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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

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ABSTRACT

The study was undertaken to evaluate the relative efficiency of doubled haploid production methods following the chromosome elimination approach utilizing maize- and *Imperata cylindrica*- mediated systems. For doubled haploid production, triticale x wheat derived recombinants in different generations comprised of F_1, F_2, F_3, F_4, BC_1F_1, BC_1F_2, BC_1F_3, BC_1F_4 and BC_2F_3 were used. The haploid embryos were cultured in the MS medium with different mineral constituents and hormones. All the generations responded for different haploid induction parameters following maize- and *Imperata cylindrica*- mediated systems. Advanced generations responded better than early generations for haploid induction through both systems. All the haploid induction parameters, *Imperata cylindrical*- were performed significantly better than maize.

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

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INTRODUCTION

Among the food grain crops of the world, wheat (*Triticum aestivum* L.) is pre-eminent regarding its antiquity and importance as a food of humankind. Globally it occupies the topmost position among the cereal crops concerning area (214.00 million hectares) and production (773.00 million metric tons) (Anonymous 2020). India ranks first in the wheat acreage (29.32 million hectares) and second in the production (107.592 million metric tonnes) in the world (Anonymous, 2020). Triticale (× Triticosecale Wittmack), the first man-made cereal as a result of a cross between wheat (*Triticum aestivum*), and rye (*Secale cereale*), may be used as a bridging species to accomplish this goal since it easily hybridizes with wheat. Because they complemented rye chromosomes, triticale's have agronomic attributes that are not found in wheat (Merker, 1984). The gene pool of triticale can thus be incorporated into the wheat background to combine uniform grain quality, production, and chapatti-making quality of wheat with disease resistance and rye nutritional quality. Careful selection of parents is required on the part of breeders for triticale × wheat hybridization programs to isolate potential recombinants from the segregating populations. The time, space, and labour requirements for raising the crosses' segregating generations render such breeding programs very costly. However, to achieve quick and desirable results, the various haploid breeding procedures have immense significance and practical utility compared to the conventional breeding approaches. In vitro haploid production through anther culture or intergeneric hybridization with Maize (*Zea mays* L.) and Cogon grass (*Imperata cylindrica*), followed by chromosome doubling by colchicine treatment leads to the production of completely homozygous plants in just a single step (1 year), whereas conventional breeding approach takes much time (7-8 years) for the isolation of stable lines from the crosses (Chaudhary et al., 2015).

Further, the gametoclonal variation arising through androgenesis may also supplement the selection program, and we also need not grow large populations of haploid plants since selection is possible based on gametic frequency. The haploid breeding approach also increases the correctness of selection of multiple crosses compared to the traditional methods besides having use in mutation breeding as the homozygous constitution of gametes allows dominant and recessive mutations to be expressed directly among haploid regenerants and the deleterious mutations are not recovered. The process of obtaining homozygous populations from the triticale ×wheat hybrids and their back-crosses can be accelerated by utilizing doubled haploid breeding (DH) following the chromosome elimination approach through *Zea mays*- and *Imperata cylindrica*-mediated systems.

MATERIALS AND METHODS

Plant Materials

Triticale × wheat-derived populations of different backcross generations were used for doubled haploid production using *Imperata cylindrica* pollen and further utilized to detect rye chromatin introgression using GISH and FISH techniques (Table 1). *Imperata cylindrica,* belongs to the family poaceae, most commonly known in English as "cogon grass" having a chromosome number of 2n=2x=20 and cross-pollinated. *I. cylindrica* is a perennial grass that varies in height (30-150 cm). The inflorescence is a white, spike-like panicle, terminal, fluffy, 5-20 cm long and up to 2.5 cm in diameter. Spikelets are numerous, 3.5-5.0 mm long, each surrounded by a basal ring of silky hairs 10 mm long. Flowering time of *I. cylindrica,* a wildly growing weedy grass available naturally around the wheat fields during every *rabi* season, coincided well with the flowering of triticale ×wheat derivatives (Chaudhary et al., 2005). However, another pollen parent viz., maize (*Zea mays*) grown during the *kharif* season, thrives well in poly house conditions and its flowering coincides with that of wheat and triticale (Chaudhary et al., 2002). Therefore, maize (Early Composite) was sown during the rabi season with seven day intervals to ensure pollen availability for a longer duration.

Wide hybridization procedure

Emasculation was done three days before anthesis by removing the anthers manually with the forceps' help without cutting lema and palea in triticale x wheat hybrids. The next day, fresh pollen from the maize and *I. cylindrica* was collected separately in petri plates and applied gently to the feathery stigma of the emasculated spikes of triticale x wheat recombinants with a fine camel hairbrush. The pollinated spikes were immediately covered with butter paper bags and tagged.

In vivo hormonal treatment

The triticale×wheat spikes pollinated with the maize and *I. cylindrical* and were injected with a 2,4-D solution of 250 mg/L concentration (Pratap and

Chaudhary, 2007) at the base of the uppermost internode using a syringe fitted with a fine hypodermic disposable needle. Petroleum jelly (Vaseline-Hindustan Lever Ltd., India) was used for sealing the injection holes. The injections were repeated for two more consecutive days to ensure proper seed and embryo formation.

Embryo rescue medium

Murashige and Skoog medium was used for the rescue of haploid embryos (Murashige and Skoog, 1962) and the MS medium was supplemented with 0.5 mg/L kinetin, 150 mg/L glutamine, 20 mg/L each arginine, cysteine and leucine. The medium was solidified with 0.8% agar.

Embryo rescue procedure

The pollinated spikes were harvested from the tiller base after 18-20 days of pollination. The embryo carrying seeds were identified by placing the seeds in a petri plate under a light source and viewed from below as per the procedure (Bains et al., 1998). The embryos were removed under strict aseptic conditions and placed on the culture medium in the test tubes.

Growth conditions

Cultured immature embryos were given cold treatment at 4 °C temperature in the dark for first 24 h. After that, they were incubated in the dark in the Plant Growth Chamber at 25±1°C for regeneration for about a week till the roots and shoots initiated. The regenerated plantlets were then transferred to the other section of the Plant Growth Chamber at 25 ± 1°C with 10/14 h light/dark profile for plants' proper development.

Rooting, hardening and chromosome doubling

The haploid plantlets were transferred to rooting medium for profuse rooting, then potted in soil mixture for hardening and later treated with 0.1 % colchicine solution for chromosome doubling. The haploid plantlets were treated with colchicine at three to five tiller stages according to the method given by Inagaki (1985) with slight modifications. Each haploid plant crown was submerged in a 0.1 % colchicine solution supplemented with 1.5 % dimethyl sulphoxide at 20 °C for 5 h. The treated plants were kept in the running tap water for 20 min, then potted in soil and maintained in the cage house up to maturity.

Recording of observations

Observations were recorded with respect to haploid induction traits on per cent were given below. These data were transformed to definite value using Arcsine transformation for further analysis (Warton and Hui, 2011).

$$\text{Pseudoseed formation frequency} = \frac{\text{Number of pseudoseed formed}}{\text{Total number of floret pollinated}} \times 100$$

$$\text{Embryo formation frequency} = \frac{\text{Number of pseudoseed carrying embryo}}{\text{Total number of pseudoseed formed}} \times 100$$

$$\text{Haploid plantlet regeneration frequency} = \frac{\text{Number of haploid plantlets developed}}{\text{Total number of embryos cultured}} \times 100$$

RESULTS AND DISCUSSION

For comparing the efficiency of *Zea mays*- and *Imperata cylindrica*- mediated systems of DH breeding data concerning each component trait of two haploid production systems were recorded on different individuals of various generations and comparisons were drawn among themselves.

Pseudoseed formation frequency

Pseudoseed formation in triticale x wheat derived lines through Zea mays- mediated system in 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 the generations, *Imperata cylindrica* performed far better than the Zea mays in respect of pseudoseed formation. This result is in accordance with the previous studies carried out by Chaudhary et al. (2005), Pratap et al. (2005) and Chaudhary (2008b) and Sharma et al. (2019).

Table. 1 Wheat and triticale lines used in the present investigation

Sl. No.	*Genotype*	*Parentage*	*Source*
	Wheat		
1	HS 295	CQT//IAS55//ALDML'S'/3/ALDML'S'/ NAFN/4/PJN'S'/PEL1276.6	CSKHPKV, Palampur
2	HPW 155	BT 2549/FATH	CSKHPKV, Palampur
3	PBW 343	ND/VG9144//KAL/BB/3/YACO'S'/4/VEE#5	CSKHPKV, Palampur
4	DH 40	Saptdhara x HW 3024	CSKHPKV, Palampur
5	DH 776	Pnfjoumee x HPW 143	CSKHPKV, Palampur
	Triticale		
1	TL 1210	-	PAU, Ludhiana
2	TL 2908	TL 2614/JNIT 141	PAU, Ludhiana
3	TL 2920	PBW 189/WHITE RYE/JNIT 128	PAU, Ludhiana
4	DT 123	TR 190/C 306/TR 239/3/TR 170/4/TR 169	IARI, New Delhi
5	TW 9335	PBW 3411/ TR 255	IARI, New Delhi
	Maize		
1.	Early Composite	Composite of Kullu Local, Abaskajar, Maize No. 8, Mex-3CB Bhodipur Yellow JMC 603, UC 1, YUZPSC-3, YUZPSC-4, YUZPSC-71C, YUZP-DC-77, YUZPSC-79C, VC2 and VL42	CSKHPKV, Palampur
2.	*Imperata cylindrica*	Wildly growing weedy grass in the surroundings of Experimental Fields at Palampur	CSKHPKV, Palampur

Table 2. 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 in triticale x wheat derivatives through *Zea mays*- and *Imperata cylindrica*- mediated systems in F_1 generation

Sl.No.	Generation/Crosses	(Triticale x wheat) × *Zea mays*			(Triticale × wheat) × *Imperata cylindrica*		
		sf (%)	ef (%)	pr(%)	sf (%)	ef (%)	pr(%)
	F₁						
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

*$P \leq 0.05$; sf= pseudoseed formation; ef= embryo formation; pr= plant regeneration

Table 4. Performance of various crosses in respect of different haploid induction parameters in triticale x wheat derivatives through *Zea mays*- and *Imperata cylindrica*- systems in F_2 to F_4 generations

S.No.	Generation/Crosses	(Triticale × wheat) × *Zea mays*			(Triticale × wheat) × *Imperata cylindrica*		
		sf (%)	ef (%)	pr(%)	sf (%)	ef (%)	pr(%)
	F_2						
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
	F_3						
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
	F_4						
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

*$P \leq 0.05$; sf= pseudoseed formation; ef= embryo formation; pr= plant regeneration

Table 5. Performance of various crosses in respect of different haploid induction parameters in triticale x wheat derivatives through *Zea mays*- and *Imperata cylindrica*- systems in BC_1F_1 to BC_1F_4 and BC_2F_3 generation

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

*P≤ 0.05; sf= pseudoseed formation; ef= embryo formation; pr= plant regeneration

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 frequency through chromosome elimination technique 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). All the recombinants present in all the generations recorded a sufficient number of embryos with *Zea mays* and *Imperata cylindrica*, they showed a significant difference, which implies that the hybrids are differentially responsive to this trait. 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 F_1, plant regeneration frequency of triticale × wheat derived lines through *Zea mays*- mediated system ranged from 11.25% to 42.57 % in F_1 (Table 1), from 0 to 22.50% in F_2, from 0 to 8.18% in F_3 and from 1.15% to 22.90% in F_4 (Table 2). 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). In all the generations, *Imperata cylindrica* performed far better than the *Zea mays* with respect to plant regeneration. 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 haploid embryo formation frequency through the *I. cylindrica* mediated- chromosome elimination technique was observed significantly higher than the *Z. mays*-mediated system in all the generations. The overall success rate was also significantly higher in the *I. cylindrica* - mediated system of chromosome elimination technique, proving it to be a highly efficient and economically more viable technique of haploid induction in triticale × wheat derivatives compared to the maize- mediated system.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by authors.

REFERENCES

Anonymous, (2020). World Agricultural Production: United States Department of Agriculture.https://www.fas.usda.gov/data/world-agricultural-production.

Bains, N.S., Mangat, G.S., Singh, K. & Nanda, G.S. (1998). A simple technique for the identification of embryo carrying seeds from wheat x maize crosses prior to dissection. *Plant Breeding*, *117*, 191-192.

Chaudhary, H.K., Sethi, G.S., Singh, S., Pratap, A.& Sharma, S. (2005). Efficient haploid induction in wheat by using pollen of *Imperata cylindrica*. *Plant Breeding, 124*(1), 96-98.

Chaudhary, H.K., Singh, S. & Sethi, G.S. (2002). Interactive influence of wheat and maize genotypes on haploid induction in winter x spring wheat hybrids. *Journal of Genetics and Breeding*, *56*, 259-266.

Chaudhary, H.K. (2008). Dynamics of wheat x *Imperata cylindrical*-a new chromosome elimination mediated system for efficient haploid induction in wheat. 11th International Genetics Symposium, Vol. 2. 647-650, University of Sydney Press, Brisbane, Australia.

Chaudhary, H.K., Badiyal, A. & Jamwal N.S. (2015). New Frontiers in Doubled Haploidy breeding in Wheat. *Agriculture Research Journal, 52* (4), 1-12.

Inagaki, M.N. (1985). Chromosome doubling of the wheat haploids obtained with crosses with *Hordeum bulbosum* L. *Japanese Journal of Breeding*, *35*, 193-195.

Kanbar, O.Z., Lantos, C., Chege, P.K., Kiss, E. & Pauk, J. (2020). Generation of doubled haploid lines from winter wheat (*Triticum aestivum* L.) breeding material using in vitro anther culture. *Czech Journal of Genetics and Plant Breeding, 56*, 150−158

Kapoor, C., Chaudhary, H.K., Relan, A., Majoj, N.V., Singh, K. & Sharma, P. (2020). Haploid induction efficiency of diverse Himalayan maize (*Zea mays*) and cogon grass (*Imperata cylindrica*) gene pools in hexaploid and tetraploid wheats and triticale following chromosome elimination-mediated approach of doubled haploidy breeding. *Cereal Research Communications, 48*, 539–545.

Kishore, N., Chaudhary, H.K., Chahota, R.K., Kumar, V., Sood, S.P., Jeberson, S. & Tayeng, T. (2011). Relative efficiency of the maize- and *Imperata cylindrica*- mediated chromosome elimination approaches for induction of haploids of wheat-rye derivatives. *Plant breeding, 130*, 192-194.

Mahato, A. & Chaudhary, H.K. (2015). Relative efficiency of maize and *Imperata cylindrica* for haploid induction in Triticum durum following chromosome elimination-mediated approach of doubled haploid breeding, *Plant Breeding, 134*, 379-383.

Merker, A. (1984). The rye genome in wheat breeding. *Hereditas*, *100*, 183-191.

Murashige, T. & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Phyosiology of Plants,* 15, 473-497.

Pratap, A., Sethi, G.S. & Chaudhry, H.K. (2005). Relative efficiency of different *Gramineae* genera for haploid induction in triticale and triticale x wheat hybrids through chromosome elimination technique. *Plant Breeding, 124,* 147-53.

Sharma, P., Chaudhary, H.K., Manoj, N.V., Singh, K., Relan, A., & Sood, V.K. (2019). Haploid induction in triticale × wheat and wheat × rye derivatives following *imperata cylindrica*-mediated chromosome elimination approach. *Cereal Research Communications, 47*, 701-713.

Warton, D.I. & Hui, F.K.C. (2011) The arcsine is asinine: the analysis of proportions in ecology. *Ecology, 92*, 3–10.