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Enhanced Production of Maize Pollen during Extreme Low Temperature for DH Wheat Development in Eastern Gangetic Plains of India

Shahnishan Tabassum[1], Amrita Singh[1] and Awadhesh Kumar Pal[1*]

[1]Department of Biochemistry and Crop Physiology, Bihar Agricultural University, Sabour-813210, India.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Wheat is one of the worlds most commonly consumed cereal grains. With the rapid increase in world population, the production of wheat will play a pivotal role in food security and the global economy. Conventional breeding programme for crop improvement involves hybridisation, selection and evaluation of plants which takes many generation cycles to reach homozygosity. Double haploid (DH) technology is a new boon for rapid development of new homozygous lines, which otherwise are achieved in more than seven years. Efficiency in pseudo-seeds production during wheat-maize hybridisation may lead to enhanced production of DH lines for varied purpose. Limitation of getting maize pollen during low temperature of winter in northern India is a challenge. Pollen of maize is shortly viable, and storage of the same at a lower temperature and high relative humidity could increase its viability up to 6 days at 4°C as proposed from earlier studies. Weather conditions greatly influence pollen shedding phenomenon. Cool, humid temperature delays the pollen shed while hot and dry conditions favor the same. Thus the present study was conducted to determine the efficiency of pollen development during extreme cold under four different conditions *viz*. open condition (under shade from top), in polyhouse, in the tunnel (inside polyhouse with hot air supply) and on the ground soil. The data were collected in terms of the health of the plants and pollen quantity. It was recorded that the plants in tunnel showed early spike emergence and pollen shedding in comparison to plants in a natural environment like in the open and on the ground. Chlorophyll content was least in plants in tunnel and highest in the plants sowed on the ground. Stem girth and plant height were also minimum in plants which were present in tunnel. In conclusion, for early availability and regular supply of pollen, the maize plants should be grown under a tunnel with hot air supply.

**Corresponding author: E-mail: awapal@gmail.com;*
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1. INTRODUCTION

Wheat is one of the world's most important food crops, contributing about one-fifth of human caloric intake [1]. With 60-70% starch, 12-14 % proteins (albumins, globulins, gliadins and glutenins), 10-15 % water, 2-3 % fibre, 1.5-2 % fat, 2-3.5 % sugar and 1.5-2 % mineral matter wheat can prove to be a candidate crop to be improved for increased production using modern means and technology [2,3]. It also contains a high amount of essential amino acids namely leucine, phenylalanine, tyrosine, methionine, cysteine, thiamine etc. Apart from this wheat is also rich in vitamin A, riboflavin, niacin, pantothenic acid, vitamin B6, folate, calcium, iron, magnesium, phosphorus, potassium, zinc and manganese [4] (USDA-National Nutrient Database for Standard 2006). The wheat grain is utilised in a variety of products, which are prepared and consumed such as chapatti, bread, cake, biscuits and pastries [5].

During 2016, with the annual global production of 749.0 million tonnes/ha, wheat was the third most produced crop after maize (875.1 million tonnes/ha) and rice 718.3 (million tonnes/ha). It is grown in an area of about 220.1 million hectares all over the globe. India invests largest area (30.2 million hectares) for wheat cultivation as compared to other nation of the world but ranks second in total production (93.5 million tonnes) after China which produces 120.6 million tonnes in just 24.1 million hectares of its land. As far as productivity is concerned India with merely 3.13 tonnes/ha ranks 49th globally. Nations like New Zealand (8.92 tonnes/ha), Netherland (8.59 tonnes/ha) and Belgium (8.30 tonnes/ha), have shown the productivity of more than double to that in India [6].

The climate of the eastern Gangetic region is conducive to growth and production of wheat, having high-yielding varieties, abundant resources and fertile land. But the growers fail to achieve the optimum potential of wheat crop owing to many constraints *viz*. spot blotch, rust, terminal heat, drought etc. The old conventional breeding method takes a long duration of about 7-10 years for getting a homozygous pure line [7]. By the time a variety attains homozygosity; the pathogens get altered and transformed into a more virulent form. So there is an utmost need to fasten the breeding procedure.

So to fasten the breeding procedure technology like doubled haploid (DH) is being used in wheat for granting pure homozygous lines in less time of 2-3 years may act as a blessing for the crop improvement programs especially in wheat. F_1 are developed from crosses involving one disease resistant parent and other high yielding are sown in pots on a regular interval of five days in synchronisation with the maize plants so that their flowering coincides with each other. China being the first to use this technology has released hundreds of wheat and rice varieties in the past 15 years [8].

In the case of wheat, DH technology has the potential to bring revolution by granting pure parent lines (homozygosity; no risk of heterozygosity) in less time of 2-3 years. It also accelerates gene pyramiding and improves efficacy and efficiency in screening for biotic or abiotic stress tolerance [7]. Haploids can be generated through pollen culture, anther culture or recently highly used uniparental chromosomal elimination technique. While pollen and anther culture has a very low yield of haploids, chromosomal elimination has gained worldwide acceptance due to the higher efficiency of obtaining haploids [9].

The wheat x maize hybridisation system is insensitive to *kr* locus incompatibility and thus finds extensive utility for DH strategy. However, during the growing season of wheat in winter, maize faces a challenging issue of pollen production that is drastically hindered during cold temperatures. So maize needs to be grown separately under controlled favourable conditions in polyhouse which adds to the cost of the breeding program. Also though maize is a potential pollen parent still its rate of haploid induction is lower as compared to that of *Imperata* as suggested by Chaudhary [10]. But *Imperata* is not present during wheat growing season in the eastern Gangetic plain. Maize (*Zea mays* L.) is a monoecious plant bears both the male (tassel) and female (silk) inflorescence separately on the individual plant. Maize depicts protandrous conditions with early pollen shed. These gametophytic cells are vital for the sexual reproductive phase of plant's life cycle White pollen in maize determined by the double recessive gene for anthocyanin has been reported to be unsuccessful for pollination [11]. Pollen fertility in maize could be up to 9-11 days if stored at 4°C with relative humidity of 90% [12]. Other studies conducted in this regards shows the maize pollen retains its viability up to 6 days with relatively increased humidity [13]. Maize is not much tolerant to cold hence growing maize during winter conditions limit the chances of anthesis that restricts its utility in double haploid production [9]. Maize crop needs to be grown in a greenhouse (winter retards the growth outside) to coincide flowering with wheat thereby increasing the cost of haploid production [9].

To overcome the unavailability of pollen from maize during winter breakthrough the current experiment was designed focusing effective anthesis in maize during low temperature. The present study was programmed to investigate proper growth condition of maize plants to produce maximum possible pollen under different conditions of temperature, humidity and photoperiod to relate with pollen outburst.

2. MATERIALS AND METHODS

Seeds of Maize (*Zea mays* L.) variety Vivek QPM, supplied from Dept. of Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bhagalpur were sown in pots of 22 cm x 20 cm (diameter x height) filled with soil: sand: compost as 2:1:1 at regular interval (grouped broadly under two dates) in five replicates. The plants after attaining one foot height were transplanted in pots and placed at varied environmental conditions as discussed below to determine the differences for different parameters (leaf colour, plant height, leaf area, anthesis etc.,) among different conditions taken into consideration. Pollen was mostly collected during morning time.

One set of plants were sown on the ground under natural condition.

Conditions studied in this study are as follows:

1) **Open Condition (pot)** - In this condition, the potted plants were placed in open conditions and faced the day sunlight and night dew directly throughout the 24 h.
2) **Open Condition (Under the shade) (pot)** - These plants sown in pots were kept at different in open natural condition but under a roof covered on the top thereby stopping any dews to fall on plants. The plants were able to get natural sunlight and photoperiod.
3) **In playhouse (pot)** - The potted maize plants were kept under control condition of polyhouses. Plants were not exposed to direct sunlight.
4) **In tunnel (inside polyhouse) (pot)** – Potted maize plants were placed in fully covered by a polytunnel inside polyhouse. Temperature and humidity inside the tunnel were maintained.
5) **On ground soil** - Maize seeds were sown in the soil on the ground. Plants were directly exposed to sunlight and night dew with optimum photoperiod.

Each condition consisted of five replicated plants which were sown and inspected regularly for their growth and anthesis. Morphological parameters like a number of leaves per plant, leaf size, stem girth, plant height and chlorophyll content were measured. Leaf size was calculated after finding the leaf area factor of maize variety used under this study. Leaf area was determined to be the length multiplied with width and leaf area factor to get the actual leaf area. Stem girth was measured using Vernier calliper. Plant height was measured using a measuring tape. Chlorophyll content was estimated spectrophotometrically as per the methods of Arnon [14]. Parameters related to pollen formation like number of branches of spikes, spike length, days of spike emergence after sowing, number of days for pollen shedding after sowing of plant and that after spike emergence was recorded in all five conditions. The second lots of parameters, i.e. related to spike were directly related to the amount and duration of pollen production to be used for DH production in wheat.

3. RESULTS AND DISCUSSION

Development of new homozygous wheat lines can be a promising technology for rapid production of wheat varieties against abruptly changing abiotic factors and frequently emerging virulent pathotypes which can drastically decline grain yield. DH technologically is a technique which can fulfil such requirement in case of wheat. During DH lines generation using wheat x maize system, availability of maize pollen during wheat growing time becomes a severe problem. In this regards five different growth conditions were evaluated for maize pollen production (Figs. 1 and 2).

A number of leaves per plant was significantly higher in protected conditions i.e. under poly tunnel and polyhouse but the size of leaves was smaller as compared to other conditions especially open conditions. The Chlorophyll content was significantly higher in plants grown on the ground followed by open conditions. Chlorophyll content significantly declined under (protected) poly tunnel and polyhouses conditions. Leaves turned pale green under protected conditions, may be due to limitations of sunlight which was scanty as per the requirement. The plant height also followed the same as the leaves traits. Plant height was significantly superior in the plants grown in open conditions. Stem girth was at par among the open conditions both plants in pots as well as on ground (Fig. 1A-E).

A number of spikes was superior in the plants sown on the ground. Among other conditions, the number of spikes per plant was at par. The branches per spike surprisingly showed significant variations among different conditions. It was maximum in potted plants in open condition and drastically decreased in the polytunnel conditions. The length of spikes was

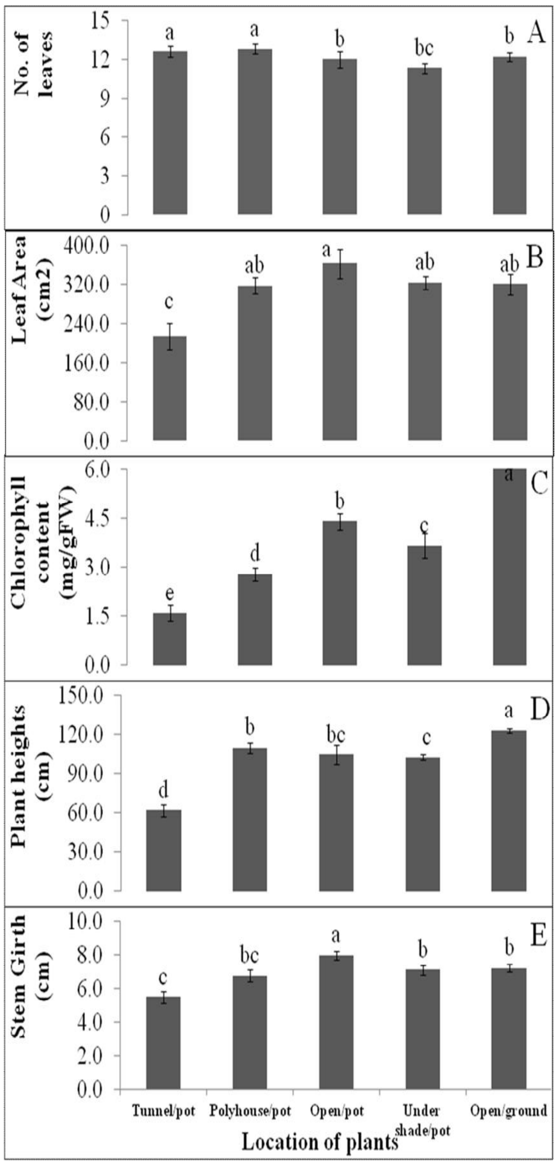

Fig. 1. Morphological parameters in maize at different growth conditions. Data represent mean of five replications and value with different alphabets show significance at P ≤ 0.5.

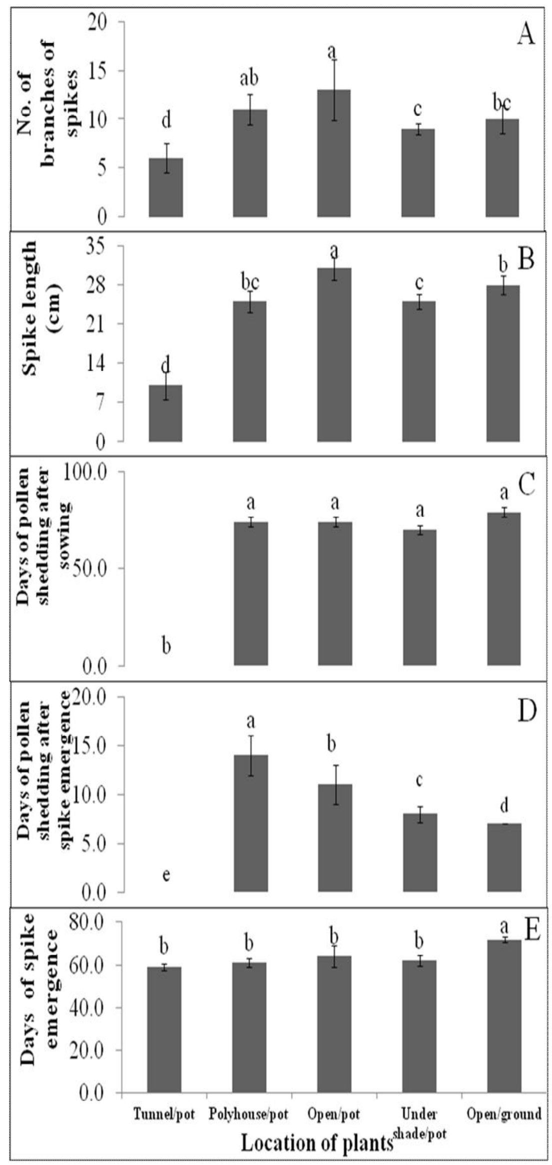

Fig. 2. Spike related parameters in maize at different growth conditions. Data represent mean of five replications and value with different alphabets show significance at P ≤ 0.5.

significantly inferior in potted plants under polytunnel followed by that in polyhouses. Spike length was superior in plants both potted and on the ground in open conditions. The plants transplanted in pots gave pollen taking no time as soon as they were shifted to polytunnel but the amount of pollen was very scanty. Among open conditions and that in polyhouses took time for shedding pollen. The duration was lesser in plants grown on the ground. This may be due to the continuity of growth that they maintained on the ground (Fig. 2A-E).

Hot, dry condition reduces pollen viability and decreases the amount of the pollen shed (weather condition influence pollen shed.) rain and dew also hinders pollen shed because anthers are wet. Cold stress at the time of tassel initiation can potentially reduce tassel branching and spikelet formation.

4. CONCLUSION

The low temperature drastically decreased the pollen formation but the time taken for pollen formation was lesser in the plants grown in protected condition conditions. The amount of pollen was significantly higher in plants grown in open conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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