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# Effect of Pollen Selection for Moisture Stress Tolerance in Maize (*Zea mays* L.)

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Abstract

In the present investigation the effect of pollen selection for moisture stress tolerance in $F_1$ generation on physiological traits and pollen number were studied. The susceptible inbred line BTM1 was crossed to drought tolerant inbred line BTM2 to produce $F_1$ seeds. One set of true $F_1$ plants were grown in pots without any stress and selfed to produce control $F_2$ population ($CF_2$). Another set was grown in pots and subjected to moisture stress during reproductive stage (40-50$^{th}$ days after sowing). The stigma of the stressed plants were painted with 50 per cent polyethylene glycol (PEG) one hour before selfing producing $F_2$ seeds labelled as pollen selection in stressed plant $F_2$ ($PSSPF_2$). These two $F_2$ populations were evaluated under moisture stress condition in the field where in physiological traits (relative water content and chlorophyll content) and number of pollen grains per anther was recorded. The $PSSPF_2$ showed higher values for relative water content (RWC), chlorophyll content and number of pollen grains per anther compared to $CF_2$ under moisture stress condition. Thus results indicate that pollen selection has improved sporophytic physiological traits like RWC, chlorophyll content and number of pollen grains per anther when grown under moisture stress condition in the field.

*Keywords*: Maize, pollen selection, RWC, chlorophyll content

Maize (*Zea mays* L.) is one of the most important cereal crop after rice and wheat, predominantly grown around the world for various purposes since the time immemorial. This crop has wider adaptability under varied agro climatic conditions ranging from sub humid to semi-arid regions. Globally it is grown over an area of 185.90 million hectares with a production of 1,075.49 million MT. In India it covers an area of 9.89 million hectares with a production of 25.90 million MT (USDA, 2018). This crop has a great production potential and regarded as queen of cereals but still there are constrains in its production due to biotic and abiotic stress. Among abiotic stresses, drought and heat stress are the major problem in maize (Deryng *et al.,* 2014). Drought causes severe yield loss of upto 50-60 per cent in maize (Daryanto *et al*., 2016). The effect of drought on crop plants depends on the stage and extent of susceptibility to moisture stress. In maize drought affects various stages of growth and development starting from germination till maturity. However, reproductive and grain filling stages are more critical as they determine the final seed set or yield in maize. Maize yield can be reduced by as much as 90 per cent if the crop is exposed to drought stress from a few days before tassel emergence to the beginning of grain filling (Awosanmi *et al*., 2016). Moisture stress during meiosis causes complete pollen sterility in maize by inhibiting the development of microspores mainly not because of desiccation of pollen grains but due to indirect consequence of water deficit in vegetative tissues (Barnabas *et al*., 2008). Although, various breeding strategies are employed to develop drought tolerant maize but they are mainly based on the selection at sporophytic phase which have certain limitations.

In plants, gametophytic selection occurs naturally where competition exists between pollens to fertilize ovary. This mechanism of screening pollen grains has been exploited in breeding by applying various selective agents or conditions on male gametophyte to eliminate unfavourable alleles. The use of gametes as a screening system is based on several reports indicating an overlap of genes that are expressed in both gametophytic and sporophytic level (Dominguez *et al.,* 2005). In maize about 72 per cent of genes expressed in pollen grains were also expressed in sporophyte (Sari-Gorla *et al.,* 1989). There are a large number of reports where pollen or gametophytic selection is effectively used to test the sporophytic progeny for resistance to biotic and abiotic stresses in various crops (Totsky and Lyakh, 2015). As the selection pressure acts on the male gametophyte, only those which have favourable allele fertilize the ovary and helps in obtaining viable recombinants (Chang *et al.*, 2010). Hence, in the present study an attempt was made to assess the effect of pollen selection for moisture stress tolerance in $F_1$ generation on the physiological traits and pollen number associated with drought tolerance in the $F_2$ generation.

## Material and Methods

Two homozygous inbred lines BTM1 (drought susceptible) and BTM2 (drought tolerant) were selected for the present study based on the susceptibility of these lines in earlier experiments (Ashwini, 2016). Drought susceptible inbred line BTM1 was crossed to drought tolerant inbred line BTM2 to produce hybrid seeds.

The $F_1$ plants were grown in pots during *kharif* 2016 at the Department of Plant Biotechnology, GKVK, Bangalore. The DNA from these plants were isolated by modified CTAB method when they were 15 days old (Lu *et al.*, 2008) and the hybridity was confirmed by using three polymorphic simple sequence repeats (SSR) primers *viz.*, bnlg161, bnlg 1035 and dupssf 19.

One set of five $F_1$ plants were used for inducing stress during reproductive development stage. The $F_1$ plants were subjected to stress by with holding water for 15 days during microsporogenesis (from 45$^{th}$ to 60$^{th}$ day after sowing in pots). Further the silks of the stressed $F_1$ plants were smeared with 50 per cent polyethylene glycol (PEG6000) using camel hairbrush and allowed to equilibrate the tissue for an hour in the morning hours. After one hour the pollen grains of the same plant were collected and dusted abundantly on the PEG treated silk and the seeds harvested from these plants constituted pollen selection in stressed plant $F_2$ (PSSP$F_2$). Another set of five $F_1$ plants were grown without any stress with regular watering and selfed to produce control $F_2$ (C$F_2$) seeds.

The PSSP$F_2$ and C$F_2$ were grown in replications under irrigation during *rabi* season of 2017-2018 at Department of Plant Biotechnology, GKVK under field conditions. The plants were subjected to moisture stress during microsporognesis by withholding irrigation for 15 days from 50$^{th}$ to 65$^{th}$ day in field condition. Seventy plants were selected randomly in PSSP$F_2$ and C$F_2$ to record observations on the chlorophyll content, RWC and number of pollen grains to compare the populations.

The leaf relative water content (RWC) of the stressed plants was estimated according to the formula given by Agami (2013) on 63$^{rd}$ day. The leaf down the canopy level was used to estimate the relative water content. Twenty-five leaf discs of 0.8 cm were taken from each plant and the fresh weight (FW) was measured immediately. Then the leaf discs were immersed in distilled water and left for 4.0 hours. The turgid weight (TW) was measured after 4.0 hours and the leaf discs were dried in an oven at 70 °C for 48 hours to record the final dry weight (DW). The RWC was calculated as follows

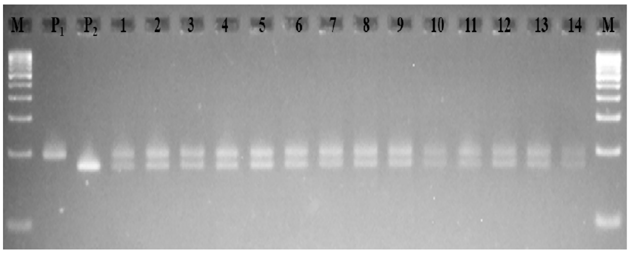
$$RWC (\%) = [FW – DW)/(TW – DW)\times 100]$$

The leaf chlorophyll content was estimated by using SPAD-502 chlorophyll meter. The SPAD chlorophyll meter reading (SCMR) were recorded from the fully expanded fourth leaf from top of the stressed plants on 65$^{th}$ day. From each plant three measurements were taken and averaged.

During anthesis, the anthers which are about to dehisce on the next day were collected from each plant from two different positions (fourth anther from top and bottom) of the single spikelet. The third spikelet from the bottom of the tassel was selected and brought to the laboratory and incubated at 70 °C in an oven for 24 hours. The anther from the selected position was then dispensed in 1.0 ml of 5 per cent tween 20 and sonicated. The pollen number was recorded under projection microscope with a magnification of 400X.

## Results and Discussion

The hybrid was produced from the cross between drought susceptible inbred line BTM1 and drought tolerant inbred line BTM2 and its hybridity was confirmed by using three polymorphic SSR primers *viz.*, bnlg161, bnlg 1035 and dupssf 19. All the hybrid plants selected produced both the parental bands




M-100 bp DNA ladder, $P_1$ -BTM1, $P_2$ -BTM2, 1-14- $F_1$ plants

Fig. 1: Confirmation of $F_1$ hybridity using SSR primers

confirming hybridity (Fig. 1). The confirmed $F_1$s were further used to produce $PSSPF_2$ and $CF_2$.

Leaf relative water content and chlorophyll content are two important physiological parameters associated with drought tolerance and can be used as surrogate for the selection of tolerant genotypes. The mean leaf relative water content was significantly higher in $PSSPF_2$ population compared to $CF_2$ population. The relative water content in $PSSPF_2$ ranged from 53.59 to 87.31 per cent with a mean of 69.34 per cent compared to $CF_2$ which showed a mean of 58.35 per cent with a range of 24.40 to 80.10 per cent under moisture stress environment (Table I). The frequency distribution was skewed towards plants

TABLE I

*Study of physiological parameters and pollen number in $CF_2$ and $PSSPF_2$ population of maize*

| Parameters | $CF_2$ | $PSSPF_2$ |
|---|---|---|
| Relative water content (%) | 58.35 | 69.34 *** |
| Chlorophyll content | 34.89 | 37.20 ** |
| Pollen number (4th from top) | 4857.14 | 6036.19 *** |
| Pollen number (4th from bottom) | 5592.38 | 6484.762 *** |

** Level of significance at 1%
*** Level of significance at 0.1%

with higher values for RWC in $PSSPF_2$ compared to $CF_2$ (Table II). This is mainly because pollen selection at $F_1$ generation increased the number of moisture stress tolerant plants with alleles that maintained higher RWC in $PSSPF_2$ compared to $CF_2$ without any pollen selection. Similar results were observed in wheat and maize. The tolerant genotypes had higher leaf RWC compared to susceptible genotypes under stress (Arjenaki *et al.,* 2012; Singh and Ravikumar, 2017). Higher relative water content maintains

TABLE II

*Frequency distribution for RWC (%) in $CF_2$ and $PSSPF_2$ under moisture stress condition.*

| RWC (%) | Frequency | |
|---|---|---|
| | $CF_2$ | $PSSPF_2$ |
| 10 - 30 | 2 | 0 |
| 30 - 50 | 8 | 0 |
| 50 - 70 | 51 | 38 |
| 70 - 90 | 9 | 32 |

protoplast hydration which is necessary for normal functioning of various biochemical process in plants (Sikuku *et al.*, 2012).

In the present study the chlorophyll content ranged from 22.57 to 46.77 with a mean of 37.20 in $PSSPF_2$ and in $CF_2$ it was 25.4 to 43.5 with a mean of 34.89. The mean chlorophyll content was significantly higher in $PSSPF_2$ compared to $CF_2$ (Table I). The more number of plants with higher chlorophyll content under moisture stress conditions was observed in $PSSPF_2$ (Table III), suggesting that pollen selection

TABLE III

*Frequency distribution for chlorophyll content in $CF_2$ and $PSSPF_2$ under moisture stress condition*

| Chlorophyll content (SCMR) | Frequency | |
|---|---|---|
| | $CF_2$ | $PSSPF_2$ |
| 20 - 25 | 0 | 1 |
| 25 - 30 | 9 | 3 |
| 30 - 35 | 28 | 17 |
| 35 - 40 | 22 | 27 |
| 40 - 45 | 11 | 18 |
| 45 - 50 | 0 | 4 |

for moisture stress improved the chlorophyll stability and content in the progenies. The selection of pollen grains with allele for moisture stress tolerance in $F_1$ generation using PEG as a selective agent on the stigma has led to increased frequency of moisture stress tolerant plants with higher chlorophyll content in $PSSPF_2$. This is mainly because under lower relative water content, reactive oxygen species are produced and they degrade chlorophyll pigments. The loss of chlorophyll pigments will eventually decrease the photosynthetic efficiency of a plant and decreases the photosynthetic assimilates making them susceptible to moisture stress. The results are consistent with the study conducted by Singh and Ravikumar (2017) in maize where heat tolerant maize inbred lines had higher chlorophyll content compared to susceptible lines.

The total pollen grains produced per tassel will determine the kernel set in maize. In the present experimentation, the pollen number at different position from a single spikelet was measured. Under moisture stress, significant (P<0.001%) reduction in number of pollen grains per anther was observed in $CF_2$ compared to $PSSPF_2$ at both the position (Table I). Population distribution for pollen number under moisture stress revealed differences in $CF_2$ and $PSSPF_2$ populations (Table IVa and IVb). The increased chlorophyll content in $PSSPF_2$ increased the tolerance leading to increased pollen number per anther under moisture stress conditions compared to $CF_2$.

TABLE IV

*Frequency distribution for number of pollens per anther collected from (a) fourth from top (b) fourth from bottom in third spikelet from base in different $F_2$ populations under moisture stress*

| Poolen Number per anther | Frequency | |
|---|---|---|
| | $CF_2$ | $PSSPF_2$ |
| <3000 | 6 | 0 |
| 3000 - 4000 | 12 | 4 |
| 4000 - 5000 | 23 | 12 |
| 5000 - 6000 | 16 | 20 |
| 6000 - 7000 | 9 | 23 |
| 7000 - 8000 | 3 | 5 |
| >8000 | 1 | 6 |

(a)

| Poolen Number per anther | Frequency | |
|---|---|---|
| | $CF_2$ | $PSSPF_2$ |
| <3000 | 2 | 0 |
| 3000 - 4000 | 6 | 1 |
| 4000 - 5000 | 18 | 11 |
| 5000 - 6000 | 18 | 15 |
| 6000 - 7000 | 14 | 18 |
| 7000 - 8000 | 9 | 18 |
| >8000 | 3 | 7 |

(b)

The results indicated that the pollen selection for moisture stress tolerance in $F_1$ generation has improved the sporophytic physiological traits like RWC, chlorophyll content and pollen number per anther in $F_2$ generation under moisture stress environment. Gametophytic selection in $F_1$ not only modifies drought tolerance of $F_2$ but also changes the genetic structure of $F_2$ population. The $F_1$ plants produce pollen grains with alternate alleles for traits associated with drought tolerance. The pollen selection by inducing stress on the stigmatic surface before pollination will be useful strategy to reduce the frequency of pollen grains carrying susceptible alleles. Similar result was observed by Totsky and Lyakh (2015) in sunflower. It is important to compare the performance of selected and control $F_2$ population for seed yield and related traits under moisture stress conditions. It is important to compare the performance of selected and control $F_2$ population for seed yield and related traits under moisture stress conditions.

The haploid state, large population size of male gametophyte and probability of selecting the complex allele combination in gametophytic phase makes gametophytic selection more reliable. Thus, gametophytic selection offers opportunities to screen large number of haploid genotypes for moisture stress which can be used as breeding tool to develop drought tolerant crops.

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