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RESEARCH ARTICLE

Relative efficiency of chromosome elimination techniques for haploid induction parameters in triticale × wheat derived advanced generation through *Zea mays*- and *Imperata cylindrica*- mediated systems

M.S. Jeberson[1*], H.K Chaudhary[2] and R.K. Chahota[2]

ABSTRACT

The study compared the effectiveness of doubled haploid generation methods using maize and Imperata cylindrica mediated systems. Triticale x wheat generated recombinants in different generations were employed for doubled haploid formation. These were grown in MS medium with various minerals and hormones. All generations reacted to maize and Imperata cylindrica-mediated haploid induction parameters. Both advanced and early generations responded well to haploid induction. Imperata cylindrical outperformed maize in all haploid induction parameters.

Keywords: Double haploid; *Zea mays*; *Imperata cylindrica*-; Triticale x wheat derived recombinants

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*Corresponding author e-mail address: samuel8142@gmail.com (M.S. Jeberson)

INTRODUCTION

Wheat (Triticum aestivum L.) is the world's oldest and most important food grain crop. In terms of area

(214.00 million hectares) and productivity (773.00 million metric tonnes), it leads all cereal crops (Anonymous 2020). India leads the world in wheat acreage (29.32 million hectares) and production (107.592 million metric tonnes) (Anonymous, 2020). Triticale (Triticosecale Wittmack), the first man-made cereal, may be utilised as a bridging species because it easily hybridises with wheat (Triticum aestivum). Triticale's agronomic features are unique to rye chromosomes (Merker, 1984). Breeders must pick parents carefully for triticale-wheat hybridization operations to separate possible recombinants from segregating populations. Raising the separate generations of crosses requires a lot of time, space, and labour. Compared to conventional breeding methods, the numerous haploid breeding procedures have tremendous relevance and practical utility. It takes one year to produce completely homozygous plants using in vitro haploid culture or intergeneric hybridization with maize and cogon grass (Imperata cylindrica) and chromosome doubling using colchicine, but conventional breeding takes 7-8 years to isolate stable lines from the clines (Chaudhary et al., 2015). We don't need to establish vast populations of haploid plants because selection based on gametic frequency can be done. To create homozygous populations from triticale-wheat hybrids and backcrosses, doubled haploid breeding (DH) using Zea mays and Imperata cylindrica-mediated methods can be used.

MATERIALS AND METHODS

Using Imperata cylindrica pollen, we doubled haploided Triticale wheat populations and used GISH and FISH to find rye chromatin introgression (Table 1). Imperata cylindrica is a cross-pollinated poaceae plant with 2n=2x=20 chromosomes. I. Cylindrica is a tall perennial grass (30-150 cm). The inflorescence is a terminal white spike-like panicle, 5-20 cm long and 2.5 cm in diameter. Spikelets are 3.5-5.0 mm long, with a 10 mm basal ring of silky hairs. I. flowering time cylindrica, a weedy grass found in the rabi wheat fields, matched the flowering of triticale (wheat derivatives) (Chaudhary et al., 2005). However, during the kharif season, maize (Zea mays) grows well in poly houses and flowers with wheat and triticale (Chaudhary et al., 2002). So, during the rabi season, maize (Early Composite) was sowed seven days apart to assure pollen availability.

In triticale x wheat hybrids, emasculation was performed three days before anthesis by manually removing anthers without severing lema or palea. The next day, maize pollen and I. It was collected separately in petri plates and gently applied to the feathery stigma of emasculated spikes of triticale x wheat recombinants. The pollinated spikes were immediately bagged and labelled. Pollinated the triticale wheat spikes with maize. cylinders were injected with a 250 mg/L 2,4-D solution using a syringe and a fine hypodermic disposable needle (Pratap and Chaudhary, 2007). The injection holes were sealed with Vaseline (Hindustan Lever Ltd., India). It was done for two days to ensure appropriate seed and embryo production.

The haploid plantlets were moved to rooting medium, hardened in soil mixture, and then treated with 0.1 percent colchicine solution to double their chromosomes. It was used to treat haploid plantlets at three to five tiller stages using Inagaki (1985) method with minor adjustments. For 5 hours, each haploid plant crown was immersed in a 0.1 percent colchicine solution with 1.5 percent dimethyl sulphoxide. The plants were treated for 20 minutes under running tap water, then potted in soil and caged till maturity. Observations on haploid induction features on percent are shown below. For further investigation, these data were Arcsine converted (Warton and Hui, 2011)

RESULTS AND DISCUSSION

Pseudoseed formation of F_1 ranged from 16.22% to 46.93% generation (Table 1), 10.86% to 19.95% in F_2, 28.77 to 62.79% in F_3, 15.13% to 53.05% in F_4 (Table 2), 33.98% to 51.99% BC_1F_1, 12.91% to 30.21 % in BC_1F_2, 45.06% to 56.41% in BC1F3, 32.09% to 46.09% in BC_1F_4 and 39.38% to 43.09% in BC_2F_3 generations (Table 3). Whereas, the pseudoseed formation frequency through *I. cylindrica*- mediated system ranged from 26.42% to 50.42% in F_1 generation (Table 1), 32.67% to 43.17% in F_2, 47.99% to 79.86% in F_3, 27.16% to 61.64% in F_4 generations (Table 2). In back cross generations pseudoseed formation frequency ranged from 43.33% to 63.77% in BC_1F_1, 34.55% to 38.36 % in BC_1F_2, 46.90% to 51.24% in BC_1F_3, 48.31% to 56.65% in BC_1F_4 and 42.54% to 43.14% in BC2F3 (Table 3). In all generations, Imperata cylindrica outperformed Zea mays in producing pseudoseed. This conclusion is in line with recent investigations by Chaudhary et al. (2005), Pratap et al (2019).

Table 1. Detail of the Triticale x wheat derived cross combinations in different generations utilized for the present investigation

S.No	*Generation/Crosses*	*S.No*	*Generation/Crosses*
	F_1		*F_2*
1	DT 123 × HPW 89	4	TL 2920 × HPW 155
2	DT 123 × PBW 343	5	TL 2920 × HS 295
3	DT 126 × C 306	6	TW9331×HPW155
4	DT 126 × DH 40		*F_3*
5	DT 126 × HPW 42	1	ITSN105/58 × HS396
6	DT 126 × HPW 89	2	TL 2908 × HS 396
7	ITSN 65 × C 306	3	TL 2920 × HS 396
8	ITSN 65 × DH 40	4	TL 2920 × C 306
9	ITSN 65 × HPW 42	5	TL 2920 × VL 858
10	ITSN 65 × HPW 89		*F_4*

11	ITSN 65 × HPW 155	1	ITSN 65 × VL 858
12	TL 1207 × CBME-IYC-16	2	ITSN 65 × Tyari
13	TL 1207 × DH 40	3	TL 1217 × C 306
14	TL 1207 × HS 240	4	TL 1217 × VL 858
15	TL 1207 × HS 295	5	TL 2908 × VL 858
16	TL 1210 × DH 40		*BC_1F_1*
17	TL 1210 × DH 776	1	ITSN 65 × HPW 155 × HPW 155
18	TL 1210 × HPW 155	2	ITSN 65 × HPW 89 × HPW 89
19	TL 1210 × HS 295	3	TL 1210 × W 5 × W 5
20	TL 1210 × HS 365	4	TL 1217 × HPW 42 × HPW 42
21	TL 1210 × HS 396	5	TL 2920 × DH 776 × DH 776
22	TL 1210 × VL 858	6	TL 2920 × HS 396 × HS 396
23	TL 1210 × VWFW 269	7	TL 2920 × W 5 x W 5
24	TL 2900 × DH 40		*BC_1F_2*
25	TL 2900 × HS 375	1	ITSN 105/58 × HS 396 × HS 396
26	TL 2900 × HS 396	2	TL 2900 × VL802 × VL 802
27	TL 2908 × HPW 42		*BC_1F_3*
28	TL 2908 × HPW 249	1	ITSN 105/58 × VL 802 × VL 802
29	TL 2920 × DH 40	2	ITSN 105/58 × HPW 89 × HPW 89
30	TL 2920 × HS 295		*BC_1F_4*
31	TL 2920 × PBW 343	1	ITSN 105/58 × VL 802 × VL 802
32	TW 9335 × HS 240	2	TL 2900 × VL 802 x× VL 802
	F_2	3	TL 2920 × VL 802 × VL 802
1	ITSN 65 × DH 40		*BC_2F_3*
2	TL 2900 × DH 776	1	ITSN 105/58 × VL 802 × VL 802 × VL 802
3	TL 2920 × CBME-IYC-16	2	ITSN105/58 × RL-14-1 × RL-14-1 × RL-14-1

Table 3. Performance of various crosses in respect of different haploid induction parameters

Generation/Crosses	(Triticale x wheat) × *Zea mays*	(Triticale × wheat) × *Imperata cylindrica*

Sl.No.		sf (%)	ef (%)	pr(%)	sf (%)	ef (%)	pr(%)
	F_1						
1	DT 123 × HPW 89	20.61	6.00	0	44.12*	25.08*	23.11
2	DT 123 × PBW 343	30.00*	6.14	0	30.00	19.54	30.00
3	DT 126 × C 306	16.22	9.00	0	48.36*	12.99	27.00
4	DT 126 × DH 40	28.91*	4.44	18.00*	38.59	22.06*	32.14
5	DT 126 × HPW 42	25.97	7.72	15.00	34.55	24.55*	45.00*
6	DT 126 × HPW89	22.54	4.43	0	41.65*	23.86*	45.00*
7	ITSN 65 × C 306	17.03	6.33	11.25	36.64	22.47*	57.47*
8	ITSN 65 × DH 40	22.40	6.02	0	36.88	22.53*	22.50
9	ITSN 65 × HPW 42	23.87	4.82	0	35.24	24.67*	28.95
10	ITSN 65 × HPW 89	22.98	4.82	0	34.74	21.30	45.00*
11	ITSN 65 × HPW155	19.54	8.03	0	29.27	23.68*	24.12
12	TL 1207 × CBMEIYC16	24.82	9.37	0	34.39	19.27	24.93
13	TL 1207 × DH 40	35.21*	11.65*	22.50*	44.97*	17.97	28.95
14	TL1207 × HS 240	19.63	0	0	30.21	21.14	22.50
15	TL 1207 × HS 295	18.39	0	0	30.86	12.64	30.00
16	TL 1210 × DH 40	26.38	2.92	0	39.99*	17.89	54.12*
17	TL 1210 × DH 776	24.22	7.15	0	31.20	20.37	30.00
18	TL 1210 × HPW 155	33.18*	7.57	15.00	37.68	18.71	20.88
19	TL 1210 × HS 295	23.94	0	0	29.00	14.77	33.75
20	TL 1210 × HS 365	34.30*	10.84*	0	38.14	20.33	15.00
21	TL 1210 × HS 396	24.09	15.60*	22.50*	29.55	25.43*	45.00*
22	TL 1210 × VL 858	19.93	19.93*	0	32.03	20.30	45.00*
23	TL 1210 × VWFW 269	25.73	13.02*	18.00*	28.64	24.14*	27.00
24	TL 2900 × DH 40	18.05	0	0	43.16*	18.09	22.55
25	TL 2900 × HS 375	18.50	6.00	0	28.45	8.39	0
26	TL 2900 × HS 396	27.75*	8.88	18.00*	30.00	22.28*	36.00
27	TL 2908 × HPW 42	21.49	6.00	0	35.27	22.85*	36.00
28	TL 2908 × HPW 249	18.98	6.00	0	26.42	14.30	30.00
29	TL 2920 × DH 40	29.51*	7.20	0	50.42*	19.75	48.10*
30	TL 2920 × HS 295	36.36*	24.45*	25.05*	36.36	24.45*	25.05
31	TL 2920 × PBW 343	19.34	6.64	0	50.8*	21.29	49.87*
32	TW 9335 × HS 240	46.93*	19.52*	42.57*	46.93*	19.52	42.57*
	Mean	24.90	7.83	6.50	36.39	20.21	32.73
	SE(m)±	1.20	1.01	1.54	1.22	0.72	2.18

Table 4. Performance of various crosses in respect of different haploid induction parameters

S.No.	Generation/Crosses	(Triticale × wheat) × *Zea mays*			(Triticale × wheat) × *Imperata cylindrica*		
		sf (%)	ef (%)	pr(%)	sf (%)	ef (%)	pr(%)
	F2						
1	ITSN 65 × DH 40	12.89	0	0	36.41	15.93	22.50
2	TL 2900 × DH 776	18.74*	10.00*	0	36.59	9.99	30.00*
3	TL 2920 × CBMEIYC16	18.43*	13.28*	22.50*	37.09	21.54*	24.93
4	TL 2920 × DH 776	16.06	7.50	0	32.67	21.35*	20.07
5	TL 2920 × HPW 155	17.02	17.02*	0	43.17*	10.72	18.25
6	TL 2920 × HS 295	10.86	0	0	41.95*	9.48	11.25
7	TW9331 × HPW155	19.95*	0	0	36.36	23.72*	26.75*
	Mean	16.28	6.83	3.21	37.75	16.10	21.96
	SE(m)±	0.66	1.40	0.70	0.72	1.23	1.23
	F3						
1	ITSN105/58 × HS396	49.15	5.05*	0	55.83	13.80	38.57
2	TL2908 × HS396	62.79*	3.78	8.18*	79.86*	8.99	38.86
3	TL2920 × HS396	39.76	2.81	0	47.99	17.58*	72.00*
4	TL2920 × C306	28.77	5.92*	0	42.48	13.83	57.86
5	TL2920 × VL858	43.25	0	0	55.21	22.83*	69.93*
	Mean	44.74	3.51	1.64	56.27	15.41	55.44
	SE(m)±	1.87	0.44	0.55	2.13	0.77	2.41
	F4						
1	ITSN65 × VL858	15.13	9.43*	16.67*	27.16	23.66*	45.00*
2	ITSN65 × Tyari	27.38	2.28	3.32	30.50	2.54	5.57
3	TL1217 × C306	53.05*	4.17	1.15	61.64*	7.31	8.75
4	TL1217 × VL858	24.50	7.51*	22.90*	29.28	12.59	33.90*
5	TL2908 × VL858	49.09*	1.32	3.10	58.52*	8.79	19.06
	Mean	33.83	4.94	9.43	41.42	10.98	22.46
	SE(m)±	1.39	0.29	0.83	1.46	0.68	1.43

Table 5. Performance of various crosses in respect of different haploid induction parameters

Sl.No	Generation/Crosses	(Triticale × wheat) × *Zea mays*			(Triticale × wheat) × *Imperata cylindrica*		
		sf (%)	ef (%)	pr(%)	sf (%)	ef (%)	pr(%)
	BC_1F_1						
1	ITSN 65 × HPW 155 × HPW 155	16.78	0	0	46.88	20.41*	50.00
2	ITSN 65 × HPW 89 × HPW 89	35.04	12.14*	13.50*	47.87	18.97*	31.10
3	TL 1210 × W 5 × W 5	51.99*	2.69	0	49.85	16.27	42.11
4	TL 1217 × HPW 42 × HPW 42	32.08	0	0	55.22*	21.17*	51.89*
5	TL 2920 × DH 776 × DH 776	50.64*	2.26	0	63.77*	15.03	49.33
6	TL 2920 × HS 396 × HS 396	33.98	6.44*	0	43.33	9.43	42.75
7	TL 2920 × W 5 × W5	40.35	0	0	59.33*	17.62	63.25*
	Mean	37.27	3.26	1.93	52.32	16.99	47.20
	SE(m)±	1.70	0.64	0.72	1.09	0.56	1.47
	BC_1F_2						
1	ITSN 105/58 x HS 396 × HS 396	12.91	0.94	2.97	34.55	12.91	19.36
2	TL 2900 × VL802 × VL 802	30.21	22.7	16.15	38.36	30.21	65.96
	Mean	34.40	11.82	9.56	36.36	21.56	42.66
	SE(m)±	0.18	1.55	0.94	0.38	1.62	4.36
	BC_1F_3						
1	ITSN 105/58 ×VL 802 × VL 802	56.41	9.51	7.72	46.9	17.37	38.60
2	ITSN 105/58 × HPW 89 × HPW 89	45.06	11.86	10.00	51.24	17.27	46.22
	Mean	50.74	10.69	6.92	49.07	17.82	42.41
	SE(m)±	0.82	0.17	0.16	0.35	0.01	0.61
	BC_1F_4						
1	ITSN 105/58 × VL 802 × VL 802	32.09	6.14	0	56.24*	25.68*	45.00
2	TL 2900 × VL 802 × VL 802	46.90*	6.97*	10.75*	56.65*	19.79	23.76
3	TL 2920 × VL 802 × VL 802	39.33	6.54	10.00*	48.31	14.87	56.08*
	Mean	39.44	6.55	6.92	53.73	20.11	41.61
	SE(m)	0.83	0.05	0.68	0.53	0.61	1.85
	BC_2F_3						
1	ITSN 105/58 × VL 802 × VL 802 × VL 802	43.09	4.19	10.38	42.54	29.51	26.61
2	ITSN105/58 × RL-14-1 × RL-14-1 × RL-14-1	39.38	2.85	8.27	43.14	27.77	51.77
	Mean	41.24	3.52	9.33	42.84	28.64	39.19
	SE(m)	0.18	0.07	0.10	0.03	0.08	1.16

Embryo formation frequency

Embryo formation frequency in triticale × wheat derived lines of different generations through *Zea mays*- mediated system in F_1 ranged for this trait from 0 to 24.45 % (Table 1), from 0 to 17.20% in F_2, 0 to 5.92% in F_3 and 1.32% to 9.43% in F_4 (Table 2). In backcrosses, BC_1 F_1 ranged for this trait from 0 to 12.14%, BC_1F_2 from 0.94% to 22.7%, BC_1F_3 from 9.51% to 11.86%, BC_1F_4 from 6.14% to 6.97% and BC_2F_3 from 2.85% to 4.19% (Table 3). The embryo formation with *I. cylindrica* in F_1 ranged for this trait from 8.39% to 25.43% (Table 1), in F_2 from 9.48% to 23.72%, in F_3 ranged from 8.99 to 22.83% and in F_4 ranged from 2.54% to 23.66% (Table 2).

In back crosses, BC_1 F_1 ranged for this trait from 9.43% to 21.17%, in BC_1F_2 from 12.91% to 30.21%, BC_1F_3 from 17.27% to 17.37%, BC_1F_4 from 14.87% and BC_2F_3 from 27.77% to 29.51 % (Table 3). In all the generations, *I. cylindrica* was performed far better than the *Zea mays* regarding embryo formation. This result is in accordance with the previous studies carried out by Chaudhary (2008) and Kishore et al. (2011) and Kapoor et al. (2020).

Haploid plant regeneration frequency

In backcrosses, BC_1F_1 plant regeneration was ranged from 0 to 13.50%, BC_1F_2 from 2.97% to 16.15%, BC_1F_3 from 7.72% to 10%, BC_1F_4 from 0 to10.75% and BC_2F_3 ranged from 8.27% to 10.38 % (Table 3).

In triticale ×wheat derived lines of different generations, plant regeneration frequency through *I. cylindrica*- mediated system in F_1 ranged from 0 to 57.47% (Table 1), 11.25% to 30.00% in F_2, 38.57% to 72.00% in F_3 and 5.57% to 45.00% in F_4 (Table 2). In back crosses, BC_1F_1 pseudo seed formation frequency ranged from 31.10% to 63.25%, BC_1F_2 from 19.36% to 65.96%, BC_1F_3 from 38.60% to 46.22%, BC_1F_4 from 23.76% to 56.08% and BC_2F_3 ranged from 26.61% to 51.77% (Table 3). This result is in corroboration with the previous studies carried out by Chaudhary (2008), Pratap et al. (2005) and Kanbar et al. (2020).

CONCLUSION

In triticale x wheat hybrids, the Imperata cylindrica mediated chromosome deletion technology produced considerably more haploid embryos than the Zea mays-based system in all generations. It also proved to be a more efficient and economically viable strategy for inducing haploids in triticale wheat derivatives than the maize-mediated system.

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