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Short Communication

Exploration of wild grass *Imperata cylindrica* for development of doubled haploids in winter x spring wheat (*Triticum aestivum* L.) hybrids accompanied with combining ability and hybrid potential estimation

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Abstract

Two spring and three winter wheat genotypes were crossed in Line x Tester manner forming six F_1's and three spring and one winter wheat genotypes were also crossed forming two three way F_1's. Combining ability studies indicated sufficient genetic variability in the breeding material. Among lines, HS542 showed good general combining ability for seed yield/plant and among testers KLE/BER/2*FL-8/DONSK-POLL was good combiner for 1000 seed weight. The cross HS542/Zander33 and HD2997/KLE/BER/2*FL-8/DONSK-POLL showed significant specific combining ability effects for 1000 grain weight. Intergeneric hybridization lead to the haploid embryo development from the crosses: HS542/China 84-40022, HS542/ KLE/BER/2*FL-8/DONSK-POLL, HS526/ID 80994W/VEE/3/CHEN/3AL X FLW13 and HD2997/ KLE/BER/2*FL-8/DONSK-POLL. These crosses also showed maximum values of heterosis for 1000 seed weight or spikelet per spike or seed yield/plant.

Key words: General and specific combining ability, Haploid induction parameters, Intergeneric hybridization

Wheat grows across a wide range of environments and is considered to have wider adaption of all cereal crop species (Briggle and Curtis 1987). In India, mainly spring wheat is grown due to its adaptability in most of the wheat growing areas. The other ecotype of bread wheat, that is, winter wheat has very limited area (75 million ha of the 220 million ha devoted to wheat worldwide) (Braun and Saulescu 2002). Winter and spring ecotypes of wheat are physiologically and genetically distinct groups, having genetic potential for reciprocal improvement. Intercrossing these groups of common wheat would bring together the two gene-pools that may lead to increase yield through combining complementary alleles for certain yield components as well as other traits. By introgressing genetic variability from winter wheat, breeders can considerably augment the yield potential of spring wheat which has already been reported by many workers (Mishra et al. 2013). In conventional wheat breeding programmes, the isolation of homozygous and homogeneous breeding lines is possible only if the breeding materials are permitted to several cycles of inbreeding and selection which is time consuming (usually 6-7 years). To speed up the breeding process, plant tissue culture methods could be used effectively. In wheat, doubled haploidy breeding through chromosome elimination was initiated with the investigations of Barclay (1975) but, was genotype specific due to the presence of the dominant crossability inhibitor genes *Kr1* and *Kr2* (Alfares et al. 2009). Later, Laurie and Bennett (1987) reported maize pollen to be insensitive to the activity of these genes

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in wheat and identified wheat × maize to be more efficient chromosome elimination technique for obtaining haploid wheat. However, the unsynchronized flowering of these two species complicates the hybridization process. Thereafter, a number of plant species *viz*., *Hordeum bulbosum*, maize, pearlmillet or sorghum are being looked for, but wheat haploids formed by crossing with *Imperata cylindrica,* a wild grass remotely related to wheat (Chaudhary et al. 2005) is the most efficient one due to its perennial nature, available in the surroundings of wheat fields and coincides well with wheat flowering and high frequency of haploid embryo formation and regeneration (Mahato and Chaudhary 2015). Taking into consideration the importance of cultivation of wheat for human consumption as well as the possibility of reducing the time required for obtaining new cultivars, the aim of this work was to obtain doubled haploid plants of bread wheat through wide hybridization with *I. cylindrica* from crosses between winter x spring wheat.

During *Kharif* 2012-13, two spring (HS542, HD2997) and three winter (ZANDER 33, China 84-40022 and KLE/BER/2*FL-8/DONSK-POLL) wheat were crossed at Indian Council of Agricultural Research-Indian Agricultural Research Institute, Regional Station, Shimla, Himachal Pradesh (India) in Line x Tester manner producing six F_1's. Also, two spring wheat (HS526 and HS536) were crossed with ID 80994W/VEE/3/CHEN/3AL during *Kharif* 2011-12 and then with FLW 13 during 2012-13 forming three way F_1's (Table 2). During *Kharif* 2013-14, half seed of these crosses was sown at Shimla and half at the Experimental Farm of the Department of Plant Breeding & Genetics, CSK HP Agricultural University, Palampur (in two replications alongwith parents). For doubled haploid development, wheat F_1's and three way F_1's plants were hand emasculated 2 to 3 days before anthesis and next morning fresh pollen from *I. cylindrica* was applied to the feathery stigmas of the emasculated florets. The uppermost internodes of wheat spikes pollinated with pollen of *I. cylindrica* were injected with 100ppm of 2,4-D solution 24 hours after pollination and the injections were repeated for two consecutive days. Crossed wheat F_1s or three way F_1's with *I. cylindrica* were harvested 18-20 days after pollination and seeds were examined for the presence of embryo which were excised and cultured as per Chaudhary *et al*. (2002). Chromosome doubling was performed according to a modified procedure of Pienaar and Lesch (1994). Observations were recorded on

Table 1. General Combining Ability and SCA effects for yield traits in winter x spring wheat crosses at Palampur

Parents	1000 seed weight	Spiklet/ spike	Seed yield/ plant
General Combining Ability			
HS 542	0.50	-0.18	0.83**
HD 2997	-0.50	0.18	-0.83**
S. E. (gi)	0.91	0.50	0.32
S.E(gi-gj)	1.29	0.71	0.46
ZANDER 33	-1.50**	0.12	-0.17
China 84-40022	-3.00**	0.02	-0.17
KLE/BER/2*FL-8/DONSK-POLL	4.50**	-0.14	0.33
S.E(gi)	0.64	0.61	0.39
S.E (gi-gj)	0.91	0.87	0.56
Specific Combining Ability			
HS 542 / ZANDER 33	2.50**	1.08	0.17
HS 542 / China 84-40022	1.00	0.18	0.17
HS 542/ KLE/BER/2*FL-8/ DONSK-POLL	-3.50**	-1.26	-0.33
HD 2997/ ZANDER 33	-2.50**	-1.08	-0.17
HD 2997 / CHINA 84-40022	-1.00	-0.18	-0.17
HD 2997/ KLE/BER/2*FL-8/ DONSK-POLL	3.50**	1.26	0.33
S.E (sca effect)	0.91	0.87	0.56
S.E (Sij-Skl)	1.29	1.23	0.79

**Significant at 1% level

haploid induction parameters *viz*., total number of florets pollinated, number of pseudoseeds, number of embryo carrying pseudoseeds and regeneration of embryos in each cross. For combining ability studies for yield traits, five plants in each F_1's and three way F_1's of each entry in each replicate were taken at random and data were recorded on three traits *viz*., 1000 grain weight, spiklets per spike and seed yield per plant. The data were subjected to ANOVA according to Gomez and Gomez (1984) which was further analyzed for general combing ability (GCA) and specific combing ability (SCA) following line x tester method (Kempthorne 1957). Heterosis was assessed over the better parent (Heterobeltiosis), mid parental value (relative heterosis) and standard variety (standard heterosis) as suggested by Mather and Jinks (1971).

Significant differences observed among wheat genotypes for the three traits studied (1000 grain weight, spiklets per spike and seed yield per plant) (Table not given) indicated sufficient genetic variability in the studied winter and spring genotypes. This is expected since parents represent diverse ecotypes of wheat. The estimates of general combining ability effects revealed that the only line HS542 was having good combining ability for seed yield/plant. Among the testers KLE/BER/2*FL-8/DONSK-POLL was good combiner for 1000 seed weight (Table 1). These two parents could contribute in subsequent development of hybrid with increased seed yield per plant and 1000 grain weight. Different parents having good combining ability in wheat has also been reported by different workers (Ahmed et al. 2013). In the present study, high value of specific combining ability in the crosses HS 542/ZANDER 33 and HD 2997/KLE/BER/2*FL-8/DONSK-POLL for 1000-grain weight (an important grain contributing trait) suggested that these crosses could be used to develop hybrids for improving grain yield of wheat through heterosis breeding program. Heterosis analysis showed that for 1000 grain weight, at Shimla the cross HD 2997/CHINA 84-40022 showed maximum value of heterosis (relative, mid parent and heterobeltosis) while at Palampur the cross HD2997/KLE/BER/2*FL-8/DONSK-POLL (mid parent and heterobeltosis) and HS542/ KLE/BER/2*FL-8/DONSK-

Table 2. Heterosis for yield related traits in wheat F_1's and three way F_1's at Shimla and Palampur

Trait	Relative heterosis		Mid parent heterosis		Heterobeltosis	
1000 grain weight	Shimla	Palampur	Shimla	Palampur	Shimla	Palampur
HS542/ZANDER33	20.41**	25.93**	20.31**	14.29	20.31**	14.29
HS 542/CHINA 84-40022	14.71**	10.71	2.63	3.33	2.63	3.33
HS 542/ KLE/BER/2*FL-8/DONSK-POLL	14.29**	26.19**	15.79**	17.78**	15.79**	17.78**
HD 2997/ZANDER 33	11.76**	10.34	13.16**	13.33	13.16**	13.33
HD 2997 / CHINA 84-40022	25.81**	23.46**	21.88**	19.05**	21.88**	19.05**
HD 2997/ KLE/BER/2*FL-8/DONSK-POLL	8.45**	16.46**	18.75**	21.43**	18.75**	21.43**
HS 526/ ID 80994W/VEE/3/CHEN/3AL X FLW13	16.22**	13.33	13.89**	9.52	13.89**	9.52
HS 536/ ID 80994W/VEE/3/CHEN/3AL X FLW13	19.23**	13.75	14.29**	5.81	14.29**	5.81
No. of Spiklets/spike						
HS542/ZANDER33	10.00**	7.49	0.00	1.14	0.00	1.14
HS 542/CHINA 84-40022	26.58**	13.74	13.64**	9.09	13.64**	9.09
HS 542/ KLE/BER/2*FL-8/DONSK-POLL	2.33	1.16	0.00	-1.14	0.00	-1.14
HD 2997/ZANDER 33	10.77**	7.67	2.86	3.57	2.86	3.57
HD 2997 / CHINA 84-40022	20.52**	12.86	10.48**	10.71	10.48**	10.71
HD 2997/ KLE/BER/2*FL-8/DONSK-POLL	5.71	9.52	5.71**	9.52	5.71**	9.52
HS 526/ ID 80994W/VEE/3/CHEN/3AL X FLW13	6.00	17.5	3.41	14.08	3.41	14.08
HS 536/ ID 80994W/VEE/3/CHEN/3AL X FLW13	13.92**	8.42	12.50**	7.32	12.50**	7.32
Seed yield/ plant						
HS542/ZANDER33	4.35	-8.7	9.09**	-8.70	9.09**	-8.70
HS 542/CHINA 84-40022	15.00**	-38.10**	4.55	-43.48**	4.55	-43.48**
HS 542/ KLE/BER/2*FL-8/DONSK-POLL	16.50**	9.52	9.09**	0.00	9.09**	0.00
HD 2997/ZANDER 33	-6.38	-17.39	-4.35	-17.39	-4.35	-17.39
HD 2997 / CHINA 84-40022	12.20	9.52	0.00	0.00	0.00	0.00
HD 2997/ KLE/BER/2*FL-8/DONSK-POLL	2.37	9.52	-6.09	0.00	-6.09	0.00
HS 526/ ID 80994W/VEE/3/CHEN/3AL X FLW13	7.84**	-23.08	-3.51	-32.20**	-3.51	-32.20**
HS 536/ ID 80994W/VEE/3/CHEN/3AL X FLW13	5.00	-33.33**	-4.55	-38.10**	-4.55	-38.10**

**Significant at 1% level

Table 3. Frequency of various haploid induction parameters obtained from wide hybridization of winter x spring wheat hybrids with *Imperata cylindrical* at Palampur

S. No.	Cross	No. of florets pollinated	Pseudoseed formation %	No of seeds with embryo %	Haploid plantlet regeneration %
1	HS 542 / ZANDER 33	866**	93.42**	3.83**	3.23
2	HS 542 / China 84-40022	558**	91.04**	3.54**	11.11**
3	HS 542/ KLE/BER/2*FL-8/DONSK-POLL	1180**	93.98**	7.39**	2.44
4	HD 2997/ ZANDER 33	654**	88.38**	8.13**	4.26
5	HD 2997 / CHINA 84-40022	273**	95.97**	4.58**	8.33**
6	HD 2997/ KLE/BER/2*FL-8/DONSK-POLL	784**	90.31**	5.37**	7.89**
7	HS526/ID 80994W/VEE/3/CHEN/3AL X FLW13	620**	84.03**	6.33**	6.06**
8	HS536/ID 80994W/VEE/3/CHEN/3AL X FLW13	1228**	88.19**	6.65**	0

**: significant difference at 1%

POLL (relative heterosis) showed maximum heterosis (Table 2). For spiklets per spike, at Shimla the cross HS542/China 84-40022 and at Palampur the cross HS526/ID 80994W/VEE/3/CHEN/3AL x FLW13 showed maximum values. For seed yield per plant, the cross HS542/KLE/BER/2*FL-8/DONSK-POLL showed maximum values at both the locations. The varieties, HS542 and HD2997 are also the highest yielding varieties which indicated that high yielding varieties involved in the crosses were predominantly responsible for enhancing the yield. High heterosis of hybrids for the traits suggested the possible exploration of winter x spring wheat hybrids to raise grain yield potential within the existing genetic variation.

In the crossed wheat F_1's and three way F_1's with *I. cylindrica,* the mean frequency of pseudoseed formation ranged from 84.03 to 95.97% in various crosses, while it was 3.54% to 8.13% for embryo formation and 0 to 11.11 % for haploid plantlet regeneration. Frequency for pseudoseed formation was highest in HD2997/CHINA 84-40022 (95.97%) followed by HS542/KLE/BER/2*FL-8/DONSK-POLL (93.98%) (Table 3). Highest frequencies of embryo formation was observed in HD2997/ ZANDER 33 (8.13%) followed by HS542/ KLE/BER/2*FL-8/DONSK-POLL (7.39%). The cross, HS542/China 84-40022 (11.11%) followed by HD2997/CHINA 84-40022 (8.33%) showed greater haploid plantlet regeneration. High frequency of pseudoseed formation in all crosses of wheat F_1's and three way F_1's with *I. cylindrica* highlighted the wide potential of the present approach over others due to its genotypic non-specific nature (Mahato and Chaudhary 2015; Patial et al. 2015). The present study therefore suggests ample scope for exploitation of heterosis for commercial production of hybrid wheat by involving winter wheat gene pool for increasing variability and getting further yield breakthrough. Also, the work highlights the efficiency of *Imperata cylindrica* for doubled haploid production for getting instant homozygosity in early generation and accelerating the varietal development process in wheat.

Authors' contribution

Conceptualization of research (MP, KVP, JK); Designing of the experiments (MP); Contribution of experimental materials (DP, HKC, MP); Execution of field/lab experiments and data collection (MP, HKC); Analysis of data and interpretation (MP); Preparation of manuscript (MP).

Declaration

The authors declare no conflict of interest.

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