

Research Article

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In vivo haploid induction potential of Himalayan maize (*Zea mays*) and cogon grass (*Imperata cylindrica*) gene pools in different segregational cycles of intra and inter-generic crosses of wheat

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Abstract

Maize and *Imperata cylindrica* have been utilized globally as a pollen source for induction of haploids in wheat through chromosome elimination technique. Pollen parents with a higher haploid induction rate are desired for recovering the high frequency of haploids in wheat and related species. The present investigation was carried out with the aim to assess haploid induction efficiency of diverse germplasm of maize and *I. cylindrica* in different generations of intra and intergeneric crosses of hexaploid and tetraploid wheat and triticale-wheat derivatives. Crosses of twenty-six lines (female) with each of two *I. cylindrica* and twenty-one maize genotypes (testers) were evaluated for four haploid induction parameters viz., pseudoseed formation frequency (PFF), embryo formation frequency (EFF), haploid regeneration frequency (HRF) and haploid formation frequency (HFF). *I. cylindrica* outperformed maize in haploid induction rate with a frequency of embryos formed with *I. cylindrica* (18.39%) were significantly higher as compared to maize (4.08%). In the case of *I. cylindrica* genotype Ic-ye identified best with mean EFF of 30.55, 14.48 and 25.43% for hexaploids, tetraploids and triticale × wheat derivatives, respectively whereas in the case of maize genotype HPMC-60 performed best with EFF of 12.61% for hexaploids, HPMC-58 (12.58%) for tetraploids and HPMC-16 for triticale × wheat derivatives with EFF of 8.91%. *I. cylindrica* genotype Ic-ye and maize genotypes HPMC-14, HPMC-53, HPMC-60, HPMC-64 with significantly positive GCA for haploid induction parameters may be utilized as efficient pollen parents for recovering higher frequency of haploids in wheat.

Introduction

Doubled haploidy is a potent plant breeding technique to significantly shorten the breeding cycle needed to achieve the 'line fixation' stage for enhancing genetic gain needed for the development of new varieties. *In vivo* haploidization through wide hybridization via chromosome elimination is an efficient approach for doubled haploids (DH) production in wheat and related species. Since the first report of the production of haploid plants in wheat (Laurie and Bennett, 1988) the technology has been utilized to produce DH derived wheat lines/varieties (Ho and Jones, 1980; DePauw *et al.*, 2011; Saulescu *et al.*, 2012; Graf *et al.*, 2013; Scheeren *et al.*, 2014; Khan *et al.*, 2017). Haploid induction potential of maize, a well-known cereal crop in wheat (Inagaki and Tahir, 1992; Inagaki and Muzeeb-Kazi, 1995; Zhang *et al.*, 1996; Verma *et al.*, 1999; Singh *et al.*, 2005; Santra *et al.*, 2017) and *Imperata cylindrica*, a rhizomatous grass (Chaudhary *et al.*, 2005; Chaudhary, 2008a, 2008b, 2013; Patial *et al.*, 2017) have been well established, however, the comparative advantage of *I. cylindrica* over maize is evident due to its potential to induce haploids not only in hexaploids but also in tetraploids, triticale × wheat and wheat × rye derivatives in which maize failed to produce desirable results (Chaudhary *et al.*, 2005; Pratap *et al.*, 2005; Chaudhary, 2008a, 2008b, 2013; Kishore *et al.*, 2011; Mahato and Chaudhary, 2015; Kapoor *et al.*, 2020). Apart from bread wheat which covers a major area under wheat, durum wheat is of economic importance and demands for genetic improvement of traits like improved agronomic performance, disease resistance and improved quality. Relatively low frequency of haploid induction in durum wheat has been achieved through wide hybridization which has been mainly attributed to the absence of D genome as well as low ploidy level as compared to hexaploids (Niu *et al.*, 2014). However, success has been achieved in recovering a higher frequency of haploids in durum wheat utilizing *I. cylindrica* as pollen source (Mahato and Chaudhary, 2015). Triticale (× *Triticosecale*) an



amphiploid of wheat and rye is a hardy cereal combining hardiness and nutritional efficiency of rye and high grain yield and nutritional qualities of wheat (Dennett *et al.*, 2013). Hexaploid triticale ($2n = 42 = AABBRR$) is commonly used as bridging species for the transfer of rye traits to wheat due to its cross-compatibility, genomic stability and better adaptation as compared to triticales of other ploidy levels (Ammar *et al.*, 2004; Oettler *et al.*, 2005; Hao *et al.*, 2013). Stable triticale \times wheat-derived lines are desirable having a combination of characters of both the species for better resilience to biotic and abiotic stresses. With increasing area and demand for triticale globally, success achieved through doubled haploidy breeding in hexaploid and tetraploid species needs to be replicated in other economic species of the wheat gene pool for deriving desirable lines in a shorter period. It has been well known that the rate of haploid induction through the chromosome elimination approach depends on the genotype of both male and female parents (Inagaki and Tahir, 1990; Verma *et al.*, 1999; Chaudhary *et al.*, 2002). Diversity in plant genetic resources provides an opportunity for breeders to select for desired traits and further utilized them for various genetic and genomic studies with wider applications. Most of the studies on DH in wheat have either utilized improved maize composites, inbred lines or hybrids as a pollen source. Due to cross-pollinated nature of both maize and *I. cylindrica*, considerable diversity exists as heterozygous populations in remote locations of North-Eastern and North-Western Himalayan region which have rarely been tested for haploid induction potential. Therefore the present study was undertaken with the aim to assess haploid induction efficiency of diverse genotypes of Himalayan maize and *I. cylindrica* in inter and intra-specific crosses of hexaploids, tetraploids and triticales.

Materials and methods

Experimental material

The material for the present investigation consisted of twenty-six lines (female) which comprised of four genotypes of hexaploid wheat, two of tetraploid, two of triticale, seven F_1 s, five BC_1F_1 s and six BC_1F_2 genotypes generated from crossing among the parental lines. The male parents (testers/pollen parents) consisted of twenty-one maize populations collected from different locations of North-Western and North-Eastern Himalayas and two genotypes of *I. cylindrica* growing locally around wheat fields (Tables 1 and 2). Lines were planted in open field conditions at three different sowing dates keeping 15 day interval for better synchronization with the male parents while maize genotypes were raised under polyhouse conditions at two different sowings maintaining 15 days interval. During hybridization period, pollen from maize plants was collected freshly in petri plates from polyhouse sowing while that of *I. cylindrica* was collected from the wild vegetation around wheat fields. All the field activities i.e. generation of F_1 s, backcross populations, wide hybridization with maize and *I. cylindrica* was conducted at the Experimental Farm while embryo culture work was done in Molecular Cytogenetics and Tissue Culture Laboratory (MCTL) of the Department of Crop Improvement, CSK Himachal Pradesh Agricultural University (CSK HPAU), Palampur, Himachal Pradesh, India. Generation advancement and hybridization work for generating intra and inter-varietal crosses was also done during off-season at Highland Agricultural Research & Extension Centre, Kukumseri (Lahaul & Spiti), Himachal Pradesh located at 2672 m amsl.

Wide hybridization work was carried out with maize and *I. cylindrica* following the protocol of Laurie and Bennett (1986, 1988) and Chaudhary *et al.* (2005), respectively.

Methodology

Two spikes of each line were crossed with each of the twenty-one maize and two *I. cylindrica* genotypes. In all total of 1196 spikes were hybridized with both the pollen species (52 spikes per pollen source). Two different concentrations of 2,4-D i.e. 100 ppm for bread wheat type and 250 ppm for durum wheat, triticale and triticale \times wheat crosses were injected in the uppermost internode of culms for three consecutive days at 24, 48 and 72 h after pollination. Doses of 2, 4-D for hexaploids, tetraploids and triticale \times wheat derivatives were standardized based on studies carried out in our lab (Chaudhary *et al.*, 2015; Badiyal *et al.*, 2016). The pseudoseeds were screened for the presence of an embryo (Bains *et al.*, 1998) under a source of light in which embryo positive seeds were retained and carried forward for embryo culture after washing with Tween -20. The embryos were excised carefully from sterilized seeds and embryo culture protocol was performed in a laminar air flow chamber under completely sterile conditions. Embryos were then transferred to test tubes containing MS medium (Murashige and Skoog, 1962) supplemented with 0.5 mg/l kinetin, 150 mg/l glutamine and 20 mg/l each of L-arginine, L-cystine and L-leucine. The pH of the medium was maintained at 5.8 and after autoclaving, the embryos were cultured in 150×20 mm glass tubes. Tubes of cultured embryos were kept at 4°C in dark for 24 h for cold treatment and thereafter shifted to the dark chamber and kept at $20 \pm 2^\circ\text{C}$ until regeneration. The regenerated plantlets were then shifted to the normal light conditions and grown at $20 \pm 2^\circ\text{C}$ with 10/14 h light/dark profile with 75 per cent relative humidity for proper development of plantlets. Plantlets were transferred to a rooting medium containing NAA (Naphthalene acetic acid) and IBA (Indole butyric acid) at three to the four-leaf stage for rooting and then to soil potting mixture for further growth.

Observations recorded and statistical analysis

Observations were recorded on four haploid induction parameters viz., pseudoseed formation frequency (PFF) (i.e. a percentage of a number of pseudoseeds formed out of a total number of florets pollinated), EFF (embryo formation frequency (EFF) i.e. a percentage of a number of pseudoseeds carrying embryo out of a total number of pseudoseeds formed), haploid regeneration frequency (HRF) (i.e. a percentage of haploid embryos regenerated out of a total number of embryos cultured) and haploid formation frequency (HFF) (i.e. a percentage of green haploid plantlets developed out of a total number of florets pollinated). Data recorded on all the four haploid induction parameters were subjected to line \times tester analysis (Kempthorne, 1957). As the data were recorded in per cent it was used for analysis after arcsine transformation (Gomez and Gomez, 1984). Two-sample *t* test (Snedecor and Cochran, 1989) was applied to determine the significant differences among frequencies of haploid induction parameters obtained upon crossing with maize and *I. cylindrica*.

Results

The results showed a variable response of both lines and testers towards haploid induction. Results of the two sample *t* test

Table 1. Parentage and source of female genotypes used for haploid induction

SNo	Genotype	Parentage	Source
<i>Bread Wheat</i>			
1	DH-40	Saptdhara/HW 3024	Molecular Cytogenetics & Tissue culture Lab, Deptt of Crop Improvement, CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh (India)
2	DH-84	VFW449/HPW147	
3	DH-86	WW24/ HW3024	
4	DH-100	WW 10/WW 24	
<i>Durum wheat</i>			
5	PDW-314	CR S-GS S// A-9-30-1/ RAJ911	ICAR-Indian Institute of Wheat & Barley Research (ICAR- IIWBR), Karnal, Haryana (India)
6	A-9-30-1	AJAI12/F3LOCAL(SEL.ETHIO.135.85)// PLATA13/3/SOMAT3/4/SMOOTY/RASCON37	ICAR-Indian Institute of Wheat & Barley Research (ICAR- IIWBR), Karnal, Haryana (India)
<i>Triticale</i>			
7	TL-2900	JNIT 128/GP 288/TL 2729	ICAR-Indian Institute of Wheat & Barley Research (ICAR- IIWBR), Karnal, Haryana (India)
8	TL-2908	TL 2614/JNIT 141	ICAR-Indian Institute of Wheat & Barley Research (ICAR- IIWBR), Karnal, Haryana (India)
<i>F₁S</i>			
9	DH-40 × PDW-314	Newly generated	Molecular Cytogenetics & Tissue culture Lab, Deptt of Crop Improvement, CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh (India)
10	DH-40 × A-9-30-1	Newly generated	
11	TL-2900 × DH-86	Newly generated	
12	TL-2908 × DH-84	Newly generated	
13	TL-2908 × DH-86	Newly generated	
14	DH-40 × DH-100	Newly generated	
15	DH-40 × DH-65	Newly generated	
<i>BC₁F₁</i>			
16	(DH-40 × DH-65) × DH-40	Newly generated	
17	(DH-40 × DH-100) × DH-40	Newly generated	
18	(TL-2900 × DH-86) × DH-86	Newly generated	
19	(TL-2908 × DH-84) × DH-86	Newly generated	
20	(TL-2908 × DH-86) × DH-86	Newly generated	
<i>BC₁F₂</i>			
21	(DH-40 × PDW-314) × DH-40	Newly generated	
22	(DH-40 × A-9-30-1) × DH-40	Newly generated	
23	(DH-40 × DH-65) × DH-40	Newly generated	
24	(TL-2900 × DH-86) × DH-86	Newly generated	
25	(TL-2908 × DH-84) × DH-84	Newly generated	
26	(TL-2908 × DH-86) × DH-86	Newly generated	

Table 2. Parentage and source of pollen parents (male) used for haploid induction

SNo	Genotype code ^a	Village/Location	Latitude	Longitude	Altitude (amsl)
<i>Imperata cylindrica</i>					
1	Ic-ye (yellow anthers)	Holta, Palampur (H.P), India (wild habitation)	32.1067°N	76.5517°E	1345 m
2	Ic-br (brown anthers)	Holta, Palampur (H.P), India (wild habitation)	32.1067°N	76.5517°E	1345 m
Maize					
1	HPMC-3	Gazmoi (Chamba H.P), India	32°31.769'	076°04.855'	1978 m
2	HPMC-11	Gadoh (Chamba H.P), India	32°17.262'	076°37.503'	2119 m
3	HPMC-12	Deol (Chamba H.P), India	32°18.124'	076°35.158'	2121 m
4	HPMC-14	Kuleth (Chamba H.P), India	32°19.464'	076°33.588'	1869 m
5	HPMC-16	Sikkim (North east), India	-	-	-
6	HPMC-18	Sikkim (North east), India	-	-	-
7	HPMC-20	Sikkim (North east), India	-	-	-
8	HPMC-21	Meghalaya (North east), India	-	-	-
9	HPMC-24	Meghalaya (North east), India	-	-	-
10	HPMC-25	Bharmour (Chamba H.P), India	32°26.618'	076°32.217'	2161 m
11	HPMC-27	Changui (L&S, H.P), India	32°27.413'	076°29.471'	1750 m
12	HPMC-28	Changui (L&S, H.P), India	32°27.413'	076°29.471'	1750 m
13	HPMC-53	Shenoor (L&S, H.P), India	32°42.020'	076°41.285'	2717 m
14	HPMC-56	Kugi (L&S, H.P), India	32°43.780'	076°38.481'	2684 m
15	HPMC-57	Shour (L&S, H.P), India	32°43.781'	076°38.478'	2500 m
16	HPMC-58	Shour (L&S, H.P), India	32°43.781'	076°38.478'	2500 m
17	HPMC-59	Chhow, Purthi, (L&S, H.P), India	32°43.781'	076°38.478'	2499 m
18	HPMC-60	Phindru(L&S, H.P), India	33°01.267'	076°25.890'	2186 m
19	HPMC-62	Karel, Kilad (L&S, H.P), India	33°05.949'	076°23.235'	2530 m
20	HPMC-63	Nighar(L&S, H.P), India	33°04.835'	076°24.457'	2613 m
21	HPMC-64	Choki, Kilad(L&S, H.P), India	33°04.889'	076°24.456'	2640 m

^aHPMC, Himachal Pradesh maize Collection; L&S, Lahaul & Spiti.

showed significant differences between both the pollen species i.e. *I. cylindrica* and maize for EFF and HFF when pooled over all the lines. Mean PFF, EFF, HRF and HFF obtained with both the pollen species is shown in Table 3. Mean PFF obtained with *I. cylindrica* and maize were 54.07 and 49.24%, respectively. Almost at par values were recorded for HRF with *I. cylindrica* and maize (33.60 and 35.09%, respectively). However, the frequency of embryos obtained with *I. cylindrica* (18.39%) was significantly higher as compared to maize (4.08%). In the case of HFF also *I. cylindrica* outperformed maize with a frequency of 3.85% as compared to 1.16% obtained with maize. The highest frequency of pseudoseeds with *I. cylindrica* were recorded in TL-2908 × DH-86 × DH-86(BC₁F₂) while in the case of maize it was with DH-84 (79.62%) and DH-100 (79.48%). No pseudoseeds were formed in triticale parental line TL-2908 and F₁s of triticale × wheat when crossed with *I. cylindrica* while the same were obtained in all the genotypes crossed with maize with very low frequency. Cross DH-40 × DH-65 (F₁) was most responsive to embryo formation (64.71%) with *I. cylindrica* followed by DH-100 (41.3%), while with maize highest percentage of embryos were obtained in genotype DH-84 (11.6%), followed by DH-86 (9.97%), PDW-314 (9.70%) and A-9-30-1 (9.67%). Genotypes DH-40,

triticales, F₁s of triticale × wheat and BC₁F₁ of (TL2900 × DH86) × DH86 remained unresponsive to embryo formation with *I. cylindrica* while embryos were recovered in all the genotypes crossed with maize except triticale genotype TL-2908. All the embryos obtained with *I. cylindrica* in crosses DH-40 × A-9-30-1 × DH-40 (BC₁F₂), DH-40 × PDW-314 (F₁) and DH-40 × A-9-30-1 (F₁) were able to regenerate, while with maize highest regeneration was recorded in embryos recovered from tetraploid genotypes PDW-314 (68.57%) and A-9-30-1 (66.08%).

When the performance of both the pollen species was compared across generations, *I. cylindrica* outperformed maize in all the haploid induction parameters (Table 3). Comparatively maximum embryo induction frequency was observed with *I. cylindrica* in hexaploid × hexaploid F₁s (+27.84%) followed by hexaploid × hexaploid BC₁F₁ (+27.36%) and in hexaploid parents (+19.58%) as compared to maize. However, F₁s of hexaploid × tetraploid and triticales showed slightly better results with maize. Comparatively higher HFF was observed with *I. cylindrica* in hexaploid × hexaploid BC₁F₁ (+5.76%) followed by hexaploid wheat parents (+5.18%). With *I. cylindrica* highest frequency of haploid plants were recovered in genotype DH-100 (11.07%), DH-86(9.56%), DH-40 × DH-65 × DH-40 (BC₁F₁)

Table 3. Mean haploid induction frequencies in different generations obtained with twenty-one maize and two Imperata genotypes (Ic)

Genotype	PFF (%)		EFF (%)		HRF (%)		HFF (%)	
	Ic	Maize	Ic	Maize	Ic	Maize	Ic	Maize
Hexaploid wheat								
DH 40	86.49	76.77	0	2.70	0	16.27	0	0.54
DH 84	73.52	79.62	36.21	11.60	28.57	48.24	8.97	3.65
DH 86	82.35	73.44	35.71	9.97	25.52	56.60	9.56	2.75
DH 100	79.40	79.48	41.30	5.42	27.83	54.37	11.07	1.95
Mean	80.44	77.33	28.31	7.42	20.48	43.87	7.40	2.22
Durum wheat								
PDW 314	45.75	42.92	35.48	9.70	32.39	68.57	4.43	1.71
A-9-30-1	57.40	64.26	26.09	9.67	34.38	66.08	5.63	3.07
Mean	51.58	53.59	30.79	9.69	33.39	67.33	5.03	2.39
Triticales								
TL 2900	0	1.96	0	4.76	0	4.76	0	0.07
TL 2908	15.51	11.95	0	0	0	0	0	0
Mean	7.76	6.96	0.00	2.38	0.00	2.38	0.00	0.04
F₁s								
DH 40 × PDW 314	42.24	77.12	2.94	3.67	100	50.79	1.46	1.47
DH 40 × A-9-30-1	80.65	71.90	1.92	3.90	100	33.10	1.61	1.26
TL 2900 × DH86	0	3.94	0	2.38	0	4.76	0	0.05
TL 2908 × DH 84	0	9.57	0	5.04	0	4.76	0	0.06
TL 2908 × DH 86	7.39	15.19	10	1.78	75.00	6.67	1.19	0.19
DH 40 × DH100	84.38	73.84	9.26	6.80	30	45.24	3.91	2.41
DH 40 × DH65	25.28	75.36	64.71	2.81	32.39	44.05	3.96	1.01
Mean	34.28	46.70	12.69	3.77	48.20	27.05	1.73	0.92
BC₁F₁s								
TL2900 × DH86 × DH86	46.32	28.83	0	2.66	0	38.10	0	0.70
TL2908 × DH84 × DH84	77.01	29.48	23.91	1.66	31.52	26.53	6.70	0.54
TL2908 × DH86 × DH86	66.41	31.48	26.67	1.92	29.17	33.33	5.47	0.53
DH40 × DH65 × DH40	86.33	73.61	25.37	4.39	31.37	51.31	9.17	2.12
DH40 × DH100 × DH40	78.17	62.02	35.71	4.92	26.97	51.79	6.38	1.92
Mean	70.85	45.08	22.33	3.11	23.81	40.21	5.54	1.16
BC₁F₂s								
DH 40 × PDW314 × DH40	18.27	47.90	22.22	0.40	58.33	11.90	2.88	0.23
DH40 × A-9-30-1 × DH40	67.91	49.07	2.22	0.82	100	23.81	1.51	0.40
TL2900 × DH86 × DH86	41.22	49.79	12.50	2.51	32.50	45.24	2.03	0.97
TL2908 × DH84 × DH84	66.20	38.65	31.11	1.55	25.00	30.95	5.36	0.52
TL2908 × DH86 × DH86	94.22	43.11	15.94	2.92	34.47	50	5.24	1.06
DH40 × DH65 × DH40	83.34	68.95	18.75	2.05	18.06	45.00	3.54	0.96
Mean	61.86	49.58	17.12	1.71	44.73	34.48	3.43	0.69
SE ±	6.15	5.09	3.34	0.61	5.93	4.00	0.66	0.20

Values in Bold are group wise mean values.

and DH-84(8.97%), while with maize maximum recovery percentage was recorded in DH-84 (3.65%) followed by A-9-30-1 (3.07%).

General combining ability (GCA) of both male and female genotypes was computed to find out the best general combiners for various haploid induction parameters (Table 4 and

Table 4. GCA effects for haploid induction parameters of maize and Imperata genotypes

Male genotypes	PFF	EFF	HRF	HFF
HPMC-3	-4.94	-2.37	-10.54	-1.42
HPMC-11	2.26*	-1.54	5.56*	0.10
HPMC-12	-5.77	-1.44	-6.44	-0.82
HPMC-14	-2.72	0.48	5.31*	1.11*
HPMC-16	1.46	-1.09	0.57	-0.17
HPMC-18	-1.51	0.36	-3.84	-0.59
HPMC-20	-0.15	-4.66	-11.07	-2.15
HPMC-21	1.88*	-4.43	-6.52	-2.09
HPMC-24	-3.43	-3.58	-8.36	-0.85
HPMC-25	-3.65	1.85	0.20	0.07
HPMC-27	-5.07	-2.17	-3.11	-0.23
HPMC-28	-10.62	-5.69	-20.95	-3.14
HPMC-53	5.32*	3.25*	8.92*	0.72
HPMC-56	0.68	-3.54	0.40	-0.73
HPMC-57	3.53*	1.39	11.84*	0.05
HPMC-58	-0.35	-1.22	-8.54	0.21
HPMC-59	1.53	-3.21	-11.18	-2.42
HPMC-60	7.58*	0.98	8.19*	1.58*
HPMC-62	2.76*	-1.85	6.49*	-0.84
HPMC-63	2.19	-0.58	16.50*	0.41
HPMC-64	2.61*	0.41	22.67*	1.39*
Ic-ye	2.87*	16.48*	3.27	6.05*
Ic-br	3.53*	12.17*	0.64	3.75*
SE ±	0.86	1.05	2.10	0.41

*Significant at 5% level of significance. Values in Bold are group wise mean values.

Supplementary Table 4). Among the testers, genotypes with significantly positive GCA for pseudo seed frequency were HPMC-11, HPMC-21, HPMC-53, HPMC-57, HPMC-60, HPMC-62, HPMC-64, Ic-ye and Ic-br. The highest values of GCA for PFF were recorded with HPMC-60 (7.58) and HPMC-53 (5.32), whereas GCA values of *I. cylindrica* genotypes for the same were 3.53 and 2.87 for Ic-br and Ic-ye, respectively. Among lines, significantly positive GCA values for PFF were recorded for DH-40, DH-84, DH-86, DH-100, A-9-30-1, DH-40 × PDW-314, DH-40 × A-9-30-1, DH-40 × DH-100, DH-40 × DH-65, DH-40 × DH-65 × DH-40 (BC₁F₁), DH-40 × DH-100 × DH-40 (BC₁F₁) and DH-40 × DH-65 × DH-40 (BC₁F₂). In the case of embryo formation, GCA of both the *I. cylindrica* genotypes was significantly higher as compared to maize (16.48 and 12.17%, respectively for Ic-ye and Ic-br) while in the case of maize only one genotype HPMC-53 was having significantly positive GCA value (3.25) for embryo formation. Among lines DH-84, DH-86, DH-100, PDW-314, A-9-30-1, DH-40 × DH-100 (F₁) and its BC₁F₁, DH-40 × DH-65 (F₁) and its BC₁F₁ were having positively significant GCA for EFF. In the case of HRF maize genotypes HPMC-11, HPMC-14, HPMC-53, HPMC-57, HPMC-60, HPMC-62, HPMC-63 and HPMC-64 possessed positive GCA while

among lines all the parental lines except DH-40, TL-2900 and TL-2908, DH-40 × PDW-314 (F₁), DH-40 × DH-100 (F₁), DH-40 × DH-65(F₁) and their BC₁F₁s, BC₁F₂s of (DH-40 × DH-65) × DH-40, (TL-2900 × DH-86) × DH-86 and (TL-2908 × DH-86) × DH-86 were having positively significant GCA. In the case of HFF tester genotypes HPMC-14, HPMC-60, HPMC-64, Ic-ye and Ic-br were having positively significant GCA with the highest values for Ic-ye (6.05) and Ic-br (3.75) while among maize significant GCA values were recorded in HPMC-60 (1.58), HPMC-64 (1.39) and HPMC-14 (1.11). Among lines DH-84, DH-86, DH-100, A-9-30-1, PDW-314, DH-40 × PDW-314 (F₁), DH-40 × DH-100(F₁), BC₁F₁ of (DH-40 × DH-100) × DH-40 and (DH-40 × DH-65) × DH-40 recorded positive GCA for HFF.

Discussion

In vivo haploid induction in wheat via chromosome elimination mediated approach has been followed globally using maize and *I. cylindrica* as an efficient pollen source. This system of haploid induction have been successful and surpassed several bottlenecks associated with in-vitro methods. Furthermore, the superiority of *I. cylindrica* over maize is evident due to its potential to induce haploids not only in hexaploid wheat but in tetraploids, triticales × wheat and wheat-rye derivatives which have been already proved in earlier studies (Chaudhary *et al.*, 2005; Kishore *et al.*, 2011; Badiyal *et al.*, 2014; Mahato and Chaudhary, 2015; Kapoor *et al.*, 2020) and further supported by our results in the present study where the frequency of haploids obtained with *I. cylindrica* are significantly higher as compared to maize. Moreover, its natural coincidence of flowering with wheat and ample pollen shed advocates its advantage over maize which has to be raised and managed under protected conditions during the winter season, especially in sub-temperate areas.

Results obtained by hybridization of diverse lines with both the pollen species indicate the superiority of *I. cylindrica* over maize in haploid induction. The mean frequency of embryos and green haploid plantlets obtained upon crossing with *I. cylindrica* were 20.34 and 3.71%, respectively as compared to frequencies of 3.90 and 1.14% obtained with maize. Although both the *I. cylindrica* genotypes (Ic-ye and Ic-br) were statistically at par in their haploid induction efficiencies but the value of mean frequencies obtained with genotype Ic-ye was relatively higher as compared to Ic-br, thus genotype Ic-ye can be utilized as a relatively efficient pollen parent over Ic-br. Genetic background of *I. cylindrica* influence the haploid induction efficiency in wheat (Rather *et al.*, 2014). Poor response of triticales lines to embryo formation may be attributed to failure to form pseudo seeds due to poor imbibition of post pollination treatment of growth hormone 2,4-D required to nurse the developing embryo. Backcross progenies obtained in triticales-wheat derivatives responded well to embryo formation as well as green haploid plantlets. A higher success rate in later segregating generations is mainly due to an increase in the frequency of D genome in triticales-wheat derived lines results in improving the morphology of plants similar to hexaploid lines. Poor response of pure triticales and triticales F₁s is due to genomic incompatibilities of wheat (AABBDD) and rye (AABBRR) genomes. D genome has been reported to be the triggering factor in chromosome elimination and haploid induction in wheat × *I. cylindrica* system (Mukai *et al.*, 2015). In a similar study, Kaila (2013) reported paternal chromosome elimination in wheat × *I. cylindrica* hybrids as the outcome of B and D

genomes of hexaploid wheats, where the relative effect of D genome is higher than the B genome of hexaploid wheat. Potential of *I. cylindrica* to induce a higher frequency of haploid embryos in durum wheat have been reported by Mahato and Chaudhary (2015). Unlike parental durum wheat lines, crosses with durum wheat were less responsive to haploid induction which may be due to the formation of F₁ pentaploid hybrids having a unique chromosomal constitution. Parental combinations used in pentaploids crosses generally influence the degree to which D genome chromosomes are retained (Padmanabhan *et al.*, 2017). On the basis of potential to induce embryos, in case of *I. cylindrica*, genotype Ic-ye identified best with mean EFF of 30.55, 14.48 and 25.43%, respectively for hexaploids, tetraploids and triticales × wheat derivatives whereas in case of maize for hexaploids HPMC-60 identified best with EFF of 12.61%, for tetraploids HPMC-58 (12.58%) and for triticales × wheat derivatives HPMC-16 performed relatively better with 8.91% EFF. Eight maize genotypes i.e. HPMC-16, HPMC-25, HPMC-27, HPMC-53, HPMC-56, HPMC-58, HPMC-60 and HPMC-64 were early flowering thereby providing an opportunity to initiate crossing work early in the season and thus prolonging the period of wide hybridization for enhancing the haploid induction efficiency. In a similar study, Kapoor *et al.* (2020) identified Himalayan maize and cogon grass genotypes with higher haploid induction efficiencies where hybridization with eight parental lines comprised of four genotypes of hexaploid wheat, two each of tetraploid wheat and triticales. Significant differences were observed in haploid induction potential among Himalayan maize genotypes in earlier studies in hexaploid wheat (Dhiman *et al.*, 2012) and in durum wheat (Mahato and Chaudhary, 2015). Significant variation for haploid induction in wheat has also been observed among maize hybrids, inbred lines and improved populations (Verma *et al.*, 1999; Chaudhary *et al.*, 2002; Kour *et al.*, 2008). On the basis of recovery of green haploid plantlets in crossed generations, BC₁F₁ generation of hexaploid × hexaploid followed by BC₁F₂ of hexaploid × hexaploid, triticales × wheat (BC₁F₂) and triticales × wheat (BC₁F₁) responded best.

Results of the study put forth superiority of *I. cylindrica* over maize in haploid induction in hexaploid and tetraploid wheat, triticales and their crosses signifying its potential to induce haploids over different ploidy groups and species. The reason seems most plausible for *I. cylindrica* to induce a higher frequency of haploids is due to better genomic compatibility with wheat including members of its gene pool and relatively longer pollen viability due to its hardy nature. Maize populations used in the study varied in haploid induction potential. Although overall maize genotypes have relatively low haploid induction potential as compared to *I. cylindrica*, maize genotypes having positive GCA for haploid induction parameters can be utilized as efficient genotypes. Sub-temperate areas where *I. cylindrica* flourishes well in the wild shall be utilized as a pollen source for wide hybridization with wheat for recovering higher frequency of haploid embryos with preference to genotype Ic-ye, whereas maize genotypes HPMC-14, HPMC-53, HPMC-60, HPMC-64 may be utilized for haploid induction through wheat × maize system of haploid induction. Maize and *I. cylindrica* genotypes with high haploid induction efficiency identified in the study are valuable genetic resources and need to be conserved both in-situ and ex-situ for long term conservation after proper characterization and documentation. Maize genotypes can be shared among workers after germplasm registration. Further cryo-preservation studies on the pollen of *I. cylindrica* may be carried out for making its viable

pollen available to labs where *I. cylindrica* is not available for carrying out wide hybridization with wheat.

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