Society for Advancement of Wheat and Barley Research
ICAR-Indian Institute of Wheat & Barley Research
Karnal - 132 001, India

Journal of Wheat Research
8(2): 43-48
Homepage: http://epubs.icar.org.in/ejournal/index.php/JWR

Research Article

Induction of haploids in wheat using Wheat x Maize system of chromosome elimination

Vikas Gupta*, Satish Kumar, Garima Singroha, Chandra Nath Mishra, Raj Kumar and Vinod Tiwari

ICAR- Indian Institute of Wheat and Barley Research, Karnal-132001, Haryana

Article history

Received: 21-11-2016
Revised : 12-12-2016
Accepted: 23-12-2016

Citation

Gupta V, S Kumar, G Singroha, CN Mishra, R Kumar and V Tiwari. 2016. Induction of haploids in wheat using Wheat x Maize System of chromosome elimination. *Journal of Wheat Research* **8**(2): 43-48.

Corresponding author
Email: *vikasdwr@gmail.com*

Abstract

The present study was undertaken with the objective to standardize the wheat x maize protocol for haploid production using different crosses (F_1s) and commercial wheat varieties. In case of varieties, caryopsis formation frequency ranged between 25.9 to 51.4 %, embryo formation frequency ranged between 4.6 to 22.4 % and plant regeneration frequency ranged between 6.4 to 63.6 %. The caryopsis formation frequency among F1's ranged between 21.4 to 60.5%, embryo formation frequency ranged between 1.3 to 21.2 % and plant regeneration frequency ranged between 9.8 to 44.4 %. A total of 100 haploid plantlets were regenerated and transferred to pots containing pot mixture for hardening and acclimatization. The surviving plants were given colchicine treatment of 0.1 % for chromosome doubling. For efficient utilization of wheat x maize protocol factors like embryo formation, induction of haploid embryo germination by cold treatment and chromosome doubling have been identified for further studies.

Key Words: Haploid, wheat x maize protocol, 2, 4 - D, embryo rescue

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the major staple cereals of the world and is grown worldwide as a source for energy, protein and fibre in human diet. It is the second most important crop after rice in India and grown on approximately 30 mha area. In India, the annual wheat production was 93.50 million tonnes during 2015-16 (Anonymous, 2016). Wheat production and productivity need to be enhanced to meet the growing demand of ever increasing population. Development of varieties with high yield potential and resistance to abiotic and biotic stresses with acceptable quality parameters is the most viable and eco-friendly approach to increase wheat yield in a sustainable manner. Most of the breeding programmes involve introduction of variation followed by selfing and selections in segregating generations, multilocational yield evaluation to identify high yielding and well adapted genotypes (Niroula and Bimb, 2009). Breeders make use of different breeding methods to fix and develop homozygous genotypes from such genetic variations. Isolation of homozygous and homogeneous genotypes through conventional inbreeding methods (single seed descent, backcrossing and selfing in the field multiple generations through use of off-season nurseries also called as shuttle breeding) requires several cycles of inbreeding and selection, making it the tedious, time consuming and expensive. Doubled haploid production can play an important role in wheat breeding programmes by reducing the breeding cycle. It also increases the selection efficiency by easier identification of the superior lines (Garcia-Llamas *et al.*, 2004). Doubled haploid provides an accelerated way of combining and fixing the desirable features of diverse wheat genotype into common genetic background (Mehta and Angra, 2000). Induction of double haploids *via* anther culture (Lashermes,1992; Saeed *et al.,* 1994; Grauda *et al.,* 2010) and chromosome elimination techniques has been demonstrated by several workers in wheat (Amin *et al.,* 2010; Barclay, 1975; Inagaki and Mujeeb-Kazi 1995; Laurie and Reymondie 1991; Suenaga *et al.*, 1997). The

wheat x maize system of doubled haploid system involving chromosome elimination technique is better compared to anther culture with respect to the number of albinos and genotype dependency. Arrival of wheat x maize system was marked by the observation of microscopic, early stage embryos in crosses between hexaploid wheat and maize (Zenkteler and Nitzsche, 1984). The presence of chromosomes of both wheat and maize during early post-pollination events in wheat x maize crosses was observed, but the maize chromosomes were eliminated during the initial cell division (Laurie and Bennett, 1986). Another noteworthy observation in this study was that in such crosses the endosperm was absent and resulted in embryo abortion before the embryo could develop to a rescueable size. However the embryo may be rescued before it aborts and haploid plants regenerated. The wheat x maize system was free from the effects of crossability alleles *Kr1* and *Kr2* (Laurie and Bennett, 1988). The system of *in vitro* culture of pollinated wheat spikelets and the first haploid plant was recovered from wheat and maize crosses (Laurie and Bennett, 1988). Further refinements in the methodology were carried out by several researchers that led to the release of double haploid cultivars in Japan (Yuichi *et al.*, 2004) and Canada (DePauw *et al.*, 2010). The present investigation was carried out with the objective to standardize Wheat x Maize protocol for double haploid production for its integration in wheat breeding programme.

2. Material and methods

Plant material consisted of six released varieties of wheat (DBW 39, Kharchia Local, DPW 621-50, HD 2967, WH 1105, CBW 38) and nine F_1s (PBW 343/DBW 14, PBW 343/HI 1563, PBW 343/HD 2864, PBW 343/K 7903, PBW 343/Raj 3765, PBW 343/NIAW 34, PBW 343/ WH 730, PBW 343/Raj 4037, HD 2329/ Kharchia 65). The set of wheat varieties were used to standardize the protocol for production of haploids using wheat x maize system. Further the standardized protocol was used for development of homozygous lines from the F1's. Maize genotype Pearl Pop Corn was used as pollinator parent. Sowing of wheat was started under controlled conditions about 15 days earlier than maize to synchronise maize and wheat flowering to carry out wheat pollinations. Sowing of wheat was done under controlled conditions in glass house maintained at 16-21°C whereas maize was planted in polyhouse to maintain high temperature for its growth and development. Emasculations were carried out in a way that anthers were removed without cutting the lemma and palea. Emasculated spikes were pollinated after 3-4 days depending upon ovary maturity, with freshly collected maize pollen as described by Hussain *et al.,* 2012. The pollinated spikes were given 2, 4-D treatment (200ppm) after 24, 48, 72 hours of pollination to sustain embryo formation (Moradi *et al.,* 2009). The caryopses were removed from the spikes, 15-18 days after pollinations. The caryopses were surface sterilized with 0.1% mercuric chloride for eight minutes followed by treatment with absolute ethanol for 2 minutes and subsequent 3-4 washings with sterile distilled water. The embryos were dissected from each caryopsis and transferred to test tubes containing half strength MS (Murashige and Skoog) medium supplemented with 40g/l sucrose and solidifying agent phytagel (3g/l) having pH 5.8. The embryos were given cold treatment for 8h and incubated in dark till germination. The regenerated plants were maintained at 25°C and a photoperiod of 8-10h. The haploid plants developed were transferred to hardening medium after 30 days of regeneration. The roots of the haploids seedlings were cut 3/4th from crown and seedlings were given colchicine treatment (colchicine 0.1% + 20ml dimethyl sulfoxide + few drops of Tween 20) for 6 hours in light. The roots were washed under running water and then transplanted in pots. Following data were recorded on the experimental plants:

Caryopsis formation frequency (CFF) =

$$\frac{\text{No. of spikelets pollinated}}{\text{No. of caryopsis formed}} \times 100$$

Embryo formation frequency (EFF) =

$$\frac{\text{No. of embryos formed}}{\text{No. of caryopsis dissected}} \times 100$$

Plantlet regeneration frequency (PRF) =

$$\frac{\text{No. of plantlets regenerated}}{\text{No. of embryos rescued}} \times 100$$

3. Results and discussion

A number of protocols have been employed for induction of haploids in wheat at present such as anther culture, ovary culture, wheat x maize system (Srivastava *et al.,* 2012), wheat x *Imperata cylindrica* (Chaudhary *et al.*, 2005)

etc. In an attempt to standardize the haploid induction protocol using wheat x maize system the present study was carried out using different commercial wheat varieties and crosses (F_1's). A total of 11374 spikelets were pollinated with maize pollen and a total of 4192 caryopsis were formed in all the varieties (Table 1). In case of varieties, caryopsis formation frequency ranged between 25.9 to 51.4 %, embryo formation frequency ranged between 4.6 to 22.4 % and plant regeneration frequency ranged between 6.4 to 63.6 %. However, highest caryopsis formation frequency (CFF) of 51.4% was observed in case of variety CBW 38 followed by WH 1105 (48.2%), HD 2967 (46.37), DPW 621-50 (44.54%, DBW 39 (41.7%) and Kharchia Local (25.9%). A total of 646 embryos were excised from the 4192 caryopsis indicating overall embryo formation frequency (EFF) of 15.4% in the varieties. The variety Kharchia local showed highest embryo formation frequency of 22.4% followed by DBW 39 (18.5%), WH 1105 (15.8%), DPW 621-50 (7.4%), HD 2967 (6.3%) and CBW 38 (4.6%).

Table 1. Summary of the caryopsis formation, embryo formation and plant regeneration in wheat (varieties) x maize crosses

S. No	Variety	Spk. Poll.	CF	CFF (%)	EF	EFF (%)	PR	PRF (%)	DH
1	DBW 39	3500	459	41.7	270	18.5	21	7.7	5
2	Kharchia Local	189	49	25.9	11	22.4	7	63.6	2
3	DPW 621-50	1210	539	44.5	40	7.4	17	42.5	-
4	HD 2967	1725	800	46.4	51	6.3	11	21.6	-
5	WH 1105	3050	1470	48.2	233	15.9	15	6.4	1
6	CBW 38	1700	875	51.4	41	4.6	14	34.1	-
	Total	11,374	4,192		646		85		

Spk. Poll – number of spikelets pollinated, CF- no. of caryopsis formed, CFF- Caryopsis formation frequency, EF- No. of embryos formed, EFF- Embryo formation frequency, PR-No. of plants regenerated, PRF- Plant regeneration frequency

Eighty five haploid seedlings were regenerated from 646 embryos (13.2%). The highest plant regeneration frequency (PRF) of 63.6% was observed in variety Kharchia Local followed by DPW 621-50 (42.5%), CBW 38 (34.1%), HD 2967

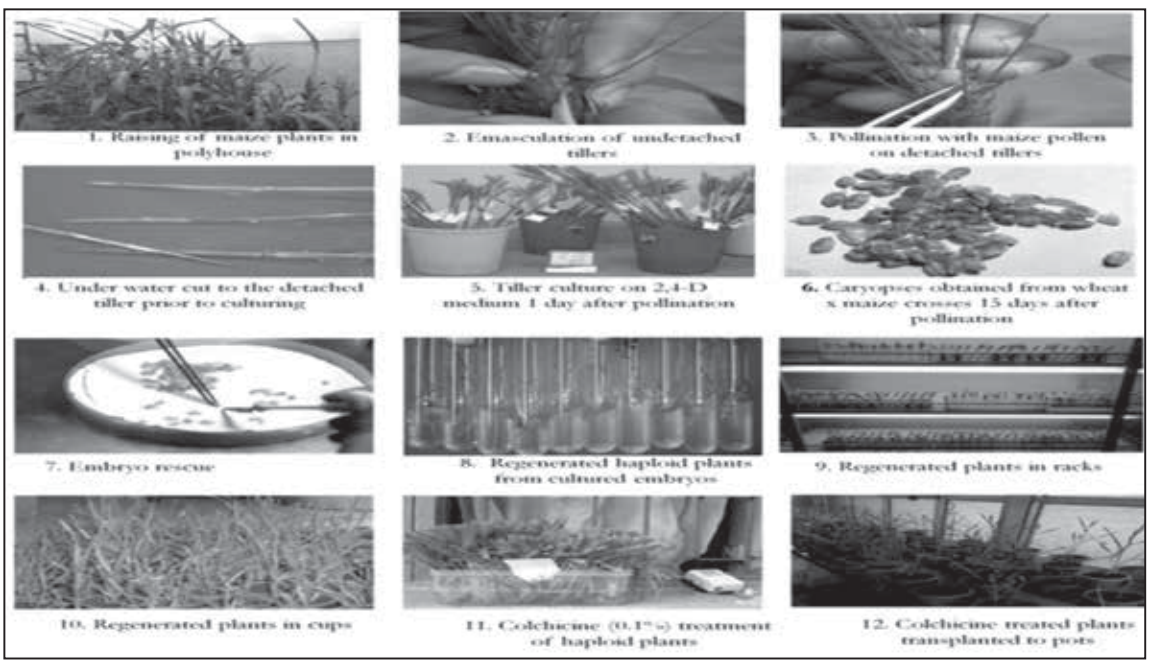

Fig. 1 Schematic representation of different steps involeved in induction of haploids in wheat using wheat x maize system

(21.6%), DBW 39 (7.7%) and WH 1105 (6.4%). All the haploid seedlings were given colchicine treatment (colchicine 0.1% + 20ml dimethyl sulfoxide + few drops of Tween 20) but only few seedlings got chromosome doubling as depicted from seed set in the treated plants. Figure 1 describes the different steps involved in the induction of haploids in wheat using wheat x maize system. Total of nine crosses (Table 2) were also used for standardization of doubled haploid induction protocol. The CFF among crosses ranged between 21.4 to 60.52%, EFF ranged between 1.3 to 21.2% and plant regeneration frequency ranged between 9.8 to 44.4% (Table 2). 934 caryopsis were formed out of 2453 spikelets pollinated (38.1%). The cross PBW 343/HI 1563, resulted in the highest CFF of 60.5% followed by the crosses PBW 343/DBW 14 (53.7%), PBW 343/NIAW 34 (48.1%), PBW 343/Raj 4037 (37.9%), PBW 343/Raj 3765 (33.3%), PBW 343/WH 730 (31.4%), HD 2329/Kharchia 65 (29.4%), PBW 343/K 7903 (25.6%) and PBW 343/HD 2864 (21.4%). The EFF was found highest for the cross HD 2329/Kharchia 65 (21.2%) followed by the crosses PBW 343/Raj 3765 (14.0%), PBW 343/WH 730 (12.1%), PBW 343/HD 2864 (6.6%), PBW 343/K 7903 (6.6%), PBW 343/HI 1563 (4.3%), PBW 343/DBW 14 (4.0%), PBW 343/NIAW 34 (1.5%) and PBW 343/Raj 4037 (1.3%). The PRF was highest for the cross PBW 343/DBW 14 (44.4%) followed by the cross PBW 343/Raj 3765 (42.8%), PBW 343/WH 730 (25.0%), PBW 343/HI 1563 (25%) and HD 2329/Kharchia 65 (9.8%). Plant regeneration was not observed in the crosses PBW 343/HD 2864, PBW 343/K 7903, PBW 343/NIAW 34 and PBW 343/Raj 4037. The regenerated plants were given colchicine treatment that resulted in chromosome doubling in few plants. The factors affecting efficient utilization of wheat x maize protocol like embryo formation, induction of haploid embryo, germination by cold treatment and colchicines treatment have been identified. These factors still need to be further investigated for optimum utilization of wheat x Maize protocol.

Table 2 Summary of the caryopsis formation, embryo formation and plant regeneration in wheat (F_1s) x maize crosses

S. No	Cross	Spk. Poll.	CF	CFF (%)	EF	EFF (%)	PR	PRF (%)	DH
1	PBW 343 x DBW 14	415	223	53.7	9	4.1	4	44.4	-
2	PBW 343 x HI 1563	152	92	60.5	4	4.3	1	25.0	1
3	PBW 343 x HD 2864	70	15	21.4	1	6.6	-	-	-
4	PBW 343 x K 7903	176	45	25.6	3	6.6	-	-	-
5	PBW 343 x Raj 3765	150	50	33.3	7	14.0	3	42.8	-
6	PBW 343 x NIAW 34	270	130	48.1	2	1.5	-	-	-
7	PBW 343 x WH 730	210	66	31.4	8	12.1	2	25.0	-
8	PBW 343 x Raj 4037	190	72	37.9	1	1.3	-	-	-
9	HD 2329 x Kharchia 65	820	241	29.4	51	21.2	5	9.8	-
	Total	2453	934		86		15		1

Spk.Poll – number of spikelets pollinated, CF- no. of caryopsis formed, CFF- Caryopsis formation frequency, EF- No. of embryos formed, EFF- Embryo formation frequency, PR-No. of plants regenerated, PRF- Plant regeneration frequency

The plant hormone 2,4-D (200 ppm) was applied to the pollinated wheat spikelets for development of caryopsis, maintenance and development of the embryos. The CFF ranged between 25.9-51.4% and 9.8- 44.4% in varieties and crosses respectively, in the present study. The dosage of 2,4-D at the concentration of 100 ppm and 213.05 ppm was suggested to be effective for caryopsis formation (Kisana *et al.*, 1993; Zhixia *et al.*, 2014). AgNO3 (120 ppm) and 2,4-D (180 ppm) was used for obtaining better haploids induction in durum wheat (Almouslem *et al.*, 1998). Haploid seedlings over pollinated florets were recorded 9.9% (Verma *et al.*, 1999) and 3.3% (O'Donoughue and Bennett, 1994).The EFF ranged between 4.6- 22.4% and 1.3- 21.2% in varieties and crosses respectively, in the present study. The caryopses obtained ranged between 8.4 and 94.8% (Mehta and Angra, 2000), whereas the

ranges of recovery of haploid embryos and seedlings ranged among 6.5 - 45.2% (Suenaga *et al.,* 1997) and 23.3 - 83.6% (Suenaga *et al.,* 1997; Polci *et al.,* 2005) respectively which were in agreement with our findings. In the present study, the PRF for varieties and crosses ranged between 6.4-63.6 and 9.8- 44.4% respectively. With regard to the percentage of haploid plants regeneration (calculated over all pollinated florets) the range observed for bread wheat varied from 0.3 and 10.1% (Riera-Lizarazu*et al.*, 1992; Bistch *et al.*, 1998; Kaushik *et al* 2004; Chaudhary *et al.*, 2005; Polci *et al.,* 2005; Verma *et al.*, 1999; Mehta and Angra, 2000) which are in conformity with the results obtained in the study. The factors affecting the proper utilization of wheat x maize have been identified and will be worked on. Since the protocol has been standardized and various constraints identified, next step is production of homozygous lines from wide crosses and development of doubled haploid mapping populations for specific traits.

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