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Variation in the Induction of Haploidy and Polyembryony in Spring Wheat through Wheat × Maize System

Farhana Sharmeen, L. Rahman, M. S. Alam and A. K. M. Shamsuddin

Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh

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Abstract

Five varieties of wheat such as Rawal, Chyria 3, Shatabdi, Sonalika and Kanchan were crossed with a maize variety Popcorn. Fertilization of wheat egg and formation and development of haploid embryos were facilitated by two post pollination treatments which include application of (i) 100 ppm 2,4-D and (ii) 100 ppm 2,4-D + 120 ppm $AgNO_3$. The haploid embryos were cultured on MS and finally regenerated haploid plants. Significant variations were observed among the wheat varieties for the development of caryopsis, formation of embryos and regeneration of haploid plants. The varieties Chyria 3 and Sonalika proved better for setting of caryopsis, production of embryos and regeneration of haploids. They yielded 8.04 and 5.03 per cent haploid plants over the pollinated florets, respectively. There were no significant variations by the application of 2,4-D and 2,4-D + $AgNO_3$ for the formation of haploid embryos and regeneration of plants from them. Some caryopsis contained more than one embryos indicating the occurrence of polyembryo in wheat when crossed with maize. The highest number of polyembryo was obtained in the variety Chyria 3 followed by Shatabdi and Sonalika.

Introduction

Haploid plants are very useful in developing dihaploid (DH) lines which could be utilized for varietal improvement specially in self pollinated crops within a short time. In addition, they can be used in producing inbred lines for hybridization and not only that they can be utilized in cytogenetic and genetic researches.

Haploids in wheat have been produced through anther and microspore culture and wide crossing. Haploids generated through anther culture occassionally face problems of being aneuploids and albino plants (Wehr and

Zeller 1990). Moreover, responses of production of haploids have higher genotypic specificity (Andersen et al. 1988; Kisana et al. 1993; Cattaneo et al. 2001). Alternatively production of haploid in wheat through its crossing with *Hordeum bulbosum*, maize, sorghum and teosinte was reported successful without the development of albino plants (Laurie and Bennett 1988; Lizarazu and Kazi 1993). In the wide crossings wheat is used as female parent, and others are used as male parents. During the development of embryos in crosses only wheat chromosomes are retained and the chromosomes of the male parents are eliminated in early stage of zygotic cell division (Laurie and Bennett 1988). Haploid plants are produced through rescue and culture of the embryos. Based on the above information, the present study was undertaken for assessing the efficiency of production of haploids in five varieties of spring wheat which are adapted and cultivated in Bangladesh.

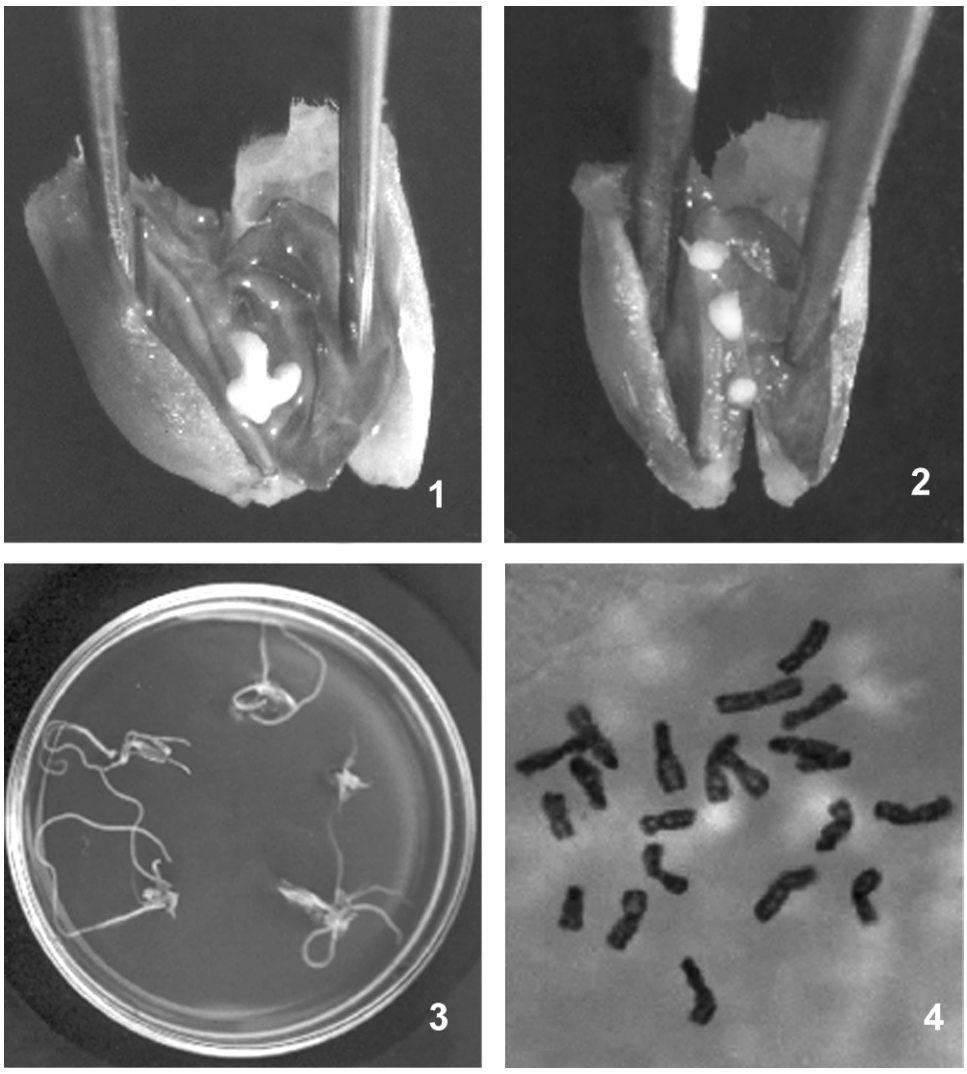
Materials and Methods

The experiment was conducted in the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh. Five varieties of spring wheat, namely Rawal, Chyria 3, Shatabdi, Sonalika and Kanchan and one variety of maize, Popcorn were grown in pots in winter of 2002-2003. They were sown in staggered to synchronize the flowering between wheat and maize varieties. Before the booting stage pots were transferred to a greenhouse where the temperature was $25/18^0$C day/night with 16h photo-period.

The wheat spikes which just emerged out of the sheath of the flag leaf were selected for crossing. The two lateral large florets of spikelets expected to reach anthesis after two days were emasculated manually and the unemasculated florets were removed. The florets were pollinated with maize pollens collected from freshly dehisced anthers. Pollination was done consecutively two days for better setting of caryopsis. Pollination was accompanied by two post pollination treatments: T_1 (100 ppm 2,4-D) and T_2 (100 ppm 2,4-D + 120 ppm $AgNO_3$). After pollination 0.5 ml of 2,4-D was injected into the uppermost internode of the pollinated spike and a drop of the same was poured as the open pollinated florets. Aqueous $AgNO_3$ was sprayed to the florets following pollination and it was continued till harvesting of the spike. The post pollination treatments caused swelling of ovary to caryopsis and retention and development of embryos.

Caryopsis at the age of 16 to 18 days were harvested and surface sterilized. The caryopsis were first sterilized with 70% ethanol for two minutes followed by treatment with chlorox (4.7% chlorine) supplemented with a few drops of Tween 20 for 15 to 20 minutes. Then the caryopsis were thoroughly washed with distilled water for 5 minutes. Embryos were excised from the caryopsis under the focal view of a stereomicroscope. The embryos observed floating in the fluid

without any endosperm were considered as haploids (Figs. 1 and 2). The excised embryos were placed in MS medium free of any hormone contained in the culture vials. The rescue and plating of embryos were done in sterile condition under the laminar airflow hood.



Figs. 1-4: 1. Single embryo in haploid caryopsis. 2. Polyembryo in haploid caryopsis. 3. Regeneration of plantlets from haploid embryos. 4. Chromosomes of haploid plants.

Vials were kept in the dark room at 25^0C for three days for germination of embryos. After germination, when the coleoptiles were about 1 - 2 cm long (Fig. 3), they were transferred in the culture room at 25^0C and 16h photoperiod provided by using fluorescent lamps. At the age of 15 to 20 days the regenerated plants were transferred in cubes containing sterile soil with sufficient organic

matter. Then for hardening the cubes were covered by transparent polyethelene bags and kept in growth chamber where temperature was maintained at 16 - 18^0C with 4500 lux light intensity. After eight - nine days bags were removed and plants were transferred in plastic pot containing normal soil and kept in greenhouse. When the plants attained 10 - 12 cm in height they were transferred in larger earthen pots with normal soil in the greenhouse at 25^0C/18^0C day/ night with 16h/8h photoperiod.

Data on a number of developed caryopsis, number of embryos, number of germinated embryos and number of regenerated haploid plants were taken in per cent. Many of the caryopsis contained more than one embryos indicating occurrence of polyembryo. Therefore, number of embryos were partitioned into single and polyembryo (Fig. 2). The regenerated plants were confirmed to be haploids by counting their somatic chromosome number (Fig. 4) and also by checking their pollen sterility. These data were transformed by arcsin transformation and analysis of variance for different parameters were performed following randomized complete block design. Differences between the treatment means were compared by the LSD test.

Results and Discussion

Analysis of variance for different parameters explaining the efficiency of production of haploids in spring wheat through wheat and maize cross is presented in Table 1. Significant variations were observed among the varieties for development of caryopsis from pollinated florets, production of total embryos from the caryopsis, proportion of single embryo to the total embryos regenerated over total embryos, regeneration of the plantlets over total embryos and pollinated florets. The result suggests that genotypic variations were present among the varieties Rawal, Chyria 3, Shatabdi, Sonalika and Kanchan for the parameters related to production of haploid in spring wheat.

Performance of the variety for the efficiency of haploid production is presented in Table 2. Chyira 3 appeared to be most promising one for development of caryopsis, formation of total and single embryos, germination of embryos and regeneration of haploid plants. Sonalika was also found better for production of single and total embryos, germination of embryos and regeneration of plants. A good number of embryos were also produced from Rawal, Shatabdi and Kanchan but their ability of regeneration of plants were comparatively lower than the other two varieties. Comparing the performance it was observed that Chyria 3 and Sonalika perfomed better for production of haploids through crossing with maize. Chyira 3 and Sonalika produced 8.04 and 5.03 per cent haploid plants, respectively when regeneration was assessed over

pollinated florets. Verma et al. (1999) obtained 9.9% and O' Donoughue and Bennett (1994 b) obtained 3.3% haploids seedlings over pollinated florets.

Effects of application of 2,4-D and 2,4-D + $AgNO_3$ as post pollination treatments to wheat spikes on the production of haploids are shown in Table 3. There were no significant differences noticed between the application of 2,4-D alone and 2,4-D + $AgNO_3$. Slight and inconsistent differences were observed between the two post pollination treatments on development of caryopsis and formation and germination of embryos (Table 3). Interaction of varieties with post pollination treatments showed Chyria 3 at both treatments and Sonalika at application of only 2,4-D produced better caryopsis than others.

Table 1. Analysis of variance for haploid production efficiency between wheat and maize cross.

Source of variance	df	Caryopsis developed from pollinated florets	Embryos obtained from caryopsis	Single embryos over total embryos	Germination of embryos over total embryos	Regeneration of plants over embryos obtained	Regeneration of plants over pollinated florets
Replication	2	97.435	2.813	113.143	81.130	23.150	4.858
Variety	4	5.870	28.697	411.459**	649.154**	374.731**	95.478**
Treatment	1	175.829*	3.859	161.843	2.506	3.724	0.363
Variety × treatment	4	70.082	7.923	27.039	159.735	128.457	13.372
Error	18		21.817	116.672	87.943	83.151	13.553

*, ** Significant at 5 and 1% probability, respectively.

Table 2. Variation among wheat varieties for efficiency of haploid plants development from wheat and maize cross.

Variety	Caryopsis developed over pollinated florets (%)	Embryos obtained from caryopsis (%)	Single embryos over total embryos (%)	Germination of embryos over total embryos (%)	Regeneration of plants over total embryos (%)	Regeneration of plants over pollinated florets (%)
Rawal	68.75b	23.99	93.06a	24.27b	17.91bc	2.25c
Chyria 3	86.86a	26.03	72.38b	54.60a	35.55a	8.04a
Shatabdi	60.07b	21.45	89.69a	20.00b	14.54c	1.87c
Sonalika	63.74b	17.33	91.46a	45.12a	34.75a	5.03ab
Khanchan	54.92b	20.51	92.05a	43.04a	24.50ab	2.76bc
Average	68.87	21.86	87.73	37.41	25.45	3.99

Values having same letter(s) are statistically identical.

2,4-D was applied to the wheat spike when crossed with maize for development of caryopsis, retention and development of the embryos (Kisana et al. 1993). The dosage of 2,4-D at the concentration of 100 ppm was suggested

effective for these purposes by many authors in both durum and bread wheat (Sun et al. 1992; Kisana et al. 1993; Matzk and Mahn 1994). Almouslem et al. (1998) obtained good result for production of haploids in duram wheat by applying 120 ppm $AgNO_3$ and 180 ppm 2,4-D. Application of $AgNO_3$ was reported to reduce the emission of ethylene, which consequently promoted increased number of embryo survival. In the present study insignificant difference between the application of only 2,4-D and 2,4-D + $AgNO_3$ suggests that emission of ethylene might not be a problem in spring wheat.

Table 3. Effects of post pollination treatments and their interactions for different parameters explaining efficiency of haploid plants regeneration

Variety	Treatment	Caryopsis developed from pollinated floret (%)	Embryos developed from caryopsis (%)	Germination of embryos over total embryos (%)	Plants regenerated from embryos (%)	Plants regenerated over pollinated florets (%)
Rawal	T_1	56.58 cd	16.62	26.15	23.01	2.17
	T_2	81.02 ab	19.45	23.14	14.81	2.33
Chyria 3	T_1	84.69 ab	23.47	57.55	33.81	6.72
	T_2	89.04 a	28.47	52.27	36.93	9.36
Shatabdi	T_1	67.20b cd	21.42	14.44	8.88	1.28
	T_2	53.28 cd	21.48	26.66	21.33	2.44
Sonalika	T_1	75.36 abc	22.35	37.63	30.10	5.07
	T_2	52.67 cd	23.27	54.92	40.48	5.00
Kanchan	T_1	59.49 cd	21.37	54.32	32.09	4.08
	T_2	50.78 d	19.60	30.00	15.71	1.56

The values having same letters are statistically identical.

During the rescue of embryos from the wheat caryopsis sometimes more than one embryos were found indicating the induction of polyembryony when crossed wheat with maize. There was significant variation for the production of polyembryo among the wheat varieties. This suggests that there were genotypic differences for production of polyembryo in wheat and maize cross.

The variety Chyria 3 produced 27.61% polyembryos over total embryos and it was the highest among the varieties (Table 4). Linacero et al. (1996) noted that 20.19% polyembryos were produced by the variety Thatcher when crossed with maize.

In the present study the varieties Chyria 3, Sonalika, Shatabdi and Kanchan showed better performance for formation of polyembryo when they were treated with both 2,4-D and $AgNO_3$. Among them, the varieties Chyria 3 and Sonalika performed better for formation of embryo and plant regeneration. These two

varieties also showed good performance for production of polyembryo. Production of higher

number of polyembryo added to their single embryos gave increased number of total embryos (Tables 2 and 4). The higher number of total embryos in varieties Chyria 3 and Sonalika consequently produced higher number of haploid plants. Therefore, it is suggested that the varieties Chyria 3 and Sonalika were potential for haploid production and they might be used as parents in haploid breeding programme.

Table 4. Effects of 2,4-D and $AgNO_3$ on formation of polyembryo in wheat x maize crosses.

Variety	Treatment	Total embryos	Polyembryos	Polyembryo over total embryos (%)	Varietal mean over treatments
Rawal	T_1	65	2	3.07	6.93b
	T_2	108	10	9.25	
Chyria	T_1	139	37	26.61	27.61a
	T_2	176	50	28.40	
Shatabdi	T_1	90	8	8.88	10.30b
	T_2	75	9	12.00	
Sonalika	T_1	93	6	6.45	8.53b
	T_2	71	8	11.26	
Kanchan	T_1	81	6	7.40	7.94b
	T_2	70	6	8.57	

The values having same letters are statistically identical.

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References

Almouslem AB, Jauhar PP, Peterson TS, Bommineni VR and **Rao MB** (1998) Haploid durum wheat production via hybridization with maize. Crop Sci. **38** : 1080 - 1087.

Andersen SB, Due IK and **Olesen A** (1988) Results with anther culture in some important Scandinavian varieties of winter wheat. Acta. Agril. Scand. **38** : 288 - 292.

Cattaneo M, Vaccino P and **Bohanec B** (2001) Dihaploid production for wheat breeding programme: The Italian experience. Biotechnological Approaches for utilization of gametic cells. Cost 824: Final Meeting. Bled. Slovonia 1 - 5 July, 2001. pp. 53-59.

Kisana NS, Nkongolo KK, Quick JS and **Johnson DL** (1993) Production of doubled haploids by anther culture and wheat × maize method in a wheat Breeding programme. Plant Breed. **110**: 96 - 102.

Laurie DA and **Bennett MD** (1988) Chromosome behaviour in wheat × maize, wheat × sorghum and barley × maize crosses. *In:* P.E. Brandham (Ed), Kew chromosome Conf. III. 167 - 177. HMSO, London.

Linacero R, Marisa G, Bilbao L, Romero C, Laurie DA and **Vazquez AM** (1996) Genotypic differences in polyembryo formation and somatic embryogenesis increment in wheat (*Triticum aestivum*), following 2,4-D treatment. Euphytica. **89** : 345 - 348.

Lizarazu OR and **Kazi AM** (1993) Polyhaploid production in the Triticeae: wheat x *Tripsacum* crosses. Crop Sci. **33** : 973 - 976.

Matzk F and **Mahn A** (1994) Improved techniques for haploid production in wheat using chromosome elimination. Plant Breed. **113** : 125 - 129.

O'Donoughue LS and **Bennett MD** (1994b) Durum wheat haploid production using maize wide crosses. Theor. Appl. Genet. **89**(5) : 559 - 566.

Sun JS, Lu H, Lu TG, Wang KA, Ren Z, Wang JL, Fang R and **Yang C** (1992) The production of haploid wheat plants via wheat × maize hybridization. Acta. Botanica. Sinica. **34** : 817 - 821.

Verma V, Bains NS, Mangat GS, Nanda GS, Gosal SS, Singh K, Verma V and **Singh K** (1999) Maize genotypes show striking differences for induction and regeneration of haploid wheat embryos in the wheat × maize system. Crop Sci. **39** : 1722 - 1727.

Wehr BF and **Zeller FJ** (1990) *In vitro* microspore reaction of different German wheat cultivars. Theor. Appl. Genet. **79** : 77 - 80.