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Maize-induced haploid production from triticale x wheat crosses

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Abstract

The system of maize induced haploidy was used to devise a rapid technique for generating D/R substitutions from F_1s of hexaploid triticale x bread wheat. Standard wheat x maize protocol was ineffective. Two refinements which included supplementing the 2,4-D (100ppm) with picloram (100 pm) for use in post pollination tiller injection and advancing the F_1s of triticale x wheat to subsequent generations via selfing and backcrossing proved effective. Caryopsis formation frequency (CFF) to the tune of 52.30%, 11.59% embryo formation frequency (EFF) and 13.10% plant regeneration frequency (PRF) were achieved. Using above modified auxin treatment the response of F_1s of triticale x wheat (Chinese spring with *Ph* suppressor) towards maize induced haploid induction was also obtained with CFF of 17.54 and EFF of 4.65 percent. The maize mediated haploid induction is proposed as a rapid technique for the genetic enhancement of triticale and wheat.

Key words: *Triticum aestivum,* triticale, doubled haploids

Introduction

Hexaploid triticale (AABBRR) x bread wheat (AABBDD) crosses can serve to create genotypes with various combinations of D and R genome chromosomes which apart from practical utility lend themselves to studies on chromosomal and genomic interactions. The plant material thus generated can be of significance for both triticale and wheat improvement [1, 2]. The D genome of bread wheat is an important source of genes for processing and grain quality, which continues to be the single most important hindrance in the commercial adoption of triticale. Hexaploid triticale x bread wheat crosses offer an opportunity of introducing D chromosome substitutions without altering the agronomically superior hexaploid status of triticale.

Alternatively, the triticales can serve as a donor of genes governing tolerance to abiotic and biotic stresses to wheat. However, such chromosome transfers can be a long drawn out process on account of several selfing generations required to obtain stable lines. Also, the selfing series has been shown to throw up a preponderance of parental genotypes [3]. To accomplish such chromosome transfers in a rapid manner, anther culture technique has been explored [4, 5] but with limited success. In wheat, the maize induced doubled haploid production has emerged as a system of choice since its first report by Laurie and Bennett [6]. Applicability of this system has been reported in triticale [7-10] though the success rate was considerably lower as compared to wheat. In the present study this technique has been fine tuned for its use on F_1s and subsequent generations of triticale x wheat crosses with a view to generate chromosome transfers between these two species.

Materials and methods

Plant material

The plant material used in the study consisted of F_1s which represent a subset of combinations between four hexaploid triticales (T3021, T 3048, T 3065 and NGSN 23) with four bread wheat genotypes (PBW 493, PBW 521, PBW 536 and PBW 343) and their subsequent selfed and backcrossed generations. Three of the triticale genotypes chosen represented the bold, amber grained genotypes developed at Punjab Agricultural University, while genotype NGSN 23 (a mutant version of the widely used CIMMYT triticale, Armadillo) was the selection from national germplasm screening nursery having good combining ability for grain characters. The selection of wheat genotypes PBW 493, PBW 521 and PBW 536 was based on their bold, lustrous grains while genotype PBW 343 is the most widely grown (~ 7 million ha.) cultivar in India.

Crossing technique

The crosses with maize were conducted under field conditions at Punjab Agricultural University, Ludhiana

(Punjab, India). The spikes that had partially emerged from the boot leaf were emasculated without clipping of florets and freshly collected maize pollen was used for pollinations. The crossed ears were retained in the field for 18-22 days till embryo rescue was done. Tiller culture technique was also used for the crosses performed during the month of March to protect the growing caryopsis from the effect of high temperature.

Hormone and colchicine treatment

Auxins and colchicine were administered to the wheat tillers pollinated with maize in a combined treatment as follows: 2,4- D (100 ppm) or 2,4- D + picloram (100 ppm each) + Colchicine (0.75 %) + DMSO (2%) at 24 and 48 hours and 2,4-D (100 ppm) + or 2,4- D + picloram (100 ppm each) at 72 hours after pollination. For this treatment two holes were pierced in the uppermost internode one just below the ear and the other above the first node. The solution was injected through the lower hole till the solution moved out from the upper hole.

Embryo rescue

The caryopses were carefully removed from the spikes, 18-22 days after pollination. The caryopses were washed under running tap water and then with Bavistin™ (Carbendazim) 2g/l and Omnatex™ (Cefotaxime) 1g/l. The caryopses were transferred to sterilized conditions under laminar air flow for surface sterilization with $HgCl_2$ (0.1%) for 10 minutes. Caryopses were then transferred to ethanol (70%) for 30 seconds followed by 2 washings with autoclaved water. Caryopses were taken out in a sterile petridish and dissected under stereo microscope. Typically the caryopsis carried a free-floating embryo but lacked endosperm. Embryos were cultured on regeneration media in glass test tubes measuring 15 x 125 mm and having 4-5 ml of solid medium. The cultured embryos were incubated in cold (4°C) for 48 hours and then at 25°C till germination, in dark. After germination, the tubes were taken out and placed in racks having 12 h light + 12 h dark, for the development of embryo into complete plantlets. The embryo rescue media included MS Basal (Duchefa™, Haarlem Netherlands), Myo Inositol (100 mg/l), Glutamine (100 mg/l), Cystine (25 mg/l), Aspargine (25 mg/l), Kinetin (0.10 mg/l), Sucrose (30 gm/l), Activated charcoal (2 gm/l) and Gelrite (3 gm/l).

Observations

In all the experiments the observations were recorded on percent caryopsis formation frequency (No. of caryopsis formed/ No. of florets pollinated), percent embryo formation frequency (No. of embryos formed/ No. of caryopsis) and plant regeneration frequency (No. of plants regenerated/ No. of embryos cultured). These parameters served as indicators of the efficiency of the maize system of haploid production being explored in the present study.

Results and discussion

The wheat x maize system has been targeted as a rapid technique for production of doubled haploid plants in wheat and triticale. In the present study this system was explored for rapid generation of combinations of D and R genomes from hexaploid triticale x bread wheat crosses.

In the first round of experiments the F_1s of seven triticale x wheat cross combinations and two wheat varieties were used for attempting crosses with maize. The protocol for maize induced haploidy is basically available for wheat [11]. The wheat controls thus served as an indicator of the normal functioning of the wheat x maize protocol. The caryopsis formation frequency (CFF) of 44.55 % was observed in the wheat controls (Table 1). However, in the F_1s of triticale x wheat a much lower caryopsis formation (6.34 %) was observed. Actually, caryopsis formation is a function of auxin treatment and caryopsis formation has been reported to occur even in the unfertilized florets upon application of auxin [9]. In wheat 2,4-D @ 100 ppm is routinely used for post-pollination treatment in the form of injections applied to the first internode below the ear and satisfactory response has been obtained [12]. This auxin treatment (2,4-D @ 100ppm) proved less effective in promoting caryopsis formation in the F_1s of triticale x wheat. Low response of triticale to maize induced haploidy has also been reported in the few studies carried out earlier. Rogalska and Mikulski [13] have reported 0.63 percent embryo formation and Wedzony *et al.* [8] have reported 2.80 percent embryo formation in crosses of triticale with maize when 2,4-D was used as an auxin treatment. Poor seed set (4.71 %) and embryo formation (1.10 %) as well as low plant regeneration frequency (0.4 %) was also reported by Inagaki *et al.* [7] in triticale x maize crosses. Further, when the caryopsis were harvested for embryo rescue 18-22 days after pollination, no embryo were found in the maize pollinated ears of triticale x wheat F_1s while in the wheat controls 23.47% embryo formation frequency was observed. The lack of embryos in the F_1s of triticale x wheat in the current study indicated that either fertilization did not take place or embryo

Table 1. Performance of the triticale x wheat F_1s in crosses with maize

Triticale x wheat F_1	Caryopsis formation frequency (%)		Embryo formation frequency (%)	
T 3048 x PBW 536	0.00	(0/42)*	0.00	(0/0)
T 3021 x PBW 521	7.54	(43/570)	0.00	(0/43)
NGSN 23 x PBW 343	9.09	(12/132)	0.00	(0/12)
PBW 343 x T3021	0.00	(0/52)	0.00	(0/0)
T 3048 x PBW 343	4.61	(13/282)	0.00	(0/13)
T 3021 x PBW 493	69.23	(9/13)	0.00	(0/9)
T 3048 x PBW 521	5.19	(35/674)	0.00	(0/35)
Mean	6.34	(112/1765)	0.00	(0/112)
PBW 493 (wheat control)	37.10	(46/124)	26.09	(12/46)
PBW 154 (wheat control)	54.17	(52/96)	21.15	(11/52)
Mean	44.55	(98/220)	23.47	(23/98)
Overall mean	10.58	(210/1985)	10.95	(23/210)

*Figures in parenthesis show actual numbers

development was stalled at an early stage. The problem seemed to be accentuated due the chromosomal imbalance in the gametes produced by triticale x wheat F_1s.

To address the problems of caryopsis formation and embryo development two modifications were made. The auxin application made for supporting caryopsis development was enhanced. Reports have indicated that in triticale the auxins picloram and dicamba led to higher embryo formation compared to other auxin analogues tested [9, 10]. Also, the 75 or 100 ppm concentration of picloram and dicamba were reported to be best when applied as droplets to the individual florets. Thus, for second phase of study, picloram was added to the injection mixture and injections applied were as 2,4-D (100 ppm) + Picloram (100 ppm) + colchicine (0.75 %) + DMSO (2%) at 24 and 48 hours and 2,4- D (100 ppm) + Picloram (100 ppm) at 72 hours after pollination.

Secondly, with the idea of improving chromosomal balance in the gametes of triticale x wheat crosses the F_1s were advanced to the subsequent generations by backcrossing to wheat or selfing. Advanced generation material (F_2, F_3, BC_2 and BC_1F_2) was used for attempting crosses with maize employing modified hormonal treatment. Fifty advance generation lines belonging to nine cross combinations were used for attempting crosses with maize. Caryopsis formation up to 95.51 % was achieved with an overall average of 52.30 %. The cross wise performance of the different cross combinations of (triticale x wheat) x maize is given in Table 2 (data on individual performance of lines not shown). Embryo formation to the tune of 42.59 % was achieved with an overall average of 11.59 %. The plant regeneration frequency observed was upto 40.00 % with an average of 13.10 %. The combined result of using backcross/selfed derivatives for crosses with maize as well as modified hormonal treatments was that the caryopsis formation frequency (52.30 %) was more than eight times as compared to the initial results (CFF 6.34 %). Moreover adequate levels of embryo formation and plant regeneration could also be obtained in this case.

Variation was, however, observed even within a set of lines derived from the same triticale x wheat cross, some lines had very low to no response to maize induced haploidy. Again the probable reason may be the imbalanced chromosome complement of these lines.

With the advancement of the material to further generations the benefit of saving time is compromised and chances of losing some elite combinations are there. By using the refined protocol a fresh attempt was made to generate D/R chromosome transfers directly from the F_1s of triticale x wheat. The F_1s were produced by crossing the triticale genotypes used earlier with a special stock 'Chinese Spring with *Ph* suppressor from *Aegilops speltiodes* [14]. It was anticipated that the promotion of homeologous pairing due to *Ph* suppressor in the genotypes with AABBDR genomic constitution would improve chromosomal balance of gametes produced and consequently success would be better. Additionally chances of recovering translocations are also there. The results revealed that an average caryopsis formation frequency of 17.54 % could be achieved (Table 2). The embryo formation frequency and plant regeneration frequencies were 4.65 % and 40.00 % respectively. In comparison to wheat control the frequencies were expectedly lower considering the chromosomally imbalanced gametes in the F_1s of triticale x wheat. The embryo formation was almost at par with the control. Subsequently satisfactory plant regeneration could also be obtained (40.00 %). Thus, the experiment revealed that the production of doubled haploids from the F_1s was possible though the relative role played by the Chinese spring with *Ph* suppressor and improved hormonal treatment needs to be assessed individually. The caryopsis formation frequencies observed here are comparable to the reports of Pratap *et al.* [15] who have reported 20 % caryopsis formation in F_1s of triticale x wheat while using higher dose of 2,4-D (250 ppm). The present studies indicated the application of hormone in the form of injections is also

Table 2. Performance of triticale x wheat derived lines and F_1s in crosses with maize using improved methodology

Triticale x wheat cross	Generation	Caryopsis formation frequency (%)		Embryo formation frequency (%)		Plant regeneration frequency (%)	
PBW 343 x T 3048	F_2	55.88	(366/655)	7.92	(29/366)	13.79	(4/29)
T3048 x PBW 521	BC_2	47.44	(361/761)	7.20	(26/361)	3.85	(1/26)
T 3021 x PBW 343	F_2	39.83	(423/1062)	21.04	(89/423)	6.74	(6/89)
T 3048 x PBW 521	F_3	65.39	(682/1043)	4.69	(32/682)	21.88	(7/32)
T 3021 x PBW 521	BC_2	59.01	(1614/2735)	14.56	(235/1614)	14.04	(33/235)
NGSN 23 x PBW 536	F_2	71.04	(287/404)	6.27	(18/287)	22.22	(4/18)
T 3021 x PBW 521	BC_2F_2	51.25	(144/281)	15.28	(22/144)	18.18	(4/22)
T 3048 x PBW 521	F_2	40.67	(146/359)	16.44	(24/146)	16.67	(4/24)
T 3048 x PBW 343	F_2	32.05	(324/1011)	8.95	(29/324)	10.34	(3/29)
Mean		52.30	(4347/8311)	11.59	(504/4347)	13.10	(66/504)
T 3048 x CSS	F_1	30.92	(81/262)	0.00	(0/81)	0.00	(0/0)
T 3065 x CSS	F_1	10.25	(49/478)	8.16	(4/49)	0.00	(0/4)
NGSN 23 x CSS	F_1	22.12	(69/312)	5.80	(4/69)	75.00	(3/4)
T 3021 x CSS	F_1	21.62	(16/74)	12.50	(2/16)	50.00	(1/2)
Mean		17.54	(215/1226)	4.65	(10/215)	40.00	(4/10)
PBW 493 (Wheat control)		27.17	(50/184)	6.00	(3/50)	66.67	(2/3)

equally effective as its application in the form of droplets to the individual florets reported earlier [9,10]. Overall, the technique has a high feasibility as a rapid technique for generating chromosome transfers between triticale and wheat.

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