



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

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Abstract

To assess relative efficiency and identify efficient pollen source among maize and *Imperata cylindrica* for haploid induction, 21 maize and two *Imperata cylindrica* genotypes were hybridized as pollen parent with each of the four hexaploid, two tetraploid wheats and two genotypes of triticale following wheat × maize and wheat × *Imperata cylindrica* system of haploid induction. Observations for haploid induction parameters showed *Imperata cylindrica* as significantly superior pollen source than maize. Mean PFF, EFF, HRF and HFF obtained with *Imperata* were 55.05%, 24.07%, 18.58% and 4.96%, respectively, whereas the frequencies recorded with maize were 53.80%, 6.72%, 39.36% and 1.71%, respectively. Triticale remained nonresponsive to embryo formation with both the pollen species. The line × tester analysis showed significant effects of both male and female genotypes on all the haploid induction parameters. Analysis of GCA revealed differences among pollen sources in their potential to induce haploids. In maize, genotypes HPMC-57, HPMC-58, HPMC-25 for EFF and HPMC-58 for HFF emerged out to be best general combiners, while in the case of *Imperata*, GCA values were high for Ic-ye genotype. The present study put forth *Imperata* as an efficient pollen source over maize for haploid induction in hexaploid and tetraploid wheats.

Keywords Doubled haploids · *Imperata* · Embryo culture · GCA

Introduction

Doubled haploidy (DH) breeding has revolutionized wheat improvement programs and has been a routine method for production of doubled haploids globally. The merit involved in this protocol is speedy fixation of alleles or alien chromatin, thereby making available homozygous lines in a single step surpassing lengthy process and duration for achieving

the homozygosity needed for varietal release and various molecular studies. Doubled haploid production through chromosome elimination technique utilizing maize as pollen source following wheat × maize system has been successfully employed for rapid generation of doubled haploids in wheat (Barclay 1975; Laurie and Bennett 1988, Niu et al. 2014). The wheat × maize system has proved to be superior to *bulbosum* method (Kasha and Kao 1970) as the maize pollen is insensitive to the dominant crossability inhibitor genes in wheat (Laurie and Reymondie 1991). However, a decade back, *Imperata cylindrica* (cogon grass) was identified as more efficient pollen source than maize (Chaudhary et al. 2005) due to its potential to induce haploids not only in bread wheat but also in durum wheat, triticale × wheat crosses and wheat–rye derivatives (Pratap et al. 2005; Kishore et al. 2011 and Mahato and Chaudhary 2015). Unlike maize, its perennial nature, wild habitation, natural coincidence of flowering with wheat and ample pollen shed during wheat season do not demand to raise pollen source under artificial

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conditions. Apart from bread wheat, durum wheat is also of economic importance and demands improvement for various limiting traits; however, the success achieved in obtaining double haploids in durum wheat is low due to its genotypic specificity (Jauhar 2003; Mujeeb-Kazi et al. 2006). Triticale (\times *Triticosecale* Wittm.), a man-made cereal, incorporates alleles from both wheat and rye allowing better adaptation to less favorable environments and also provides better biomass and quality forage. There is a vast scope for improvement in wheat and its related species through doubled haploidy breeding for generating new varieties and also developing triticale \times wheat derivatives. Most of the studies on DH in wheat have utilized maize hybrids and improved composites and inbred lines for haploid induction. Scanty research work has been done on elucidating the potential of local maize germplasm (OPVs) for haploid induction in wheat and its related species. Due to cross-pollinated nature of both the pollen sources, highly heterogeneous populations of maize are available in farmers' field and of cogon grass in wild. Therefore, this study has been undertaken to assess relative efficiency of both maize and cogon grass populations for haploid induction and locate most responsive genotypes in both the pollen sources for the same.

Materials and methods

The experimental material for the present study consisted of eight female genotypes which comprised four genotypes of hexaploid wheat, viz. DH40, DH84, DH86 and DH100, two of tetraploid wheat, i.e., PDW314 and A-9-30-1, and two of triticale, i.e., TL2900 and TL2908 (Table 1). Two genotypes of *I. cylindrica* (Ic-ye and Ic-br, with yellow- and brown-colored anthers, resp.) and 21 maize populations (OPVs) collected from different locations of northwestern and northeastern Himalayan region were utilized as pollen source (Table 2). The hybridization program of maize and *Imperata* pollen with wheat lines was executed at the

experimental farm of Crop Improvement, CSK Himachal Pradesh Agriculture University, Palampur, India. The female lines were raised in open-field conditions at three different sowing dates to synchronize flowering with the pollen source. Maize populations were raised artificially under polyhouse conditions to avoid cold injury in open field during winter month while pollen of *Imperata* was collected from the wild vegetation around wheat fields.

Two spikes of each of the eight female lines were crossed with each of the 21 maize and two *I. cylindrica* pollen parents during 2015–2016 and 2016–2017 as per protocol of Chaudhary et al. (2005). The pollinated spikes were injected with 100 ppm of 2,4-D in the case of hexaploid wheat and 250 ppm for durum and triticale in the uppermost internode of the wheat culms for three consecutive days at 24 h, 48 h and 72 h post-pollination for in vivo embryo and pseudo-seed formation. The pollinated spikes were harvested after 18–20 post-pollination from the base of the tiller and then screened under light for the presence or absence of embryo (Bains et al. 1998). The embryo-carrying seeds were then carried forward for embryo rescue. These embryos were then excised and cultured in MS media (Murashige and Skoog 1962) supplemented with 0.5 mg/l kinetin, 150 mg/l glutamine and 20 mg/l each of L-arginine and L-leucine under aseptic conditions in a laminar airflow maintaining pH of the culture media at 5.8 and then autoclaved before culturing. Embryo-containing culture tubes (150 \times 20 mm) were first placed under cold temperature (4 °C) for 24 h and then incubated at 20 °C in a 10-h light and 14-h dark photoperiod until embryos regenerate. The regenerated plantlets were transferred to rooting media containing NAA (naphthalene acetic acid) and IBA (indole butyric acid) and then shifted to potting mixture for further growth. Observations were recorded for various haploid induction parameters, viz. PFF (pseudoseed formation frequency), i.e., endospermless seeds with embryo floating inside 2,4-D injected solution (number of pseudoseeds formed \times 100/number of florets pollinated), EFF (embryo formation frequency), i.e., number of

Table 1 Parentage and source of diverse female genotypes

S. no	Genotype	Ploidy	Parentage	Source
1	DH40 (bread wheat)	Hexaploid	Saptadhara/HW 3024	Molecular cytogenetics & Tissue Culture Laboratory, Dept. of Crop Improvement, CSK HPKV, Palampur (H.P)
2	DH84 (bread wheat)	Hexaploid	VWFW449/HPW147	
3	DH86 (bread wheat)	Hexaploid	WW24/HW3024	
4	DH100 (bread wheat)	Hexaploid	WW 10/WW 24	
5	PDW314 (durum wheat)	Tetraploid	CR S-GS S//A-9-30-1RAJ911	ICAR-IIWBR, Karnal, Haryana
6	A-9-30-1 (durum wheat)	Tetraploid	AJAIA12/F3LOCAL (SEL.ETHIO.135.85)// PLATA13/3/OMAT3/4/SMOOTY/RAS-CON37	ICAR-IIWBR, Karnal, Haryana
7	TL2900 (Triticale)	Hexaploid	JNIT 128/GP 288/TL 2729	ICAR-IIWBR, Karnal, Haryana
8	TL2908 (Triticale)	Hexaploid	TL 2614/JNIT 141	ICAR-IIWBR, Karnal, Haryana

Table 2 Source of *Imperata cylindrica* and maize genotypes used as pollen parents

S. no	Genotype code*	Village/location	Latitude	Longitude	Altitude (amsl)
<i>I. cylindrica</i>					
1	Ic-ye	Holta, Palampur (H.P.) (naturally growing around wheat fields)	32° 11.054'	76° 53.62'	1268
2	Ic-br	Holta Palampur (H.P.) (naturally growing around wheat fields)	32° 11.054'	76° 53.62'	1268
<i>Maize</i>					
1	HPMC-3	Gazmoi (Chamba H.P)	32° 31.769'	076° 04.855'	1978 m
2	HPMC-11	Gadoh (Chamba H.P)	32° 17.262'	076° 37.503'	2119 m
3	HPMC-12	Deol (Chamba H.P)	32° 18.124'	076° 35.158'	2121 m
4	HPMC-14	Kuleth (Chamba H.P)	32° 19.464'	076° 33.588'	1869 m
5	HPMC-16	Sikkim (northeast)	–	–	–
6	HPMC-18	Sikkim (northeast)	–	–	–
7	HPMC-20	Sikkim (northeast)	–	–	–
8	HPMC-21	Meghalaya (northeast)	–	–	–
9	HPMC-24	Meghalaya (northeast)	–	–	–
10	HPMC-25	Bharmour (Chamba H.P)	32° 26.618'	076° 32.217'	2161 m
11	HPMC-27	Changui (L&S, H.P)	32° 27.413'	076° 29.471'	1750 m
12	HPMC-28	Changui (L&S, H.P)	32° 27.413'	076° 29.471'	1750 m
13	HPMC-53	Shenoor (L&S, H.P)	32° 42.020'	076° 41.285'	2717 m
14	HPMC-56	Kugi (L&S, H.P)	32° 43.780'	076° 38.481'	2684 m
15	HPMC-57	Shour (L&S, H.P)	32° 43.781'	076° 38.478'	2500 m
16	HPMC-58	Shour (L&S, H.P)	32° 43.781'	076° 38.478'	2500 m
17	HPMC-59	Chhow, Purthi, (L&S, H.P)	32° 43.781'	076° 38.478'	2499 m
18	HPMC-60	Phindru(L&S, H.P)	33° 01.267'	076° 25.890'	2186 m
19	HPMC-62	Karel, Kilad (L&S, H.P)	33° 05.949'	076° 23.235'	2530 m
20	HPMC-63	Nighar(L&S, H.P)	33° 04.835'	076° 24.457'	2613 m
21	HPMC-64	Choki, Kilad (L&S, H.P)	33° 04.889'	076° 24.456'	2640 m

*Ic-ye yellow-colored anthers; Ic-br brown-colored anthers; L&S Lahaul & Spiti; H.P. Himachal Pradesh; HPMC Himachal Pradesh Maize Collection

pseudoseeds carrying embryo $\times 100$ /total number of pseudoseeds formed, HRF (haploid regeneration frequency), i.e., number of haploid embryos regenerated $\times 100$ /total number of embryos cultured and HFF (haploid formation frequency), i.e., number of haploid plantlets developed $\times 100$ /total number of florets pollinated. The data recorded on various parameters were subjected to line \times tester analysis as per Kempthorne (1957) after arcsine transformation (Gomez and Gomez 1984).

Results

Analysis of variance for line \times tester design indicated that mean squares due to crosses, females and male genotypes were significantly different for all the four haploid induction parameters when tested against error mean squares (Table 3) signifying significant variability and also differential response to the haploid induction parameters. Significant

variation was observed for haploid induction frequencies for both male and female genotypes. Both the *Imperata* genotypes were statistically at par for all the haploid induction efficiencies; however, the mean frequencies of haploid induction parameters obtained with Ic-ye were higher as compared to Ic-br (Table 4). The mean frequencies of PFF, EFF, HRF and HFF obtained with Ic-yeas pollen source were 55.78%, 26.29%, 22.53% and 6.55%, respectively, while the frequencies obtained with Ic-br were 54.32%, 21.85%, 14.64% and 3.37%, respectively. In all, the mean frequencies obtained with *I. cylindrica* as pollen source were 55.05%, 24.07%, 18.58% and 4.96% for PFF, EFF, HRF and HFF, respectively. The highest PFF was recorded in DH40 (86.49%) followed by DH86 (82.35%), while the highest EFF was recorded in DH100 (48.91%) followed by DH86 (43.75%) and DH84 (41.38%). Maximum regeneration frequency was obtained with durum wheats A-9-30-1 and PDW314 (34.37% and 32.38%, respectively), whereas maximum haploid formation frequency was observed in

Table 3 Line × tester analysis for pseudoseed formation, embryo formation, haploid regeneration and haploid formation efficiency in wheat and triticale using *I. cylindrica* and maize as pollen sources

MS					
Source	df	Pseudoseed formation frequency	Embryo formation frequency	Haploid regeneration frequency	Haploid formation efficiency
Crosses	183	1111.83**	163.75**	1208.68**	8.38**
Female genotypes	7	22747.68**	638.20**	16516.99**	60.87**
Male genotypes	22	434.78**	302.30**	1383.63**	18.86**
Female × male interaction	154	225.10**	122.39**	487.86**	4.50**
Error	920	183.47	12.08	158.75	0.91

df degree of freedom; *P: ≤ 0.05 and **: ≤ 0.01 (tested against EMS)

Table 4 Frequency of haploid induction parameters in various female genotypes using *I. cylindrica* genotypes

S. no.	Genotypes	PFF (%)		EFF (%)		HRF (%)		HFF (%)	
		Ic-ye	Ic-br	Ic-ye	Ic-br	Ic-ye	Ic-br	Ic-ye	Ic-br
1	DH 40	90.63*	82.35	0.00	0.00	0.00	0.00	0.00	0.00
2	DH 84	76.32	70.73	46.55	36.21	33.33	23.81	11.84*	6.10*
3	DH 86	82.35	82.35	51.79*	35.71	31.03	20.00	13.24*	5.88
4	DH 100	76.67	82.14	56.52*	41.30*	34.62	21.05	15.00*	7.14*
5	PDW 314	48.44	43.06	25.81	35.48	37.50	27.27*	4.69	4.17
6	A-9-30-1	58.70	56.10	29.63	26.09	43.75	25.00	7.61	3.66
7	TL 2900	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	TL 2908	13.16	17.86	0.00	0.00	0.00	0.00	0.00	0.00
	Mean	55.78	54.32	26.29	21.85	22.53	14.64	6.55	3.37
	SE±	11.78	11.20	8.51	6.56	6.72	4.36	2.23	1.06

hexaploid genotype DH100 (11.07%) followed by DH86 (9.56%). Mean embryo formation frequencies (EFFs) with hexaploids and tetraploids were 33.51% and 29.25%, respectively, whereas triticales remained nonresponsive to embryo formation with *Imperata*.

In the case of hybridization with maize, DH84 (79.62%) was most responsive to PFF followed by DH100 (79.48%) and DH40 (76.77%), while in the case of EFF, DH84 was most responsive (11.60%). In triticale, genotype TL2900 embryos were formed at frequency of 4.76%. The highest regeneration frequency was observed in durum wheat genotypes PDW314 and A-9-30-1 (68.57% and 66.08%, respectively). Maximum frequency of green haploid plants was recorded in DH84 (3.65%) followed by A-9-30-1 (3.07%) (Table 5).

Maize genotypes differ in their haploid induction efficiency when pooled over all the wheat genotypes. Mean frequencies of PFF, EFF, HRF and HFF obtained with maize were 53.08%, 6.73%, 39.36% and 1.72%, respectively (Table 6). PFF ranged from 41.28% to 74.19%, and the highest frequency was obtained with maize genotype HPMC-60. The lowest EFF was recorded with HPMC-21 (3.03%) while highest with HPMC-57 (16.34%) followed by HPMC-58 (14.70%), HPMC 25 (11.13%) and HPMC53 (10.16%). The highest regeneration frequency was recorded with HPMC-57 (64.58%) and HPMC-62 (58.33%). Maximum green haploid

Table 5 Mean performance of various female genotypes for response to various haploid induction parameters pooled over all the maize genotypes

S. no.	Genotypes	PFF (%)	EFF (%)	HRF (%)	HFF (%)
1	DH 40	76.77	2.70	16.27	0.54
2	DH 84	79.62	11.60	48.24	3.65
3	DH 86	73.44	9.97	56.60	2.75
4	DH 100	79.48	5.42	54.37	1.95
5	PDW 314	42.92	9.70	68.57	1.71
6	A-9-30-1	64.26	9.67	66.08	3.07
7	TL 2900	1.96	4.76	4.76	0.07
8	TL 2908	11.95	0.00	0.00	0.00
	Mean	53.80	6.72	39.36	1.71

plants recovered in crosses with HPMC-58 (5.83%) followed by HPMC-25 and HPMC-24 (2.91% each).

Both maize and *Imperata cylindrica* differ in their general combining ability (GCA) for various haploid induction parameters (Table 7). The highest positive GCA in the case of testers for PFF was recorded in HPMC-60 (20.28) followed by HPMC-58 (8.66) and HPMC-18 (7.38). In the case of EFF, the highest positive GCA was recorded in crosses with Ic-ye (18.05) followed by Ic-br (13.61). Genotypes HPMC-57 (8.11), HPMC-58 (6.46), HPMC-25 (2.90) and

Table 6 Mean performance of various maize genotypes for response to various haploid induction parameters pooled over all the wheat genotypes

S. no	Genotypes	PFF (%)	EFF (%)	HRF (%)	HFF (%)
1	HPMC-3	49.07	5.82	21.04	1.43
2	HPMC-11	49.72	5.74	42.71	1.64
3	HPMC-12	60.10	7.03	28.27	1.46
4	HPMC-14	56.84	5.64	33.19	1.84
5	HPMC-16	42.40	3.33	40.63	1.13
6	HPMC-18	61.29	8.06	29.17	1.38
7	HPMC-20	54.94	3.77	30.68	0.98
8	HPMC-21	41.28	3.03	27.08	0.62
9	HPMC-24	55.71	7.78	48.58	2.91
10	HPMC-25	47.02	11.13	32.29	1.10
11	HPMC-27	51.94	8.31	34.62	2.91
12	HPMC-28	57.88	3.93	17.71	0.56
13	HPMC-53	60.00	10.16	44.38	2.60
14	HPMC-56	58.42	3.97	52.08	1.80
15	HPMC-57	48.37	16.34	64.58	1.03
16	HPMC-58	62.57	14.70	41.37	5.83
17	HPMC-59	52.13	4.50	36.31	0.59
18	HPMC-60	74.19	5.10	52.08	1.88
19	HPMC-62	45.97	3.22	58.33	0.77
20	HPMC-63	47.26	5.34	39.38	1.35
21	HPMC-64	52.71	4.39	52.08	2.21
	Mean	53.80	6.72	39.36	1.71

HPMC-53 (1.92) were having positive GCA for EFF. The highest HRF was recorded in crosses with HPMC-57 (27.03) followed by HPMC-62 (20.78). In the case of haploid formation frequency, the highest GCA was recorded in Ic-ye (4.55) followed by HPMC-58 (3.83) and Ic-br (1.37). Positive GCA for HFF was also recorded in HPMC-24 (0.92), HPMC-27 (0.91), HPMC-53 (0.60) and HPMC-64 (0.21). All lines except PDW314, TL2900 and TL2908 have positive GCA for PFF. In the case of EFF, positive GCA was recorded in DH84 (5.95), DH86 (4.67), DH100 (0.97), PDW314 (3.28) and A-9-30-1 (3.02). The same genotypes have positive GCA for HRF. Genotypes with positive GCA for HFF were DH84 (2.12), DH86 (1.34) and A-9-30-1 (1.29).

Discussion

Doubled haploidy breeding in wheat has been followed globally utilizing pollen of maize and *Imperata cylindrica*; however, the potential of *Imperata* for haploid induction in wheat was explored by Chaudhary et al. (2005) and also proved to be successful in inducing haploids in tetraploids, triticale × wheat and wheat × rye derivatives (Pratap et al. 2005; Kishore et al. 2011; Mahato and Chaudhary 2015).

Table 7 GCA effects of frequencies of pseudoseed formation, embryo formation, haploid regeneration and haploid formation efficiency in wheat, triticale, *Imperata* and maize genotypes

Genotypes	PFF	EFF	HRF	HFF
<i>Testers</i>				
HPMC-3	−4.84	−2.42	−16.51	−0.57
HPMC-11	−4.19	−2.50	5.15	−0.36
HPMC-12	6.20	−1.21	−9.28	−0.54
HPMC-14	2.93	−2.60	−4.36	−0.15
HPMC-16	−11.51	−4.90	3.07	−0.87
HPMC-18	7.38	−0.18	−8.39	−0.62
HPMC-20	1.03	−4.47	−6.87	−1.02
HPMC-21	−12.62	−5.21	−10.47	−1.38
HPMC-24	1.80	−0.46	11.03*	0.91*
HPMC-25	−6.88	2.90*	−5.26	−0.89
HPMC-27	−1.97	0.07	−2.93	0.91*
HPMC-28	3.97	−4.30	−19.85	−1.43
HPMC-53	6.10	1.92	6.82	0.60
HPMC-56	4.51	−4.27	14.53*	−0.20
HPMC-57	−5.53	8.11*	27.03*	−0.96
HPMC-58	8.66	6.46*	3.82	3.83*
HPMC-59	−1.78	−3.73	−1.24	−1.41
HPMC-60	20.28*	−3.14	14.53*	−0.12
HPMC-62	−7.94	−5.01	20.78*	−1.23
HPMC-63	−6.65	−2.90	1.82	−0.64
HPMC-64	−1.20	−3.84	14.53*	0.21
Ic-ye	1.87	18.05*	−15.02	4.55*
Ic-br	0.41	13.61*	−22.91	1.37*
SE(±)	4.78	1.22	4.45	0.34
<i>Lines</i>				
DH 40	23.70*	−5.77	−22.70	−1.51
DH 84	25.18*	5.95*	8.97*	2.12*
DH 86	20.31*	4.67*	16.34*	1.34*
DH 100	25.56*	0.97	14.50*	0.74*
PDW 314	−10.74	3.28*	27.87*	−0.05
A-9-30-1	9.75*	3.02*	25.77*	1.29*
TL 2900	−52.12	−3.89	−33.21	−1.94
TL 2908	−41.65	−8.24	−37.55	−2.00
SE(±)	2.82	0.72	2.62	0.19

*Significant at 5 percent level of significance

The present study undertaken with the objective of comparing the efficiencies of both the pollen sources for haploid induction in three species has shown superiority of *Imperata* over maize in haploid induction efficiency. Embryos were formed in both hexaploid and tetraploid wheats with both the pollen species indicating the insensitivity of both maize and *Imperata* pollen to crossability inhibitor genes in wheat. However, embryos could not be induced in triticales indicating genomic incompatibilities of these species and major role of rye genome for poor response to haploid embryo formation. Higher frequency of EFF and HFF was

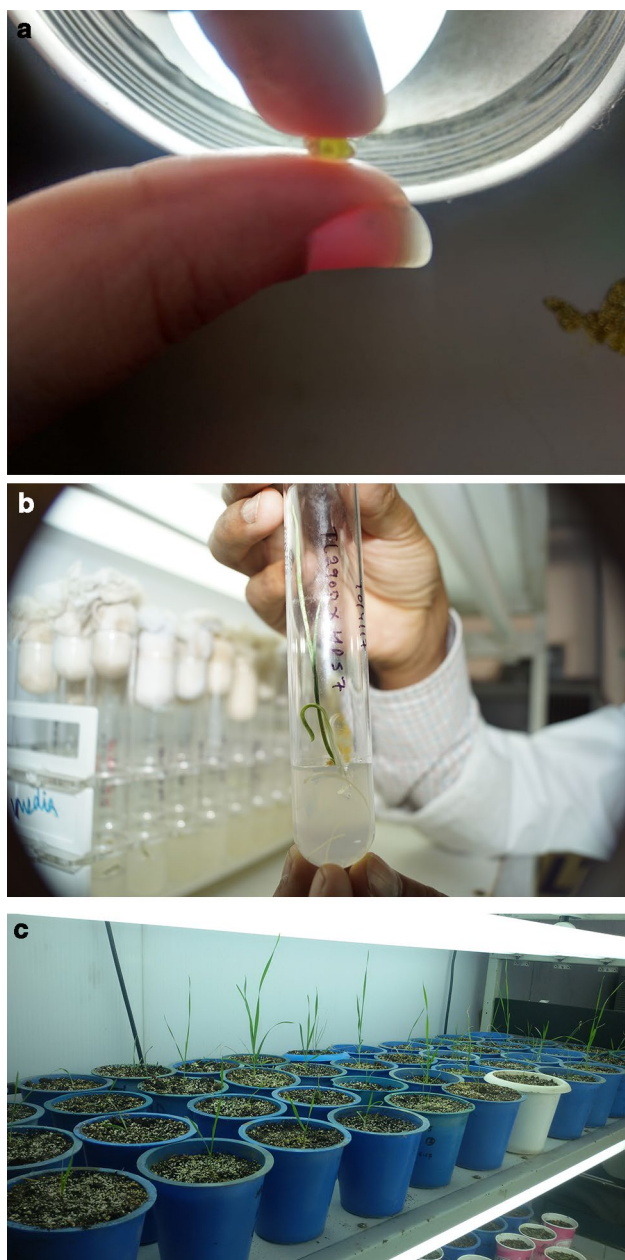


Fig. 1 a Embryo-carrying pseudoseed in wheat. b Regenerated haploid embryo in culture tube. c Regenerated haploid plantlets in wheat

recorded for hexaploid wheats as compared to tetraploids when crossed with *Imperata*, which is mainly attributed to the presence of D genome in hexaploids triggering chromosome elimination of the paternal parent (Mujeeb-Kazi et al. 2006; Dogramaci-Altuntepe and Jauhar 2001; Kaila 2013; Mukai et al. 2015). Reason for relatively low embryo formation with maize is possibly due to the influence of number of genetic and environmental factors affecting maize pollen unlike *Imperata* which has the ability to overcome fertilization barriers in embryo formation (Laurie and Bennett 1990). Furthermore, *Imperata* chromosomes are eliminated

in the first cell division unlike maize which gets eliminated in 2–3 cell divisions resulting in low stability. Diverse genotypes of maize and *Imperata* utilized as pollen source in the study showed significant differences for haploid induction efficiency. GCA is an important parameter for selection of best combiners, i.e., best male and female parents for higher embryo and green haploid plantlets formation. Relatively higher values of GCA for EFF and HFF were recorded in crosses attempted with the *Imperata*. GCA values for haploid induction were low in the case of crosses with maize; however, genotypes HPMC-57, HPMC-58, HPMC-25 were relatively superior in haploid induction. Among female lines, hexaploid wheat genotypes DH84 and DH86 emerged out to be the best general combiner for all the four haploid induction parameters, while in tetraploid wheats, A-9-30-1 proved to be superior in the case of green haploid plantlets recovered. Based on overall study, *Imperata* emerged out to be an efficient pollen source than maize in hexaploid and tetraploid wheat, while triticale remained nonresponsive to both the pollen sources. The study reveals that *Imperata* can be successfully utilized for enhancing the recovery of haploids in wheat, especially in areas where *Imperata* thrives well under natural conditions (subtemperate areas), whereas in subtropical areas or areas where *Imperata* does not survive, maize genotypes HPMC-25, HPMC-53, HPMC-57 and HPMC-58 can be utilized as pollen source. Moreover nine genotypes, viz. HPMC-16, HPMC-56, HPMC-57, HPMC-58, HPMC-59, HPMC-60, HPMC-62, HPMC-63 and HPMC-64, were earliest to flower (75 days under polyhouse conditions), thereby providing an opportunity to initiate wide hybridization work early in the season about 15–20 days in advance of *Imperata* flowering prolonging hybridization work for attempting crosses with wheat (Fig. 1).

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