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Use of asynchrony in flowering for easy and economical polyhaploid induction in wheat following *Imperata cylindrica*-mediated chromosome elimination approach

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Abstract

Wheat × *Imperata cylindrica*-mediated approach of doubled haploidy breeding requires hand emasculation followed by pollination with *I. cylindrica* pollen. The pace of this endeavour can be enhanced by utilizing asynchronous flowering of wheat spikes by direct pollination without emasculation followed by morphological marker–assisted screening of selfed and crossed seeds. The emasculated and un-emasculated spikes of 13 spring and six winter wheat genotypes and two triticale × wheat derivatives were pollinated with *I. cylindrica* pollen. The response of different genotypes for production of crossed and selfed seeds with direct pollination varied significantly within and between groups for spring and winter wheats, whereas triticale × wheat derivatives responded similarly to each other but significantly different from spring and winter wheats. Although, the proportion of pseudoseed formation was lower in case of direct pollination, yet in some genotypes, it was comparable to that of pollination after emasculation. Moreover, the response for haploid embryo induction frequency was similar in both the cases. The method of direct pollination can be utilized for easy and economical induction of haploids.

Key words: asynchronous anthesis — *Triticum aestivum* — emasculation — *Imperata cylindrica* — haploid induction

Hybridization programme in self-pollinated crops mainly cereals such as wheat is time-consuming, laborious and cost-intensive. The floral biology of wheat plant makes hybridization work more tedious due to hand emasculation and pollination. The inflorescence of wheat is a composite ear or spike. The main axis (rachis) has a number of spikelets borne alternately on opposite sides. Each spikelet bears two to five florets. The sexual organs (three stamens and a pistil with feathery stigma) and two small lodicules (responsible for opening of floret) are covered by flowering glumes, lemma and palea. The maturity of anthers and stigma are in phase with each other so that while protruding at maturity, the anthers shed some of the pollen on the receptive stigma. Therefore, hand emasculation is must in wheat spike before anthers mature for carrying out hybridization. The optimum stage for emasculation arises when the spike is still under flag leaf and anthers are green in colour. Asynchrony for anthesis in wheat genotypes exists among different tillers of a plant, individual spike and even a spikelet (Percival 1921, De Vries 1971, 1973) with variable frequency (Lukac et al. 2011). The first sign of anthesis within a wheat spike appears in the middle of an ear, and then, flowering proceeds in both directions (Waines and Hegde 2003). Such a phenomenon of wheat flowering can be utilized in accelerating the wide hybridization programmes especially leading to haploid production in this crop without additional efforts. Recently, *Imperata cylindrica*-mediated chromosome elimination approach invented in the Molecular Cytogenetics & Tissue Culture Lab, Department of Crop Improvement, CSK HP Agricultural University, Palampur, Himachal Pradesh, India, has been identified as most efficient of all the systems (Chaudhary et al. 2002, 2005, Pratap et al. 2005, Chaudhary 2008a,b, Chaudhary 2010, Kishore et al. 2011). As there is no endosperm formation during wide hybridization between wheat and *I. cylindrica,* it can act as a morphological marker to identify the hybrid and the selfed seed (Komeda et al. 2007). Hence, this new system of DH breeding can further be accelerated by utilizing the asynchronous flowering behaviour of wheat spikes for direct pollination without emasculation. During the end of the wheat season, most of the genotypes flower simultaneously and exhibit quick maturity, which makes emasculation and pollination more cumbersome, and hence, the gap between emasculation and pollination decreases. The pollen of *I. cylindrica* ($2n = 20$), however, remains available till late flowering season of wheat, but the unavailability of emasculated spikes of wheat becomes a major hindrance in carrying out hybridization work. The asynchrony in maturity of wheat spikes can serve as potential tool for overcoming this hurdle by direct pollination with *I. cylindrica* pollen without emasculation. This can also reduce the cost of labour involved in hand emasculation and hence saves time during peak crossing season for production of haploids in wheat following *I. cylindrica* system.

Keeping this in view, the present investigation was carried out to study the potential of asynchronous flowering in eliminating the emasculation step and hence enhancing the haploid production in wheat.

Materials and Methods

Twenty-one diverse genotypes of wheat encompassing 13 spring wheats (all released varieties), six winter wheats (three landraces, one released variety, two winter × spring wheat–derived doubled haploids) and two triticale × wheat–derived genotypes were hybridized with *I. cylindrica* as pollen source at the Experimental Farm of the Department of Crop Improvement, CSK HP Agricultural University, Palampur, India. The protocol given by Chaudhary et al. (2005) was used for developing haploids using *I. cylindrica*-mediated chromosome elimination approach. Ten spikes that were supposed to dehisce anthers the next day were selected randomly from primary or secondary tillers, in each genotype, and divided into two sets *viz.,* without emasculation (treatment) and

with emasculation (control). Only two florets were kept by removing the central florets in each spikelet of each spike. Lemma and palea of the florets were not clipped from the top, whereas the awns were cut from the top to support bagging in both emasculated as well as unemasculated spikes. Pollination of each floret having well-receptive stigma was exercised individually with camel brush especially in the morning hours. The 2,4-D solution of 100 mg/l concentration was injected at the base of uppermost internode of the emasculated as well as unemasculated spikes pollinated with the *I. cylindrica* 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 the two more consecutive days to ensure proper seed and embryo formation *in vivo*. The spikes were harvested after 18 –20 days after pollination and screened for number of selfed and pseudoseeds (crossed) produced. The number of pseudoseeds and haploid embryos produced per spike were counted individually, and the average frequency of pseudoseed and haploid embryo formation was worked out on the basis of 100 florets pollinated. The relative proportions of each of the haploid induction parameters *viz.* pseudoseed and haploid embryo formation were used for comparison between different groups of wheat genotypes. The selfed seeds were identified with the help of a morphological marker, that is, the presence of endosperm in selfed seeds, whereas the hybrid seeds were devoid of endosperm and the embryo floats in transparent liquid. Hence, visual observation assisted in the quick identification of crossed and selfed seeds (Figs 1 and 2). The parameters of haploid induction were analysed statistically for difference between treatment and control using *Z* test of significance. Statistical analysis for within- and between-group differences with respect to frequency of pseudoseed and haploid formation was carried out using chi square (for difference of multiple population proportions) and *Z* (for difference of two population proportions) tests of significance (Rangaswamy 2006).

Fig. 1: Selfed seeds (left) and pseudoseeds (right) harvested from wheat spikes hybridized (without emasculation) with *Imperata cylindrica* pollen

Results

The results obtained after crossing pure/stable line(s) from each group of wheat (spring wheat, winter wheat and triticale × wheat derivatives) with *I. cylindrica* were compiled, and average frequency of pseudoseed and haploid embryo production was worked out and compared. The pseudoseeds could be easily distinguished as small, shrivelled and fluid-filled seeds in comparison with the bold selfed seeds that were filled with milky endosperm (Figs 1 and 2). The average frequency of pseudoseed and haploid embryo formation recovered in spring wheats when no emasculation was carried out varied from 10% to 59% and 4% to 23%, respectively, whereas in case of control, it ranged from 42% to 96% and 16% to 37%, respectively (Table 1). In case of winter wheats, the per cent pseudoseed formation varied from 21 to 61 and 55 to 79 in treatment and control, respectively, whereas the per cent haploid embryos per florets varied from 9 to 27 and 23 to 33 in treatment and control, respectively. Among triticale × wheat derivatives, the per cent pseudoseed formation and haploid embryo formation varied from 16 to 19 and 5 to 6, respectively, in treatment, whereas in control, 55 to 64 and 16 to 20, respectively.

The response of different genotypes within each group for pseudoseed and haploid embryo formation (without emasculation) was tested statistically using chi square test of significance, and significant difference was observed within the genotypes of spring and winter wheat groups (Table 1). Whereas both the genotypes in triticale × wheat group were found at par for both the haploid induction parameters, using *Z* test of significance. Among spring wheats, genotype UP 2418 was found most responsive with per cent pseudoseed and haploid embryo formation of 59 and 23, respectively. In case of winter wheats, genotype DH 150 exhibited maximum per cent pseudoseed formation (61) and haploid embryo formation frequency (27).

Significant difference for pseudoseed formation, in case of treatment (without emasculation), was also observed between the groups, and it was confirmed statistically using *Z* test of significance. Among the three groups of wheat, the mean response of per cent pseudoseed and haploid embryo formation was found highest in spring wheats (39 and 16) followed by winter wheats (30 and 13) and triticale × wheat derivatives (17 and 5) (Table 1). The response of individual genotypes of all the three groups for per cent pseudoseed formation in treatment and control was tested statistically using *Z* test, and all the genotypes except HPW 249, HS 375 and KWS 29 exhibited significantly higher per cent pseudoseed formation when emasculation was carried out as compared with the treatment (Table 1). The geno-

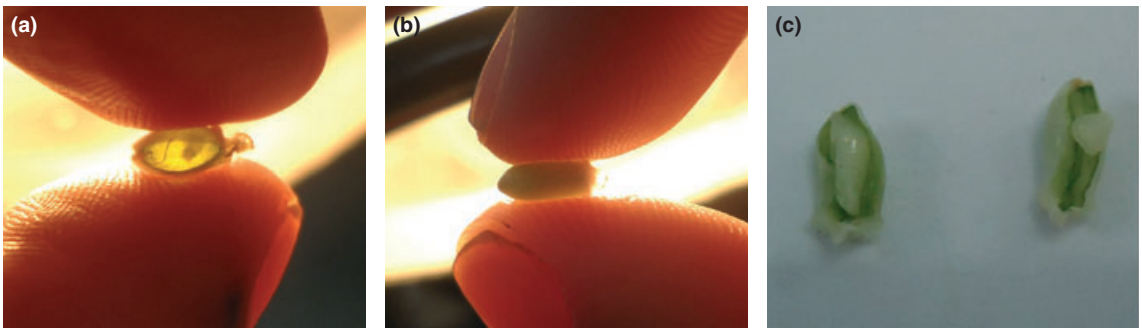

Fig. 2: (a) Embryo carrying pseudoseed of wheat. (b) Endosperm carrying selfed seed of wheat. (c) Longitudinal section of selfed seed of wheat showing endosperm (left) and embryo (right)

Table 1: Pseudoseed and haploid embryo formation frequency obtained in different groups of wheat (with and without emasculation) after hybridization with *Imperata cylindrica* pollen

Genotypes	Number of florets pollinated		Seeds obtained							Haploid embryos/ Pollinated florets (%)		
			Not Emasculated			Emasculated						
	Not Emasculated	Emasculated	Pseudoseeds/ Pollinated florets (%)	Selfed seeds/ Pollinated florets (%)	Total seeds/ Pollinated florets (%)	Pseudoseeds/ Pollinated florets (%)	Selfed seeds/ Pollinated florets (%)	Total seeds/ Pollinated florets (%)	Z (0.05) = 1.96[1]	Not Emasculated	Emasculated	Z (0.05) = 1.96[2]
A. Spring Wheat												
C- 306	112	117	10	53	64	49	0	49	7.11	4	19	3.65
Chinese Spring	118	104	41	30	72	66	0	66	3.83	16	25	1.63
HPW- 89	102	112	39	46	85	96	0	96	11.13	15	37	3.75
HPW- 249	114	107	47	15	63	42	0	42	−0.65	19	16	−0.48
HPW- 251	113	102	25	45	70	54	0	54	4.56	9	20	2.22
HS- 295	116	102	19	63	83	47	0	47	4.41	7	20	2.72
HS- 375	110	108	50	19	70	62	0	62	1.67	22	25	0.55
HS- 403	96	108	29	58	87	79	0	79	8.35	11	31	3.62
HS- 420	95	102	36	46	83	69	0	69	4.87	13	27	2.44
HW- 3024	120	96	46	13	60	66	0	66	3.02	19	26	1.20
KWS- 29	119	112	47	21	68	58	0	58	1.68	21	24	0.56
PBW- 343	114	102	51	19	71	85	0	85	5.74	22	34	1.88
UP- 2418	112	96	59	16	76	84	0	84	4.14	23	35	1.94
Mean	–	–	**39 (43)**	–	–	**66 (69)**	–	–		**16 (17)**	**26 (27)**	
χ^2 (0.05, 12) = 21.03[3]			**95.43**			**158.95**				**31.04**	**29.32**	
B. Winter Wheat												
DH- 114	104	96	21	50	71	65	0	65	7.07	9	27	3.25
DH- 150	112	104	61	24	85	79	0	79	3.01	27	33	0.95
Saptdhara	102	98	21	40	61	59	0	59	5.86	10	26	2.91
Tyari- 1	100	102	28	49	77	57	0	57	4.50	12	24	2.34
Tyari- 2	92	96	27	50	77	55	0	55	4.08	11	23	
Tyari- 3	104	94	22	55	77	64	0	64	6.70	9	25	2.98
Mean	–	–	**30 (31)**	–	–	**63 (62)**	–	–		**13 (14)**	**26 (26)**	
χ^2 (0.05, 5) = 11.07[3]			**45.46**			**13.84**				**16.05**	**2.28**	
C. Triticale × wheat derivatives												
TW-6-109	108	102	16	52	69	64	0	64	8.09	5	20	3.29
TW-6-210	110	112	19	52	71	55	0	55	6.04	**6**	16	2.50
Mean	–	–	**17 (19)**	–	–	**59 (64)**	–	–		**5 (6)**	**18 (20)**	
***Z* (0.05) = 1.96[4]**	–	–	**0.44**	–	–	**1.40**	–	–		**0.25**	**0.67**	

Figures in parenthesis represent mean of absolute count.
[1]Calculated values of Z for comparison of pseudoseed frequency with and without emasculation.
[2]Calculated values of Z for comparison of haploid formation frequency with and without emasculation.
[3]Calculated values of chi-square for comparison of genotypes with respect to pseudoseed and haploid embryo formation frequencies within a group.
[4]Calculated values of Z for comparison of two genotypes with respect to pseudoseed and haploid embryo formation frequencies within a group.

types HPW 249, HS 375 and KWS 29 (spring wheats) showed similar response for per cent pseudoseed formation with and without emasculation. For per cent haploid embryo formation, the genotypes 'Chinese spring', HPW 249, HS 375, HW 3024, KWS 29, PBW 343 and UP 2418 (spring wheats), DH 150 (winter wheat) responded similarly in both the cases. In triticale × wheat group, both the genotypes were showing poor response to haploid induction parameters when no emasculation was carried out in comparison with control.

Discussion

In wheat spikes, maturity of florets is initiated from the middle of the spike and proceeds bidirectionally. In wheat × *I. cylindrica*-mediated system of chromosome elimination, the pseudoseeds can be easily distinguished as small and fluid-filled seeds acting as a morphological marker in comparison with the milky endosperm carrying bold selfed seeds. It was observed that the selfed seeds were mostly present on upper middle area, whereas the top most and basal region of the spike produced more pseudoseeds. The different genotypes within two of three groups (spring wheat and winter wheat) showed significant difference for pseudoseed and haploid embryo formation frequency when no emasculation was carried out, conferring that the asynchronous behaviour of flowering is different in different genotypes. Among the spring wheats, the genotype UP 2418 showed significantly superior response to pseudoseed and haploid embryo formation frequency in comparison with other genotypes. In case of winter wheats, genotype DH 150 was showing highest pseudoseed and haploid embryo formation frequency in comparison with others. The different groups also exhibited significant difference for pseudoseed formation with each other in case of direct pollination that is without emasculation. Among the three groups, spring wheats followed by winter wheats responded better for pseudoseed and haploid embryo formation under direct pollination. Although, the haploid embryo formation frequency computed on the basis of number of florets pollinated was significantly higher when emasculation was carried out in all the genotypes except Chinese spring, HPW 249, HS 375, HW 3024, KWS 29, PBW 343, UP 2418 and DH 150, yet the results are quite appreciable with this technique of direct pollination during the end of the season of wheat flowering, when the spikes mature quickly leading to dehiscence of anthers while exercising emasculation. In such case, it can be advantageous to go for direct pollination instead of pollination carried out after emasculation in the spikes that are non emasculable. Moreover, during peak flowering season of wheat, we can save time and reduce the labour required for emasculation by employing direct pollination with *I. cylindrica* pollen. Strategically, it will also be advantageous where the wheat breeding materials including purelines and precious segregants to be used for induction of haploids are limited.

References

Chaudhary, H. K., 2008a: Dynamics of wheat × *Imperata cylindrica*- a new chromosome elimination mediated system for efficient haploid induction in wheat. In: R. Appels, R. Eastwood, E. Lagudah, P. Langridge, M. Mackay, L. McIntyre, and P. Sharp (eds), 11th International Wheat Genetics Symposium, Vol. 2, 647—650. University of Sydney Press, Sydney, NSW, Australia.

Chaudhary, H. K., 2008b: Dynamics of doubled haploidy breeding and molecular cytogenetic approaches in bread wheat: focus on north- west Himalayan regions. In: K. Taniguchi, and X. Zhang (eds), Adv. Chromosome Sci. **3**, 67—69.

Chaudhary, H. K., 2010: Dynamics of doubled haploidy breeding in bread wheat *vis-à-vis* Chromosome elimination process- a boon or bane for alien introgression in wheat, 110—111. Proc. 4th Asian Chromosome Colloquium, Beijing, China.

Chaudhary, H. K., S. Singh, and G. S. Sethi, 2002: Interactive influence of wheat and maize genotypes on haploid induction in winter × spring wheat hybrids. J. Genet. Breed. **56**, 259—266.

Chaudhary, H. K., G. S. Sethi, S. Singh, A. Pratap, and S. Sharma, 2005: Efficient haploid induction in wheat by using pollen of *Imperata cylindrica*. Plant Breed. **124**, 96—98.

De Vries, A. P., 1971: Flowering biology of wheat, particularly in view of hybrid seed production – a review. Euphytica **20**, 152—170.

De Vries, A. P., 1973: Some aspects of cross pollination in wheat (*Triticum aestivum* L.). 2. Anther extrusion and ear and plant flowering pattern and duration. Euphytica **22**, 445—456.

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

Komeda, N., H. K. Chaudhary, G. Suzuki, and Y. Mukai, 2007: Cytological evidence for chromosome elimination in wheat × *Imperata cylindrica* hybrids through fluorescence *in situ* hybridization. Genes Genet. Syst. **82**, 241—248.

Lukac, M., M. J. Gooding, S. Griffiths, and H. E. Jones, 2011: Asynchronous flowering and within- plant flowering diversity in wheat and the implications for crop resilience to heat. Ann. Bot. **109**, 843—850.

Percival, J., 1921: The Wheat Plant. Duckworth, London.

Pratap, A., G. S. Sethi, and H. K. Chaudhary, 2005: Relative efficiency of different Gramineae genera for haploid induction in triticale and triticale × wheat hybrids through the chromosome elimination technique. Plant Breed. **124**, 147—153.

Rangaswamy, R., 2006: A Text Book of Agricultural Statistics, 102—119. New Age International (P) Ltd., Publishers, New Delhi, India.

Waines, J. G., and S. G. Hegde, 2003: Intraspecific gene flow in bread wheat as affected by reproductive biology and pollination ecology of wheat flowers. Crop Sci. **43**, 451—463.