize is grown in diverse environmental conditions where various kinds of environmental stresses impair growth and development at different stages, namely germination, seedling growth, vegetative development, reproductive establishment, grain yield and quality (Payero *et al.* 2006; Cattivelli *et al.* 2008). An increase of 1°C temperature could result in decrease in maize yield by ~7.4% globally, a decrease that is highest among the cereals (Zhao *et al.* 2017). Maize is highly susceptible to heat stress particularly at reproductive stage, specifically the male reproductive system seems to be the most affected by heat stress resulting in reduction in grain yield (Thakur *et al.* 2010; Prasad *et al.* 2017; Lizaso *et al.* 2018). A reduction in the number of healthy pollen grains has gained attention as a cause of yield loss under heat stress. High temperature conditions at flowering stage caused wide spread instability across many

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production areas in maize (Barnabás *et al.* 2008). Many studies have suggested that stress exposure during microgametogenesis leads to microspore abortion and associated male sterility (De Storme and Geelen 2014; Rieu *et al.* 2017; Begcy and Dresselhaus 2018). In fact, high temperature stress impedes the germination of pollen as well as the growth of pollen tube both *in vivo* and *in vitro* conditions (Herrero and Johnson 1980; Song *et al.* 2015; Singh *et al.* 2016). Earlier reports highlighted that the pollens are highly sensitive to heat stress but a large genetic variation for pollen viability exists that can help in reducing heat stress-induced yield losses in crops like cotton (Kakani *et al.* 2005), pearl millet (Djanaguiraman *et al.* 2018), tomato (Paupière *et al.* 2017), sorghum (Sunoj *et al.* 2017) and wheat (Bheemanahalli *et al.* 2019).

Selection pressure for different stresses has been applied during the gametophytic generation in several studies (Bajaj *et al.* 1992; Frova *et al.* 1995; Chi *et al.* 1999; Clarke *et al.* 2004; Domínguez *et al.* 2005) and proved successful in increasing the frequency of tolerant progenies (Mohapatra *et al.* 2020). The efficiency of pollen selection was reported in tobacco, chickpea and maize (Touraev *et al.* 1995; Frascaroli and Songstad 2001; Ravikumar *et al.* 2007) for simply inherited traits. The effect of one, two and/or three cycles of pollen selection (cyclic selection) tested in F_2, F_3 and F_4 populations for heat stress tolerance resulted in improved performance of progenies in succeeding generations (Singh *et al.* 2020a). Thus, high level of resistance can be achieved through repeated number of pollen selection cycles.

The response of pollens to heat stress was studied in *in vitro* and significant variations in *per se* performance of the progenies were observed in our studies (Singh *et al.* 2020b). Moreover, combination of cyclic pollen selection could be explored to achieve a high level resistance in less time. This prompted a systematic investigation to demonstrate the positive response of cyclic pollen selection on successive generations in maize. In this work we reported the effect of cyclic pollen selection for heat tolerance in F_1 and F_2 generations on the performance of F_3 generation progenies in terms of seed yield and yield contributing traits under natural heat stress conditions. Further, the positive effect of selection pressure for heat tolerance was also confirmed through the SSR marker analysis in the F_2 generation.

Material and methods

Plant material

The heat susceptible (BTM4 as female parent) and tolerant (BTM6 as male parent) inbred lines of maize were crossed to produce F_1 (Singh *et al.* 2017). The true F_1 plants were confirmed by genotyping with SSR markers (table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/). Sixty F_1 seeds were sown and grown under field conditions during rabi season at GKVK, Bengaluru. The freshly dehisced pollen grains from each F_1 plant were collected separately and immediately brought to laboratory. Proper safety measure was taken so that the pollens were not mixed with each other. The pollen grains from each individual plant were uniformly spread in a Petri plate. The pollen grains in each Petri plate of 30 individual F_1 plants were exposed separately to heat stress by incubating them in a growth chamber maintained at 36°C and at 70% relative humidity (RH) for 3 h. The pollen grains of the remaining individual 30 F_1 plants were kept at 22°C for 3 h as control. After the heat treatments, the pollen grains were used to self the F_1 plants to produce F_2 generation seeds. The F_2 seeds produced from the heat stress treated pollen grains were termed as pollen selected F_2 (G) and the F_2 seeds produced from the plants pollinated with pollen grains stored at 22°C were termed as control F_2 (C).

The control F_2 (C) and pollen selected F_2 (G) were grown in the field during kharif season at GKVK, Bengaluru and three different groups of F_3 progenies were produced: (i) the pollen selected F_2 (G) plants were selfed with heat stress treated pollen grains (36°C for 3 h with 70% RH) to produce F_3 (GG) progenies; (ii) one set of control F_2 (C) plants were selfed with heat stress treated pollen grains (36°C for 3 h with 70% RH) to produce F_3 (CG) progenies; (iii) another set of F_2 (C) plants were selfed with normally treated (22°C for 3 h) pollen grains to produce F_3 (CC) progenies. The seeds produced from a cob selfing by treated pollens (either heat stress or control) in individual plant were considered as a progenies for next generation. Fifteen progenies each from GG, CG and CC were randomly selected and assessed for their heat tolerance at the Agricultural Research Station (ARS), Bheemarayanagudi, Yadgir district, Karnataka during summer season. It is a recognized hotspot for heat stress screening of maize. Each F_3 progeny was grown in a single row of 5 m during summer at ARS Bheemarayanagudi and the plants were exposed to high temperature 40–45°C (table 2 in electronic supplementary material) stress during reproductive stage. Five plants each of GG, CG and CC along with parental lines were randomly chosen to record the observation on physiological and seed-yield traits such as SPAD value (greenness index) using SPAD meter, anthesis to silking interval (ASI), pollen quantity and quality using 1% Tween 20 method (Singh *et al.* 2020a), cob length (cm), number of seed rows per cob, number of seeds per cob and five cob seed weight (g). Three field grown plants each from GG, CG and CC F_3 progenies were selfed to produce F_4 generation progenies which was termed as GGG, CGG and CCC, respectively. The experimental details at each location and pollen treatment given in table 3 in electronic supplementary material. Phenotypes of parental genotypes were studied in the same location in 2017 (table 4 in electronic supplementary material; Singh *et al.* 2017).

Evaluation of F_4 progenies for seedling heat tolerance

Two contrasting F_4 progenies, namely GGG (pollen selection in F_1, F_2 and F_3 generations) and CCC (no pollen selection in F_1, F_2 and F_3 generations) derived from F_3 plants grown at ARS Bheemarayanagudi were selected to study the effect of pollen selection on seedling heat tolerance. The seedling heat tolerance was tested using the protocol proposed by Nieto-Sotelo *et al.* (2002) with little modifications. About 300–350 seeds from each GGG and CCC F_4 progenies were incubated in dark at 28°C in growth chamber in enamel trays containing paper towels saturated with 0.1 mM $CaCl_2$ and wrapped in aluminum foil. The three-day old germinated seedlings with uniform root length were transferred to sterile flasks containing 80 mL 0.1 mM $CaCl_2$ and preincubation treatment of seedlings was carried out for 1 h at 28°C in a rotary shaker at 60 rpm. This experiment was performed in three replications with 50 seedlings in each replication. After 1 h the flasks were incubated at 40°C for 1 h in water bath for induced temperature treatment of heat stress followed by incubation at 28°C for 1 h in a rotary shaker at 60 rpm. The second heat shock treatment was given at 50°C for 1 h in water bath. Soon after the heat stress treatment, the seedlings were transferred to trays at 28°C for recovery. After three days, the seedlings were transferred to a tray containing sand for the establishment and allowed to grow for six days in the greenhouse. Another set of seedlings which were not given any heat shock treatment and considered as a control. The establishment percentage, root length and shoot length were measured on 9th day after treatment (Nieto-Sotelo *et al.* 2002; Singh *et al.* 2020a). The establishment percentage was calculated by number of seedlings survived out of total seedlings exposed to heat stress. Further, the per cent reduction in establishment, root and shoot growth under heat stress treatment with reference to their respective control was determined.

$$\text{Reduction over control } (\%) = \frac{\mathrm{C} - \mathrm{R}}{\mathrm{C}} \times 100.$$

Where R is establishment / root length / shoot length of heat stress treated seedlings. C is establishment / root length / shoot length of control seedlings.

Genotyping of selected (G) and control (C) F_2 population using SSR markers

The genomic DNA from young leaves of parents (BTM4 and BTM6) along with 100 plants each from selected F_2 (G) and control F_2 (C) was extracted by modified CTAB method (Doyle and Doyle 1987). Totally 85 SSR primers were used to screen the parental lines to identify polymorphic markers. Of these, five primers recorded polymorphism between parents on agarose gel (table 5 in electronic supplementary material). These five primers were used for genotyping individual 100 plants of control and selected F_2 individuals. Three types of banding pattern for each primer were observed and evaluated as homozygous male, homozygous female and heterozygote.

Statistical analysis

One-way analysis of variance (ANOVA) was performed to compare three groups of F_3 progenies namely GG, CG and CC. The monogenic segregation pattern (1:2:1) of each of markers in control F_2 (C) and selected F_2 (G) was tested by chi-square (χ^2) test.

Results and discussion

Evaluation of F_3 and F_4 progenies for heat stress tolerance

The success of pollen selection for heat tolerance is determined by a high temperature tolerance of the progenies derived through pollen selection compared to progenies obtained without pollen selection. Three groups of F_3 progenies, namely pollen selection for heat tolerance in F_1 and F_2 generations (GG), pollen selection only in F_2 generation (CG) and no pollen selection (CC) were compared for their heat tolerance by growing them under high temperature stress conditions at ARS, Bheemarayanagudi, Yadgir district of Karnataka. The date of sowing of the progenies were adjusted so that all the plants were uniformly exposed to more than 40°C during the flower initiation and microsporogenesis stage (figure 1 in electronic supplementary material). The analysis of variance showed significant differences between the three groups of F_3 populations, namely GG, CG and CC for SPAD values at 65 and 85 DAS (chlorophyll count), number of pollen grains per anther, pollen sterility per cent, cob length, number of seed rows per cob, number of seeds per cob and five grain cob weight (table 1).

Male gametophyte grows and acquires nutrition from the surrounding tissue through signal exchange with their surroundings (Beaudry *et al.* 2020). It has also been reported that different sets of genes are expressed during different stages of male gametophyte development (Honys and Twell 2004; Tang *et al.* 2010; Warman *et al.* 2020). The abiotic stress environment affects gametophytic development and reproductive performance. Continues high temperature stress during plant growth and reproductive initiation stage reduces the pollen production and competitive ability (Greenway and Harder 2007). The genetic overlap between the gametophytes and sporophytes suggest that the heat stress tolerant genotypes produce superior gametophytic performance resulting in superior pollen grains. In the present study, three groups of F_3 progenies significantly differed for the number of pollen grains per anther and pollen sterility under heat stress (figure 2 in electronic supplementary material).

The total number of pollen grains per anther were higher in GG F_3 lines (1180.44 ± 24.11) followed by the CG (849.33 ± 25.22) and CC (605.33 ± 20.47) lines. Contrastingly, pollen sterility was significantly highest in F_3 CC (35.64 ± 2.02%) compared to CG (21.94 ± 1.21%) and GG (10.24 ± 1.00%) progenies. It is interesting to note that the F_3 (CC) progenies were significantly different from CG and GG for the total number of pollen grains per anther and pollen sterility per cent (table 2). The pollen quality and quantity of the progenies derived through two cycles of pollen selection (GG) were significantly improved over a single cycle or no pollen selection under the heat stress condition at flowering stage. The progenies derived from moderate or intense gametophytic selection in maize resulted in higher seedling weight, longer roots and heavier kernels (Ottaviano *et al.* 1982, 1986). Even though maize can produce overabundance of pollen, the reduced pollen number under high temperature stress resulted in reduced kernel number and grain yield (Westgate *et al.* 2003). Heat stress also induced reduction in starch granule number and size in maize pollen, resulted in pollen sterility and lower seed set and yield (Wang *et al.* 2019). As in the present study, the cyclic pollen selection improved the performance of progenies in succeeding generations in terms of pollen numbers per anther and seed yield contributing traits are evidenced in maize (Mohapatra *et al.* 2020). Hence recurrent pollen selection can be combined with phenotypic selection in breeding to select stress tolerant plants.

It is expected that the higher pollen number in GG progenies might be due to healthy male gametophytic tissue and

Table 1. Mean sum of squares of seven characters for three sets of F_3 progenies (F_3 (CC), F_3 (CG) and F_3 (GG)) tested at ARS, Bheemarayanagudi.

df		SPAD value (65 DAS)	SPAD value (85 DAS)	ASI	NPG/A	PS	CL	S/RC	S/P	FGW
Population	2	194.96**	274.76**	2.69	1249808.40**	2426.00**	38.95*	78.21*	75624.13**	107,24.344**
total	44									

*Significant at $P < 0.05$ and **significant at $P < 0.01$. SPAD value (65 DAS), SPAD chlorophyll meter reading recorded at flowering stage (65 DAS); SPAD value (85 DAS), SPAD chlorophyll meter reading recorded at maturity stage (85 DAS); ASI, anthesis to ailking interval, NPG/A, number of pollen grains per anther; PS, pollen sterility (%); CL, cob length (cm); S/RC, number of seeds per row of cob; S/P, number of seeds per plant; FGW, five cob seed weight (g); CC, F_3 progenies derived without any pollen selection; CG, F_3 progenies derived from pollen selection in F_2 generation and without any pollen selection in F_1 generation; GG, F_3 progenies derived from pollen selection in both F_1 and F_2 generations.

Table 2. Performance of three groups of F_3 progenies (CC, CG and GG) for physiological and quantitative traits under high temperature condition at ARS, Bheemarayanagudi.

	Mean value			LSD (mean difference) between selected F_3 population		
Trait	CC	CG	GG	CG-CC	GG-CC	GG-CG
SPAD (65 DAS)	43.35 ± 1.52	40.55 ± 1.47	47.71 ± 0.88	2.80	4.36*	2.19
SPAD (85 DAS)	32.84 ± 1.82	30.93 ± 1.40	39.11 ± 1.62	1.90	6.27*	8.18**
ASI	3.53 ± 0.53	3.53 ± 0.83	2.80 ± 0.42	0.0	0.73	0.73
NPG/A	605.33 ± 20.47	849.33 ± 25.22	1180.44 ± 24.11	244*	574.67**	331.11*
PS	35.64 ± 2.02	21.94 ± 1.21	10.24 ± 1.00	13.70**	25.41**	11.70*
CL	7.96 ± 1.52	10.95 ± 0.85	10.49 ± 0.82	2.99	2.53	0.46
S/RC	7.31±1.65	9.57 ± 1.36	11.87 ± 1.27	2.27	4.57*	2.3*
S/P	110.95 ± 10.70	177.48 ± 17.90	252.87±18.30	66.53	141.92**	75.38
FGW	101.33 ± 31.50	124.97 ± 27.40	214.67 ± 24.30	22.99	112.69**	89.70*

*Significant at $P < 0.05$, **significant at $P < 0.01$. SPAD value (65 DAS), SPAD chlorophyll meter reading recorded at flowering stage (65 DAS); SPAD value (85 DAS), SPAD chlorophyll meter reading recorded at maturity stage (85 DAS); ASI, anthesis to silking interval; NPG/A, number of pollen grains per anther; PS, pollen sterility (%); CL, cob length (cm); S/RC, number of seeds per row of cob; S/P, number of seeds per plant; FGW, five cob seed weight (g); CC, F_3 progenies derived without any pollen selection; CG, F_3 progenies derived from pollen selection in F_2 generation and without any pollen selection in F_1 generation; GG, F_3 progenies derived from pollen selection in both F_1 and F_2 generations

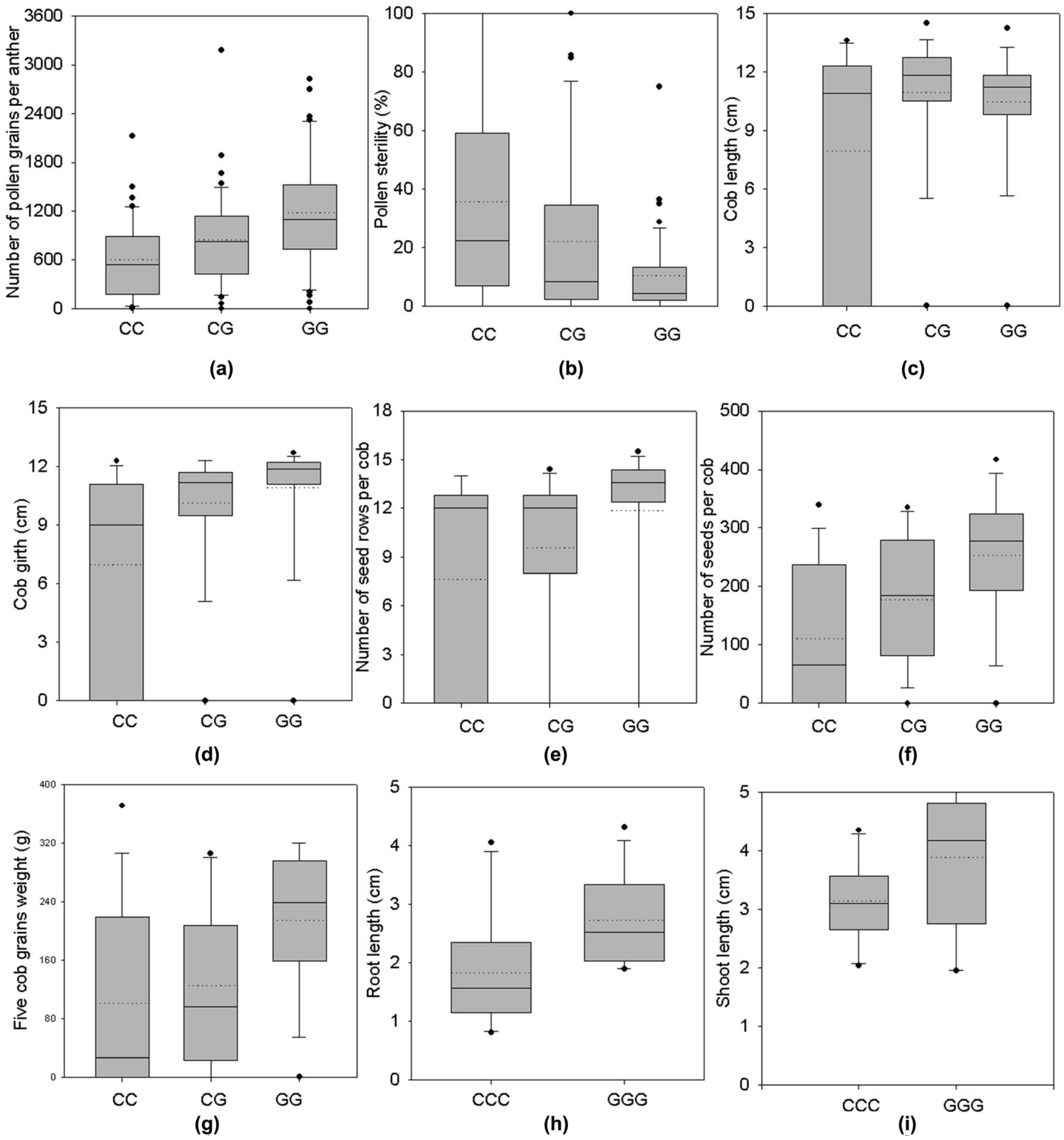

Figure 1. Distribution pattern of F_3 and F_4 progenies of maize for physiological and yield attributes. Three F_3 populations (CC, CG and GG) parameters: (a) number of pollen grains per anther, (b) pollen sterility (%), (c) cob length (cm), (d) cob girth (cm), (e) number of seed rows per cob, (f) number of seeds per cob, (g) five cob grain weight. Two F_4 population (CCC, GGG) seedling parameters: (h) root length (cm), (i) shoot length (cm); solid line in box plot: median of population, dash line in box plot: mean of population. CC, F_3 progenies derived without any pollen selection; CG, F_3 progenies derived from pollen selection in F_2 generation and without any pollen selection in F_1 generation; GG, F_3 progenies derived from pollen selection in both F_1 and F_2 the generation; GGG, F_4 progenies derived from pollen selection in F_1, F_2 and F_3 the generation; CCC, F_4 progenies derived without any pollen selection in F_1, F_2 and F_3 the generations.

surroundings. The GG progenies had significantly higher chlorophyll content under heat stress (table 2). The SPAD value at 65 DAS was significantly higher in GG group (47.71 ± 0.88) than the other two groups of progenies (CG and CC). Similar trend was observed for SPAD values recorded at 85 DAS. It indicated that the GG group of progenies derived from the pollen selection in F_1 and F_2 generations maintained higher chlorophyll content under heat stress (table 2). Higher chlorophyll content in tolerant progenies than susceptible ones under both green house and field conditions is a clear indication that the resulted progenies recorded more chlorophyll content and vigour suggesting their potentiality to yield higher under stress condition.

The higher chlorophyll content in the progenies derived through pollen selection for heat tolerance in both F_1 and F_2 generations (GG) may have resulted in higher seed yield and seed yield related traits followed by the progenies derived from one generation of pollen selection (CG) compared to control progenies (CC) (figure 1). More chlorophyll content, more number of pollen grains per anther and less pollen sterility of GG progenies led to improved seed yield and higher values for yield contributing traits under high stress compared to progenies obtained from one cycle of pollen selection (CG) and progenies derived through without pollen selection (CC). These results clearly indicated that pollen selection in each generation improves the performance of progenies in succeeding generations under heat stress. Similar kind of results was reported earlier in maize (Singh *et al.* 2020a). Ottaviano *et al.* (1986) compared the progenies derived through intense gametophytic selections for plant vigour in maize and showed that they were characterized by higher seedling dry weight, longer roots and heavier kernels. The pollen selection for pollen competitive ability resulted in improvement of seed yield in maize (Landi *et al.* 1989). Petolino *et al.* (1990) reported that agronomic performance of the gametophytically selected progenies was found to be superior as evidenced by higher grain yield, greater seedling vigour, and reduced stalk and root lodging. A correlation between pollen selection at low temperatures and the response of the sporophyte under the same conditions was observed (Zamir *et al.* 1981; Landi *et al.* 1989). The lines derived through gamete selection for cold tolerance resulted in stronger response than the original parents observed in maize (Kovács and Barnabás 1992). The direct effect of pollen selection was reported on several quantitative traits like plant vigour and other associated with grain yield (Winsor *et al.* 1987), reproductive performance (Quesada *et al.* 1996) and disease resistance (Chikkodi and Ravikumar 2000). The cyclic pollen selection for moisture stress tolerance was found effective in producing progeny with better stress tolerance with higher seed yield and yield related components in sorghum (Patil *et al.* 2006), chickpea (Clarke *et al.* 2004), tomato (Domínguez *et al.* 2005) and sunflower (Shobha and Ravikumar 2006).

The contrasting F_4 progenies, namely GGG (pollen selection in F_1, F_2 and F_3 for heat tolerance) and CCC (no pollen selection) were compared for heat tolerance at seedling stage. High temperature stress tolerance at seedling stage can be measured using temperature induction response (TIR) as demonstrated in rice (Devi *et al.* 2013); ragi (Babu *et al.* 2013) and maize (Rajesh *et al.* 2011; Dar *et al.* 2016). The GGG progenies recorded significantly higher establishment percentage and root length over the CCC progenies under heat stress treatment (table 3). Under no stress conditions the per cent establishment of seedlings was same in both GGG and CCC progenies. The mean root and shoot length of GGG F_4 progenies was higher than the CCC F_4 progenies (table 3; figures 1 & 2). Thus the above results clearly showed that the pollen selection could combine the rare favourable allelic combinations that would not be detected under sporophytic selection (Landi *et al.* 1989; Singh *et al.* 2020a). In maize, it was proved that more number of repeated cyclic selections achieved a high level of resistance for low temperature (Kovács and Barnabás 1992). The selected F_4 progenies (GGG) showed the improved performance under no heat stress condition indicating no deleterious effect of directional pollen selection on other desirable traits. Such positive stress tolerance between gametophytic and sporophytic tolerance for pollen selection across generations showed that adaptive recurrent selection leads to genetic improvement for high temperature in maize without affecting the quantitative traits. The genetic variation favoured in gametophytic phase will have positive effect on the sporophytic tolerance to several biotic and abiotic stresses (Peters and Weis 2018).

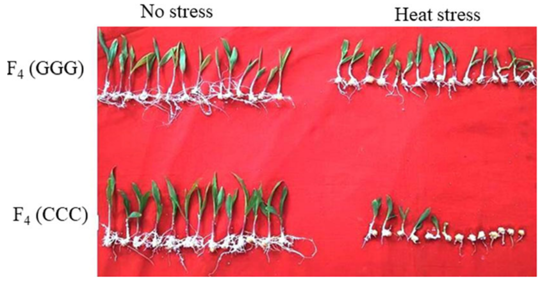

Figure 2. Effect of heat stress on seedlings of F_4 (CCC) and F_4 (GGG) progenies of maize. GGG, F_4 progenies derived from pollen selection in F_1, F_2 and F_3 the generations; CCC, F_4 progenies derived without any pollen selection in F_1, F_2 and F_3 the generations.

Table 3. Mean seedlings performance of F_4 progenies in GGG and CCC treatments under heat stress.

	No stress		Induced treatment (50°C-1 h)	
Parameter	CCC	GGG	CCC	GGG
Establishment (%)	100.00 ± 0.00	100.00 ± 0.00	21.25 ± 2.27 (78.75)	39.92 ± 1.64* (60.08)
Root length (cm)	7.04 ± 0.43	8.02 ± 0.52	1.83 ± 0.30 (74.17)	2.73 ± 0.22* (65.96)
Shoot length (cm)	7.62 ± 0.20	8.21 ± 0.45	3.14 ± 0.21 (58.87)	3.88 ± 0.35 (52.74)

Values in parenthesis are per cent reduction values over the control. *Significant at $P < 0.05$ and **significant at $P < 0.01$. GGG, F_4 progenies derived from pollen selection in F_1, F_2 and F_3 generations. CCC, F_4 progenies derived without any pollen selection in F_1, F_2 and F_3 generations.

Table 4. Number of plants in control (C) and selected (G) F_2 population showing parental type and hybrid type SSR segregation banding pattern.

	Primer name	Control F_2 (C) BTM4	Hybrid	BTM6	χ^2 (1:2:1)	Selected F_2 (G) BTM4	Hybrid	BTM6	χ^2 (1:2:1)
1	bnlg1526	25 (25)	47 (50)	28 (25)	NS	15 (25)	42 (50)	43 (25)	S
2	umc1894	31 (25)	43 (50)	26 (25)	NS	17 (25)	53 (50)	30 (25)	NS
3	umc1781	31 (25)	41 (50)	28 (25)	NS	13 (25)	44 (50)	43 (25)	S
4	phi097	28 (25)	43 (50)	29 (25)	NS	12 (25)	40 (50)	48 (25)	S
5	bnlg1413	26 (25)	47 (50)	27 (25)	NS	17 (25)	39 (50)	44 (25)	S
Total		141 (125)	221 (250)	138 (125)		74 (125)	218 (250)	208 (125)	

Values in parenthesis are number of plants expected to show either any of the parent or hybrid type marker segregation; NS, nonsignificant; S, significant at 5%.

Molecular comparison of pollen selected (G) and control (C) F_2 population

The effect of pollen selection for heat tolerance in F_1 generation on the segregation of genomic SSR markers was studied in F_2 population and compared with parental lines. All the five markers showed expected monogenic segregation of 1:2:1 in the control population (no pollen selection) (table 4). The results are in accordance with the established fact of that for bi-parental population, SSR markers showed the Mendelian inheritance pattern of 1:2:1 ratio in the F_2 generation. On the contrary, four of five SSR markers showed significant deviation from the expected monogenic ratio of 1:2:1 in the pollen selected F_2 population. It is interesting to note that the marker homozygous for the male parent (BTM6) alleles were significantly higher than expected which showed deviation from the expected 1:2:1 ratio. It provides strong evidence that pollen selection is effective in increasing the frequencies of SSR alleles from male parent tolerant heat stress into the progenies. The pollen selection disproportionately increases the frequency of SSR alleles in the F_2 and succeeding generation. The pollen selection improved the heat tolerance in pollen grains to survive and fertilize the ovule resulted in significant deviation from Mendelian ratio in F_2 and higher tolerance in plants.

The deviation from normal Mendelian segregation in selected F_2 and skewness towards the alleles selected from male parent provides evidence that pollen selection increases the frequency of tolerant male parent alleles is being selectively passed to the succeeding progeny. The increased frequency of heat tolerant plants in F_3 and F_4 generations observed under field and laboratory condition demonstrated the practical benefits of pollen selection for heat tolerance. The deviation in expected segregation ratio in gamete selection derived progenies demonstrated the utility of gamete selection (Ottaviano *et al.* 1988; Ravikumar *et al.* 2013). The results clearly suggested that, it could be possible to rapidly transfer resistant alleles with heat stress into elite germplasm by subjecting the heterozygous plants to pollen selection. We are extending this study and trying to identify the candidate genes that are responsible for reproductive heat stress tolerance in maize.

Conclusion

We conclude from the above findings that pollen selection through growth chamber could be used as an alternative in selecting for heat stress tolerance. The response of pollen selection persisted across the generations and repeated selection pressure resulted in more improvement in the sporophytic performance of progenies. There is growing evidence that selection of parental alleles during gametophytic stage of the plant life cycle may have desirable effect at the gene level to select the favourable alleles altering the sporophyte. Therefore, the selection for favourable parental alleles through gamete selection in the two phases of plant life cycle contributes to faster adaptation during stress.

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