Effect of Wheat Parent and Embryo Age on Haploid Formation in Wheat x Maize Crosses

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Introduction

The aim of wheat breeding is to improve the quality, sustainability and productivity of wheat. The improvement of wheat can be achieved through wide hybridization. Wide hybridization is considered to be a useful tool in plant breeding for creating new species, gene transfer or induction of haploids. Techniques to produce haploids that have been used in wheat include anther culture, microspore culture and intergeneric crosses (Laurie and Bennett, 1986; Mujeeb-Kazi *et al.,* 2006). Doubled haploid production by means of hybridization between hexaploid wheat and maize is potentially of great value to wheat breeding programme because it may reduce the time required to achieve homozygosity in breeding lines. The relative insensitivity of maize to the action of Kr_1 and Kr_2 alleles of wheat suggests that wheat × maize crosses could be a valuable alternative to other techniques for the production of wheat haploids (Daniel *et al.,* 2005; Guzy-Wrobelska *et al*., 2007). In wheat x maize crosses, the embryo soon aborts; however, exogenous treatment with synthetic auxin 2, 4-D promotes seed and embryo development until the embryo can be excised and plated onto a synthetic medium for continued growth and plant regeneration.

Haploid production efficiency is also influenced by genotype of the wheat parent and the proportion of haploid embryos which germinate and develop into plantlets. One factor which is likely to influence the germination success of haploid embryos is the time of embryo rescue. In most recent reports, haploid embryos have been rescued from 2 to 3 weeks after pollination. Therefore, the present investigation was undertaken to study the effect of wheat genotypes on embryo formation, embryo germination and haploid plantlet production as well as to investigate the effect of age of embryo rescue on embryo germination.

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Materials and Methods

The experimental material consisted of four wheat F_1s UP2113 × UP2338 (W1), PBW396 × UP2338 (W2), PBW396 × UP2425 (W3) and UP2113 × UP2425 (W4). For making inter-generic crosses between wheat and maize, maize variety 'Surya' with good pollen shedding ability was chosen. The following crosses were made: W1 × Surya; W2 × Surya; W3 × Surya; W4 × Surya. Emasculation of wheat spikes was done by cut glume method; while spikes were still half inside the flag leaf sheath. The emasculated spikes were covered with cellophane bags and tagged. Two days later, when the stigma was feathery and receptive, the pistils were hand-pollinated by dusting with pollen from the dehiscing anthers of the male parents and spikes were covered with glassine paper bag to avoid any pollination by undesired foreign pollen. After one day of pollination, the spikes were sprayed with 100 ppm 2, 4-D solution, one shot along each side. This was done for three consecutive days and the spikes were left as such for about 15-18 days. For embryo culture, after 15-18 days of pollination spikes were collected and seeds (or enlarged ovaries) were taken out with the help of the forcep. Number of seeds were counted and recorded for each of the wheat genotype. A total of 122 embryos were excised from the enlarged ovaries with the help of sterilized scalpel and cultured on the surface of the modified MS media (MS + 0.5 mg/l BAP). After this, culture tubes were closed and transferred to a refrigerator at 4°C in the dark after recording the number of embryos cultured. After 5-6 days, cultures were transferred to incubator at 25°C temperature and 16/8 light and darkness cycle. The germination and growth of the embryos were then observed. Confirmation of haploidy was done by counting the chromosomes of the root tip at metaphase stage.

Experiment 1*: Influence of wheat genotype on embryo formation, embryo germination and haploid production in wheat × maize crosses.*

To reveal the effect of wheat genotype on embryo formation, embryo germination and haploid production, all the four hybrids (W_1-W_4) were compared. The pollinated ears were collected after 15-18 days of pollination and the number of pollinated florets, the number of seed like structures formed, embryos obtained and embryos germinated were recorded using the following formulae:

$$\text{Seed set (\%)} = \frac{\text{Number o f seeds obtained}}{\text{Number o f florets pollinated}} \times 100$$

$$\text{Embryo formation (\%)} = \frac{\text{Number o f embryo containing seeds}}{\text{Number o f seeds obtained}} \times 100$$

$$\text{Embryo germination (\%)} = \frac{\text{Number o f embryos germinated}}{\text{Number o f embryos obtained}} \times 100$$

$$\text{Haploid formation (\%)} = \frac{\text{Number o f haploids obtained}}{\text{Number o f embryos obtained}} \times 100$$

Experiment 2*: Influence of age of embryo on haploid plantlet production in wheat × maize crosses*

In order to investigate the effect of age of embryo to be rescued on embryo germination, the immature embryos from young wheat spikeletes were collected after 11-20 days of pollination and cultured on modified MS (Murashige and Skoog, 1962) media (MS+ 0.5 mg/l BAP). The cultures were maintained at 4°C in the dark. After 5-6 days, cultures were transferred to 25°C and 16/8 light/dark cycle.

Results and Discussion

Experiment 1: The mean comparison for per cent embryo formation among the four F_1s showed that, W_3 showed highest per cent of embryo formation (9.62%). Mean values for the trait have been shown using Table 1. The grand average of per cent embryo formation over all the four wheat F_1 and replications was 6.83 per cent. Analysis of variance showed a highly significant F value for the differences among wheat hybrids suggesting that the response of these four hybrids for per cent embryo formation in crossing with maize was different (Table 2). Several other workers have also reported the genotypic effect of hexaploid wheat parent on percentage of embryo formation. (Suenaga *et al.,* 1989; Ahmed *et al.,* 2005; Mujeeb-Kazi *et al.,* 2006).

Analysis of variance showed no significant difference among the four hybrids for per cent embryo germination and per cent haploid formation, which means that the genotypes of wheat did not affect germination of haploid embryos and haploid formation (Table 2). Since, the F value for this trait was not significant, there was no necessity of mean comparison but for the demonstration of individual performance of the genotype, mean have been presented in Table 3. In case of euploid or normal wheat, once a seed is formed properly, the germination of that seed is not affected by the genotype of maternal parent. In case of haploid embryos, it can be expected that a complete or bipolar embryo on the nutritional media should be able to germinate and produce a plantlet, but if not, it can be due to its developmental stage and other conditions (media composition, seasonal variations etc.) rather than the genotype of wheat parent. Campbell *et al.* (2000) reported the effect of media composition, seasonal variations, etc. on recovery of haploid plants. Daniel *et al.* (2005) reported the effect of wheat varieties and colchicines treatment on the regeneration of wheat embryos to plants.

Experiment 2: Influence of embryo age on haploid plantlet production in wheat × maize crosses

The stage of development of the hybrid embryos at the time of culture was found to be very important as their growth was strongly influenced by their age. Once the abortion process began (in the absence of endosperm), they were difficult to grow. The best age of the embryo was found to be 15-17 days after pollination in which plantlets were healthy and showing growth. The younger embryos i.e. 12-14 days had a tendency to stop growing after an initial increase in size and they were difficult to

Table 1. Influence of wheat F_1s for seed set and embryo formation

Wheat genotype (%)	No. of florets pollinated	No. of seed set (%)	No. of embryo formed(%)
W_1	492	430 (87.39)	31 (7.20)
W_2	478	402 (84.10)	14 (3.48)
W_3	536	478 (89.17)	46 (9.62)
W_4	518	476 (91.89)	31 (6.51)
Total	2024	1786 (88.24)	122 (6.83)

Table 2. Analysis of variance for embryo formation, embryo germination and haploid formation

Source of Variation	Degree of Freedom	Embryo Formation (%)*		Embryo Germination (%)#		Haploid Formation (%)##	
		SS	F value	SS	F value	SS	F value
Genotype	3	3.78	8.45**	157.99	0.910 ns	45.07	0.11 ns
Error	16	2.39		925.11		2108.26	
Total	19	6.18		1083.11		2153.34	

* CV= 14.73 † CV=16.97 †† CV=43.72

Table 3. Effect of wheat genotypes on percent embryo germination and per cent haploid formation

Wheat Genotype	Embryos Obtained and Cultured	No. of Embryo Germinated (Per cent)	No. of Haploid Formed (Per cent)
W_1	31	16 (51.61)	6 (19.35)
W_2	14	5 (35.71)	1 (7.14)
W_3	46	26 (56.52)	10 (21.73)
W_4	31	15 (48.33)	6 (19.35)
Total	122 (6.83)	62 (50.81)	23 (18.85)

isolate and culture. The older embryos 18-20 days started shriveling and showed signs of degeneration as the process of abortion had already set in by that age. Such embryos either aborted on the spikelet itself or often dried in the culture medium.

The two basic stages of embryo growth exist with regard to nutritional independence. The heterotrophic stage of growth is the period during which the embryo depends on the endosperm for its nutrition; it extends from the fertilization to approximately the heart stage. The autotrophic phase starts from the late heart stage. This stage is significant for the *in vitro* culture as the embryo becomes sufficiently independent of the endosperm for the subsequent growth. As the embryo matures, its growth requirements for the artificial culture become less complex. Older, healthy embryos are, therefore, easier to culture than the younger ones. The culture of young embryos is also more difficult due to their size, damage during desiccation and sensitivity to osmotic shock.

Suenaga and Nakajima (1989) found that larger embryos tended to show better germination. Embryo size not only depends on their age but also on the fitness of the donor plant, amount of auxin applied and other factors. Chen *et al.* (1996) cultured 10-14 days old embryos and found that most of the embryos larger than 400 µm germinated and produced normal plantlets because the smaller ones failed to germinate. Kammholtz *et al.* (1996) reported that embryos rescued 12-15 days after pollination were large and easily rescued and also had a significantly higher germination (51-80%) than embryos rescued 9, 17, 19 or 21 days after pollination (6.4-31%). Wedzony and Lammeren (1996) observed that when excised at 14 days after pollination, haploid embryos were rescued by *in vitro* culture from 14% of the pollinated florets. Campbell *et al.* (2000) reported that 16-day old embryos resulted in improved frequency of haploid plant formation in wheat x maize system. Ahmad *et al.* (2005) reported low embryo viability in tetraploid wheat compared to hexaploid wheat because of the size of embryos.

Thus, from the present investigation and the reports given by various workers, it can be inferred that the right age for transferring the hybrid embryos to the culture medium varies from genotype to genotype and needs to be standardized in each case separately. In the present case, the optimum time of rescue was when the embryos were 15-17 days old.

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