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An insight into wheat haploid production using wheat x maize wide hybridization

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ABSTRACT

The present study was carried out with six wheat and four maize genotypes from Mahyco Research Centre, Dawalwadi, Jalna, MH to develop wheat double haploid lines by wheat x maize crossing system. The following steps were followed while conducting the experiment: emasculation of wheat spikes, crossing or pollination of emasculated spikes with maize pollens, treating pollinated spikes with 100 ppm 2,4-D solution, excision of embryo and subsequent culture on half strength MS media. In total, 296 spikes were emasculated and pollinated with randomly collected maize pollens after 24 hrs. Success was obtained in rescuing a total of 27 embryos from 575 caryopses among two genotypes *i.e.* MW2 and MW3. Percentage of embryo formation varied in the range of 0-10 per cent owing to non-standard conditions. However, the frequency of embryo formation could be increased by focusing on the most important of all culture conditions *i.e.* temperature (18-21 °C).

1. INTRODUCTION

Wheat (*Triticum aestivum L.*) is widely grown cereal crop in the world. It is consumed worldwide as major staple food. To serve rapidly growing human population, we need to boost up wheat production from the available inadequate resources. Conventional breeding approach require several generations for achieving homozygous wheat lines. Double haploid technology has the potential to achieve homozygous wheat lines in a single generation.

Several haploid production systems have been reported in wheat including anther culture [1, 2, 3], microspore culture [4,5,6,7] and intergeneric crosses with maize [8,9,10], sorghum [11], *Hordeum bulbosum* L. [12] and pearl millet [13]. The haploid wheat embryo production was reported for the first time by crossing between wheat and maize [14]. The wheat x maize crossing system may be applied either to detached/cut tillers or intact plants grown in the field. Wheat x maize crossing system is applicable to all wheat genotypes [15]. The anther culture is highly genotype dependant with frequently observed albinos as anther lacks chloroplasts while microspore culture needs complex medium. Anthers provide synergistic conditions to microspores in the culture medium therefore are more responsive. Wheat x maize crossing system is superior to the anther and/or microspore culture in wheat for double haploid production due to relatively straight forward procedures and predictable out comes [16,17]. In wheat x maize system, wheat is being used as female parent and maize as male parent for the production of double haploid. The haploid embryo development occurs due to the preferential chromosome elimination of pollen parent.

The developed embryos may be aborted when left to develop on the spike itself due to the absence of nutrient media [9, 18]. To defeat this problem, the haploid embryos are rescued *in vitro* and cultured on the artificial nutrient media to obtain the haploid plantlets. These haploid plants necessitate doubling of chromosome number to become fertile doubled haploids. The spontaneous chromosome doubling may occur in wheat but at very low frequency [19].

Thus to enhance the efficiency, artificial chromosome doubling can be done with colchicine treatment at the seedling stage. The wheat x maize system can also be employed in production of double haploid plants for other traits like disease resistant [15]. Taking into account the effectiveness of wheat x maize system over anther and/or microspore culture, we have conducted this study to extend the technique of wheat x maize system for the production of wheat haploid lines.

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2. MATERIALS AND METHODS

2.1 Plant Materials

Six wheat genotypes (MW-1, MW-2, MW-3, MW-4, MW-5 and MW-6) and four maize genotypes (MM-1, MM-2, MM-3 and MM-4) were selected from the Mahyco Research Centre, Dawalwadi, Jalna, MH India. Wheat plants were raised in a greenhouse under controlled conditions of temperature (30 °C-35 °C) and maize plants were planted in staggered sowing in the field (35 °C) to synchronize wheat anthesis and sufficient maize pollen availability for crossing.

2.2 Emasculation and Pollination

Healthy wheat spikes were selected for emasculation at the optimum stage of crossing. All the anthers were removed manually with fine forceps without damaging the stigma. After emasculation, the spikes were covered with butter paper bags to avoid contamination and moisture loss. The emasculated wheat florets were pollinated with freshly collected mixed pollens depending on the availability in Petri-dish after 24 hrs of emasculation.

2.3 Plant Hormone (2, 4-D) Treatment

The day after pollination, a solution of 2, 4-D (100 mg/l) was applied to the pollinated florets by a drop method with plastic Pasteur pipette. It was reported that 2, 4-D solution facilitate the embryos to remain alive [15].

2.4 Embryo rescue

After 14-16 days of pollination, the spikes were collected and caryopses were removed from maize pollinated spikes (Figure 1). The caryopses were surface sterilized with 70% ethanol for a minute and washed three times with sterile distilled water followed by 4% sodium hypochlorite for 10-15 min and again washed three times with autoclaved distilled water. The embryos were dissected out from the sterilized caryopses under stereomicroscope inside the laminar air-flow hood.

The excised haploid embryos were cultured on half strength MS based media without phyto-hormones with 30 gm/l sucrose and 0.7% agar [10, 20] (Figure 1). The cultured plates were kept at 25±2 °C temperature and 16 hrs photoperiod until the development of 3-5 cm plantlet. The plantlets were sub-cultured on same media in test tubes for further regeneration (Data not shown). After 14 days, the plantlets attained the size around 10 cm; hardening was followed for further plantlet development.

3. RESULTS AND DISCUSSION

At present, a numbers of techniques have been employed for the production of double haploid plants in cereals, particularly in wheat, such as anther/microspore culture, ovary culture, and chromosome elimination *via* wide hybridization. In this study, six wheat genotypes and four maize genotypes have been used to optimize the wheat x maize embryo rescue protocol for the development of wheat haploid lines. In total, 296 spikes were emasculated among all the six wheat genotypes (Table 1).

Table. 1: Details of wheat x maize wide hybridization technique used to develop haploid lines in wheat.

Wheat Genotype	No. of Spikes used for Pollination	No. of Caryopsis obtained	No. of Haploid embryo rescued
MW-1	51	150	-
MW-2	54	200	07 (3.5%)
MW-3	58	200	20 (10.0%)
MW-4	4	25	-
MW-5	58	-	-
MW-6	71	-	-
Total	**296**	**575**	**27 (4.69%)**
Average	**49.33**	**143.75**	**13.5**
SEM	**9.49**	**40.00**	**3.30**

'-' indicates no development was observed in corresponding genotype.
( ) indicates percent embryo rescued

Each of the wheat genotype was crossed with maize pollens the day after emasculation. The 24 hrs of period in between emasculation and pollination showed consistent embryo production as compared to 48 and 72 hrs intervals [9]. The time interval of 24 hrs was preferred in between the emasculation and pollination for better results. Further, haploid embryo formation was found notable around 25 °C and lower down significantly with increase in temperature [9]. After 24 hrs of pollination, 2, 4-D solution (100 mg/l) was applied to the pollinated floret for development of haploid embryos. Spikes were collected and caryopses removed after 14-16 days of pollination and dissected out to rescue developing embryos (Figure 1).

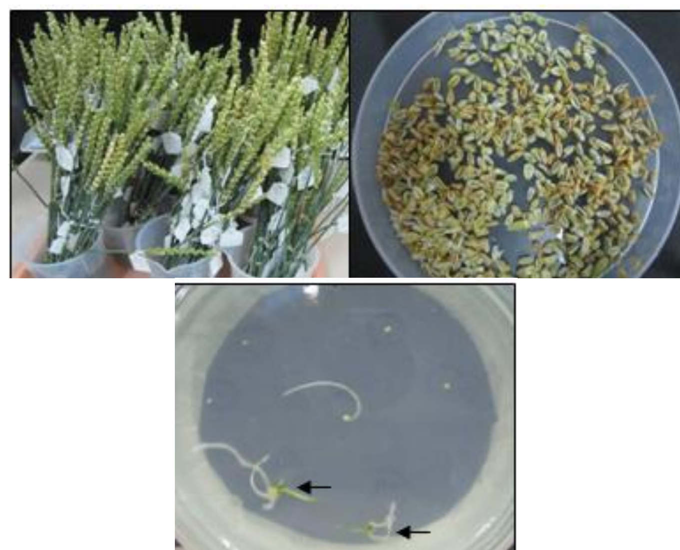

Fig. 1: Steps involved in wheat x maize system to develop haploid lines in wheat a) Spikes collected from the field b) Caryopses removed from the collected spikes c) Cultured embryos on half strength MS media (without growth regulator), arrow indicates embryos showing shoot and root development.

Failure of development of caryopses was also observed in case of many spikes of the studied genotypes. Out of six genotypes used, the caryopses development was observed in four genotypes *i.e.* MW-1, MW-2, MW-3 ans MW-4. While dissecting

the caryopses, it was observed that only two genotypes, MW-2 and MW-3 responded to the development of embryos due to prevailing high temperature i.e. 30 °C in greenhouse and 35 °C in the field

In total, 27 embryos (4.69% of Caryopses) were rescued from the 575 caryopses (Table 1) which is found lower as compared to the 211 embryos rescued (26% of caryopses) [15]. This may be due to the high temperature (~35 °C). The rescued embryos were cultured on plates containing half strength MS medium without growth hormones (Figure 1). The number of embryos rescued from the genotypes ranged in between 07-20, average being 13.5 embryos per genotype. The highest number of embryos rescued were observed in genotype MW-3 (20 embryos) while the lowest was in genotype MW-2 (7 embryos) as mentioned in Table1. The cultured plates of rescued embryos were kept at 25±2 °C temperature with 16 hrs photoperiod until the formation of 3-5 cm wheat plantlet.

In this study, the percent embryo rescued was found to be varied from 3.5% to 10.0%. These results of percent embryo rescued matches with that obtained by detached tiller method in the range of 2.22 to 9.52 [9]. The higher embryo formation frequency in the range of 12-56% was observed as compared to embryo formation frequency in the present study [20]. Freshly collected pollens from maize genotypes were used for crossing of six wheat genotypes. The cultured embryos grew well and formed normal shoots and roots. The plantlets were sub-cultured on same media in test tubes for further regeneration. The regeneration of plantlets was observed only in single wheat genotype i.e. MW-3. Out of 20 rescued embryos of MW-3, only two showed response to the regeneration in to plantlets with the frequency of 10%. The plants regenerated from embryos without endosperm were haploids only. The standard protocol of wheat x maize system was performed with field grown wheat genotypes under natural environmental conditions but the efficiency of embryo formation and plantlet regeneration was identified very low in field condition due to variation in field temperature (up to 40 °C), therefore not discussed here. The ideal temperature for wheat DH should be around 21 °C. We have also calculated the SEM (Standard Error of the Mean) for number of spikes pollinated, caryopses obtained and haploid embryo rescued which was found as 9.49, 40.00 and 3.30 respectively (Table 1) and graphically represented in figure 2.

Non standard condition discussed in the present article involves field and greenhouse. However, embryo rescue from field grown genotypes were very poor and therefore not included here. For the sake of brevity, DH techniques are rarely successful in field conditions where the prevailing temperature is very high (35 °C-40 °C). Therefore, it is our endeavor to point out that such efforts hardly yields any fruitful result and should be discouraged.

Gamborg's B5 medium without growth regulators showed the percentage of embryo formation in the range of 1-8.4% [21]. As compared to these results, higher percentage of embryo formation (3.5-10.0%) was observed in our study on half strength MS media without growth regulators. Wheat x maize hybridization system was successfully applied to regenerate the plantlets from rescued embryos in wheat genotypes, which confirms the potential of using this system for the development of haploid lines in wheat. Remarkable embryo formation was observed on half strength MS media without growth hormones. However, the frequency of embryo formation can further be increased by focusing towards the most important of all environmental conditions i.e. temperature (18-21°C). This technology may be used to reduce the time for release of new wheat varieties. In summary, wheat x maize system of double haploid development could be the method of choice for wheat breeding programs.

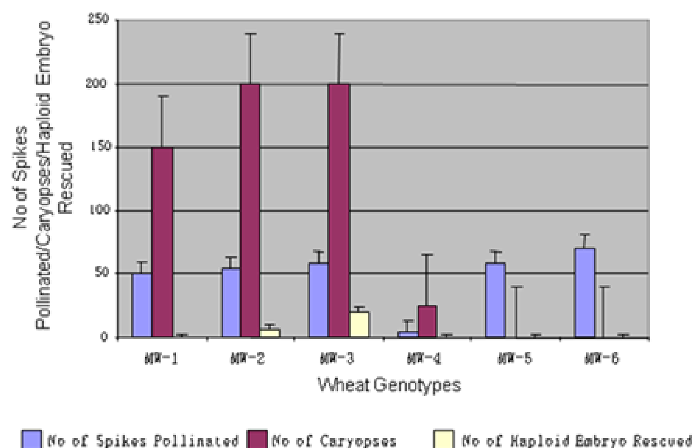

Fig. 2: Graphical representation of number of spikes pollinated, caryopsis and haploid embryo rescued in the present study (X-axis: Wheat genotypes, Y-axis: No. of spikes pollinated/caryopsis/haploid embryo rescued)

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