Influence of Different Wheat and *Imperata cylindrica* Genetic Backgrounds on Haploid Induction Efficiency in Wheat Doubled Haploid Breeding

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Abstract

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Four Indian and one Japanese accession of *Imperata cylindrica* were assessed for their influence upon haploid production in F_1 generations of 21 wheat crosses (winter × spring, spring × spring and winter × winter) to find an efficient pollen source for haploid induction, which would enhance doubled haploid breeding in bread wheat. The frequency of haploid induction was influenced differently by the wheat and the *I. cylindrica* genotypes, indicating both maternal and paternal genetic influence on haploid induction. The gene actions controlling the inheritance of haploid induction appeared to be non-additive. Haploid formation efficiency was closely associated with other haploid induction parameters, i.e. pseudoseed formation, embryo formation and haploid regeneration. Amongst wheat F_1 groups, spring × spring wheats exhibited the highest potential for haploid induction. General combining ability for haploid production was highest for the, *I. cylindrica* genotype Ic-Aru, native to the northeastern Himalayas, which appears as a potential pollen source for efficient haploid induction in bread wheat.

Keywords: general combining ability; haploid induction; *Imperata cylindrica*; interactive influence; wheat

Doubled haploid (DH) breeding offers unique advantages for rapid genetic improvement of bread wheat in a single generation, reducing the time required to achieve absolute homozygosity and enhancing the selection efficiency. The remarkable discovery that haploid embryos and plants can be produced by culturing anthers of Datura (Guha & Maheshwari 1964, 1966) brought renewed interest in haploid breeding. This was quickly attempted in many species but the frequencies were very low, relative to the large number of pollen grains per floret. Then, Kasha and Kao (1970) reported haploid production in barley following wide hybridization, and the subsequent preferential elimination of the wild species chromosomes during early embryogenesis. Subsequently, this technique was initiated in wheat with the investigations of Barclay (1975), who recovered wheat haploids in crosses between the wheat variety Chinese Spring and *Hordeum bulbosum*. The technique was, however, genotype specific due to the presence of dominant crossability inhibitor genes *Kr1*, *Kr2*, *Kr3* and *Kr4* in wheat, which are expressed in many wheat varieties and located on 5B, 5A, 5D and 1A chromosomes, respectively (Zheng *et al.* 1992). Later on, Laurie and Bennett (1987) produced a wheat × maize system of haploid production that was genotype non-specific because of the insensitivity of maize pollen to the action of crossability inhibitor genes, thereby rendering the chromosome elimination technique more efficient and of practical value. Recently, a wheat × *Imperata cylindrica* approach (Chaudhary *et al.* 2005) was discovered as an efficient alternative to the existing ones for obtaining a high frequency of haploid and

doubled haploids in wheat and triticale. Further, this new approach was applied to recover haploids from triticale × wheat (Pratap *et al.* 2005) and wheat × rye derivatives (Kishore *et al.* 2011).

The frequency of production of wheat haploids is influenced by the genetic makeup of both wheat and the pollen parent in the wheat × maize-mediated system of doubled haploid breeding (Singh *et al.* 2004). The diversity, evident in *I. cylindrica* (Chou & Tsai 1999), has made it imperative to search for potential pollen sources for efficient polyhaploid induction in bread wheat, so that the efficiency of the system can be further enhanced by utilizing the more responding *I. cylindrica* genotype for haploid induction. Thus, the present investigation was carried out to assess the interactive influence of diverse genotypes of *I. cylindrica* and wheat on the haploid induction parameters, in order to find out and identify an efficient *I. cylindrica* genotype as a pollen source for haploid induction in bread wheat and establish the gene action for various haploid induction parameters.

MATERIAL AND METHODS

Twenty-one wheat F_1s (16 winter × spring, two each of winter × winter and spring × spring and one spring × winter wheat derivative) were generated out of eight elite and diverse genotypes of winter and spring wheat ecotypes (Table 1). Five diverse *I. cylindrica* genotypes from different geographical locations (Table 2) were used to pollinate the wheat F_1s. The wheat F_1s were raised in a completely randomized design, each sown individually in two rows, 1.5 m long with 25 cm row × row spacing. Three exotic collections of *I. cylindrica* genotypes, *viz.* Ic-Aru, Ic-Sri and Ic-Jp, were grown in pots (50 pots for each collection) filled with soil, sand and vermicompost at the ratio of 2:1:1, whereas the local genotypes, i.e. Ic-Pbr and Ic-Pye, were grown naturally.

Table 1. Parentage and source of different wheat genotypes used for generating F_1s

No.	Genotype	Parentage
Winter wheats		
1	DH-100	WW 10/WW 24
2	DH-114	VWFW 452/WW 24
3	Saptdhara	Selection from Atou (Cappelle/Garnet)
4	Tyari-1	Landrace from Lahaul Spiti (North West Himalayan regions)
Spring wheats		
5	DH-40	Saptdhara/HW 3024
6	HPW-155	BT 2549/FATH
7	HS-295	CQT//IAS55//ALDML'S'/3/ALDML'S'/ NAFN/4/PJN'S'/PEL1276.6
8	KWS-29	Amargas/Veery 'S'

Source – Molecular Cytogenetics & Tissue Culture Lab, Dept. of Crop Improvement, CSK HPKV, Palampur, India

Haploid induction was carried out as per the wheat × *I. cylindrica* mediated approach of doubled haploid breeding proposed by Chaudhary *et al.* (2005). Ten spikes of each wheat genotype (F_1) were hybridized with each *I. cylindrica* genotype in a line (wheat hybrid) × tester (*I. cylindrica* genotype) fashion under natural conditions. After 24 h post-pollination of the emasculated wheat spikes with *I. cylindrica* pollen, a 2,4-D solution of 100 ppm concentration was injected at the base of the uppermost internode of each pollinated spike for three consecutive days in order to ensure pseudoseed and embryo formation in each cross. The crossed spikes were harvested from the tiller base after 18–20 days of pollination. The embryo carrying pseudoseeds were identified under a source of light (Bains *et al.* 1998). The embryo was seen floating in the fluid (aqueous solution) instead of the solid endosperm found in selfed seeds. The embryos were then excised from surface sterilized pseudoseeds. These excised embryos were transferred to test tubes containing Murashige and Skoog (MS) medium (Murashige & Skoog 1962) supplemented with essential amino acids. Cultured embryos were given a cold treatment at 4°C in the dark for 24 h immediately after embryo rescue followed by incubation in the dark at 20 ± 2°C till regeneration of roots and shoots. The regenerated plantlets were then shifted to a growth chamber at 20 ± 2°C with 10-h day length regime and 75% relative humidity (RH), until they developed properly into complete green plantlets. The green haploid plantlets developed through embryo culture were then subjected to rooting medium (liquid) for profuse rooting at the three- to four-leaf stage. The haploid plantlets at the four- to five-leaf stage were subsequently subjected to colchicine treatment (0.1% solution + 1.5% dimethyl sulfoxide) for five hours and transplanted into pots and maintained up to maturity.

Observations were recorded with respect to various haploid induction parameters, *viz.* pseudoseed formation, embryo formation, haploid regeneration and haploid formation in each cross. The information was used to generate data with respect to pseudoseed formation frequency (number of pseudoseeds obtained per 100 wheat florets pollinated with *I. cy-*

Table 2. Geographic location of different *Imperata cylindrica* genotypes used for haploid induction in bread wheat

No.	*I. cylindrica* genotype*	Geographic location	Region	Latitude and longitude
1	Ic-Pbr**	Palampur, Himachal Pradesh, India	North-west Himalayas	32.12°N and 76.53°E
2	Ic-Pye***	Palampur, Himachal Pradesh, India	North-west Himalayas	32.12°N and 76.53°E
3	Ic-Sri	Pulwama, Jammu & Kashmir, India	North-west Himalayas	33.88°N and 74.92°E
4	Ic-Aru	Pasighat, Arunachal Pardesh, India	North-east Himalayas	28.07°N and 95.33°E
5	Ic-Jp	Osaka Kyoiku Dai Mai, Osaka, Japan	Eastern Asia	34.66°N and 135.52°E

*Ic – *Imperata cylindrica*; Pbr – Palampur brown; Pye – Palampur yellow; Aru – Arunachal Pradesh; Sri – Srinagar; Jp – Japan; **_I. cylindrica_ collection having spike with brown anthers; ***_I. cylindrica_ collection having spike with yellow anthers

lindrica), embryo formation frequency (number of embryo-carrying seeds per 100 pseudoseeds), haploid regeneration frequency (number of green haploid plantlets developed per 100 embryos cultured) and haploid formation efficiency (number of green haploid plantlets developed per 100 wheat florets pollinated with *I. cylindrica*). The data obtained from ten spikes/genotype/tester in each haploid induction parameter was computed as per the completely randomized design (CRD), so as to work out the analysis of variance. The data on all the crosses with respect to various haploid induction parameters were subjected to line × tester analysis according to Kempthorne (1957) after arcsine transformations (Gomez & Gomez 1984). Correlation coefficients (*r*) between various haploid induction parameters were calculated and tested according to the formula suggested by Chandel (1965).

RESULTS

Analyses of variance of the data in respect of various haploid induction parameters revealed that mean squares due to crosses, wheat, *Imperata cylindrica* genotypes and wheat × *I. cylindrica* interaction were significantly different for all the haploid induction parameters when tested against error mean squares. The wheat genotypes also showed significant differences for all the haploid induction parameters when tested against mean squares due to wheat × *I. cylindrica* interaction. Whereas, the *I. cylindrica* genotypes showed significant differences when tested against mean squares due to wheat × *I. cylindrica* interaction for two haploid induction parameters, *viz.* embryo formation frequency and haploid formation efficiency (Table 3).

Estimation of general combining ability (GCA) of lines and testers for various haploid induction parameters was worked out in order to determine the genetic control of haploid induction and identify the most efficient wheat and *I. cylindrica* genotypes. Among the wheat F_1s, the highest positive GCA effect for pseudoseed formation and embryo formation was exhibited by DH 100 × KWS 29 and DH 114 × KWS 29, respectively, whereas the highest positive GCA effect was revealed by HPW 155 × KWS 29 for haploid regeneration and haploid formation efficiency (Table 4).

Table 3. ANOVA for various haploid induction parameters in wheat × *Imperata cylindrica* crosses

Source of variance	df	Mean sum of squares (MS)			
		pseudoseed formation	embryo formation	haploid regeneration	haploid formation
Crosses	104	312.52*	194.54*	148.41*	142.59*
Wheat genotypes	20	688.28*#	384.13*#	293.60*#	366.03*#
I. cylindrica genotypes	4	363.36*	1461.73*#	164.12*	454.45*#
Wheat × *I. cylindrica* interaction	80	216.03*	83.79*	111.33*	71.14*
Error	945	0.97	1.20	0.99	1.98
$(\sigma^2_D/\sigma^2_A)^{1/2}$		6.87	3.97	7.94	4.53

*Significant at $P = 0.05$ (tested against EMS); #significant at $P = 0.05$ (tested against MS (wheat × *I. cylindrica*)); σ^2_A = additive genetic variance; σ^2_D = dominance variance; df – degree of freedom

Table 4. General combining ability (GCA) effects of wheat genotypes with respect to various haploid induction parameters

No.	Wheat genotypes	Haploid induction parameters			
		pseudoseed formation	embryo formation	haploid regeneration	haploid formation
1	Saptdhara × DH 40	–7.46*	–4.86*	–1.27*	–4.48*
2	Saptdhara × HPW 155	–2.11*	–2.74*	–1.87*	–2.61*
3	Saptdhara × HS 295	–2.04*	–0.59*	–0.25	–0.75*
4	Saptdhara × KWS 29	–2.51*	0.49*	–3.36*	–1.75*
5	Tyari 1 × DH 40	–1.37*	0.34*	–0.06	–0.20
6	Tyari 1 × HPW 155	–2.85*	–0.02	1.43*	–0.14
7	Tyari 1 × HS 295	2.47*	2.19*	0.57*	1.73*
8	Tyari 1 × KWS 29	–0.66*	–2.01*	0.31*	–0.99*
9	DH 100 × DH 40	–2.72*	–3.14*	–0.80*	–2.50*
10	DH 100 × HPW 155	–2.32*	2.41*	–3.54*	–1.00*
11	DH 100 × HS 295	1.24*	3.93*	4.43*	4.27*
12	DH 100 × KWS 29	7.59*	1.93*	2.60*	3.96*
13	DH 114 × DH 40	3.83*	–1.31*	0.04	0.00
14	DH 114 × HPW 155	0.42*	3.60*	–0.06	1.62*
15	DH 114 × HS 295	0.26	1.62*	–0.48*	0.79*
16	DH 114 × KWS 29	6.48*	5.09*	1.80*	4.69*
17	Saptdhara × Tyari 1	–2.19*	–2.22*	–3.60*	–3.03*
18	Saptdhara × DH 114	–2.85*	–3.51*	–1.43*	–2.85*
19	DH 40 × DH 100	4.68*	–2.80*	–1.82*	–1.25*
20	HS 295 × DH 40	–2.24*	–1.22*	1.48*	–0.56*
21	HPW 155 × KWS 29	4.36*	2.82*	5.89*	5.08*
CD (5%)		0.39	0.42	0.40	0.55

*Significant at $P = 0.05$; CD – critical difference

Among diverse pollen sources, the highest positive GCA effect for pseudoseed formation and haploid regeneration was exhibited by the genotypes Ic-Pye and Ic-Pbr. The genotype Ic-Aru possessed the highest positive GCA effect for embryo formation as well as haploid formation efficiency (Table 5).

Table 5. General combining ability (GCA) effects of *Imperata cylindrica* genotypes with respect to various haploid induction parameters

No.	*Imperata cylindrica* genotypes	Haploid induction parameters			
		pseudoseed formation	embryo formation	haploid regeneration	haploid formation
1	Ic-Pbr	–2.20*	–1.40*	1.47*	–0.51*
2	Ic-Pye	1.17*	1.40*	–0.05	0.95*
3	Ic-Aru	0.75*	3.97*	–0.05	2.07*
4	Ic-Sri	–0.07	–1.66*	–0.66*	–1.13*
5	Ic-Jp	0.35*	–2.30*	–0.70*	–1.37*
CD (5%)		0.18	0.21	0.20	0.27

*Significant at $P = 0.05$; CD – critical difference

Table 6. Mean performance of various wheat F_1 groups for response to various haploid induction parameters pooled over all the *Imperata cylindrica* genotypes

S. No.	Wheat groups	Haploid induction parameters				
		f	sf	ef	r	h
1	winter × spring wheat	24526	75.88 (18653)	40.51* (7644)	45.97 (3548)	14.36
2	winter × winter wheat	3187	72.40 (2299)	34.83 (803)	41.91 (337)	10.62
3	spring × spring wheat	3057	77.45* (2374)	41.00* (983)	52.31* (531)	17.15*
Mean		10256.67	75.24	38.78	46.73	14.04
CD (5%)			0.86	1.14	1.07	1.03

*Significant at $P = 0.05$; f – total number of florets pollinated; sf – pseudoseed formed; ef – embryo formation; r – embryo regeneration; h – haploids formed; figures in parenthesis represent the number obtained; CD – critical difference

Table 7. Estimates of correlation coefficients between various haploid induction parameters

Correlation coefficient	Haploid induction parameters			
	pseudoseed formation	embryo formation	haploid regeneration	haploid formation
Pseudoseed formation	–	0.36*	0.30*	0.65*
Embryo formation	–	–	0.24*	0.78*
Haploid regeneration	–	–	–	0.71*
Haploid formation	–	–	–	–

*Significant at $P = 0.05$

The mean response of various wheat F_1 groups over all the testers to pseudoseed formation, embryo formation, haploid regeneration and haploid formation ranged from 72.40 to 77.45%, 34.83 to 41%, 41.91 to 52.31% and 10.62 to 17.15%, respectively. The spring × spring wheats exhibited the highest mean response to all the haploid induction parameters (Table 6).

For all the haploid induction parameters, the magnitude of variance in respect of the dominance component (σ^2_D) was higher than in the additive component (σ^2_A) rendering the degree of dominance $(\sigma^2_D/\sigma^2_A)^{1/2}$ greater than one (Table 3). Positive and significant correlations were found between all the haploid induction parameters. The correlation coefficient (r) was highest for embryo formation followed by haploid regeneration and pseudoseed formation when observed against haploid formation efficiency (Table 7).

DISCUSSION

The wheat × *I. cylindrica*-mediated approach of chromosome elimination has proved to be the most efficient alternative to the existing systems of doubled haploid production in bread wheat (Chaudhary *et al.* 2005). Keeping in view the diversity apparent in *I. cylindrica* (Chou & Tsai 1999), and to further enhance the efficiency of the system, it was imperative to search potential pollen sources for efficient polyhaploid induction in bread wheat. From the analyses of variance, all the wheat genotypes exhibited significant differences in response to various haploid induction parameters. These results are in accordance with the findings of Chaudhary *et al.* (2005), who found significant effects of diverse wheat genotypes on different haploid induction parameters when crossed with *I. cylindrica*.

GCA plays an important role in the selection of potential parents on the basis of their genetic value which can further be utilized for the production of desirable hybrids. Among the wheat F_1s, DH 100 × KWS 29 and DH 114 × KWS 29 were identified as being the best general combiners for pseudoseed and embryo formation frequency, respectively, whereas, the genotype HPW 155 × KWS 29 was identified as the best general combiner for both haploid regeneration frequency and haploid formation efficiency. Among the *I. cylindrica* genotypes, Ic-Pye and Ic-Pbr were recognized as the best general combiners for pseudoseed formation and haploid regeneration frequency, respectively. Genotype Ic-Aru was identified as the best general combiner for both embryo formation frequency and haploid formation efficiency. The prevalence of non-additive (dominance) gene

action governing the inheritance of all the haploid induction parameters infers that the haploid induction frequency can be enhanced by using diverse maternal and paternal parents.

The parameter haploid formation efficiency gave the highest significant correlation with the remaining parameters, *viz*. pseudoseed formation, embryo formation and haploid regeneration. Based on haploid formation efficiency, spring × spring wheat F_1s and HPW 155 × KWS 29 were identified as potential maternal parents, whereas the Ic-Aru (*I. cylindrica* collection from Pasighat, Arunachal Pradesh, India, representing northeastern regions of the Himalayas) was identified as the potential pollen source for efficient haploid induction.

In conclusion, the present investigation revealed that the genetic constitution of maternal and paternal genotypes affects the induction of haploids through main and interactive effects. The promising genotypes involved in intergeneric hybridization mediated haploid induction must be identified on the basis of haploid formation efficiency as it is highly associated with other haploid induction parameters. Moreover, the work also revealed that *I. cylindrica* predominant in other areas of the world is also capable of inducing haploids in wheat. A preponderance of non-additive gene action governing the inheritance of all the haploid induction parameters suggests that wheat and *I. cylindrica* genotypes utilized for haploid induction must be diverse in nature.

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