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# Exploring the Potential of *Imperata Cylindrica*, a Wild Grass for Development of Doubled Haploids in Wheat

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**Abstract:** *Different inter-specific/inter-generic hybridization methods are used for haploid production in wheat of which* Imperata cylindrica *mediated approach is most economical and efficient one due to its genotypic non specificity, simplicity, efficacy, lack of somaclonal variation and albino plants, time saving ability and higher regeneration rate coupled with low cost. Intergeneric hybridization of different wheat cross combinations with* Imperata cylindrica *showed variable frequencies of the haploid induction parameters and resulted in forty one haploid plants. These haploids were treated with colchicine for doubled haploid production. The plant mortality after colchicine was more and only seven DH plants were recovered. The paper highlights the potential of I. cylindrica for DH production in wheat and figures the standardization of colchicine treatment for increasing the frequency of DHs.*

**Keywords:** *Wheat, Imperata cylindrica, haploid.*

## INTRODUCTION

Wheat is an important staple food crop which occupies 25 per cent of the world's cultivated area (Velu and Singh [1]). Over the last fifty years, breeding programmes worldwide have achieved significant genetic gains in wheat leading to an increase yield of 0.7 t/ha/decade (BSPA [2]). But, growing population complemented with the changing climatic conditions forces us to augment the wheat production. Thus, there is an urgent need to develop new varieties as quickly as possible.

The *traditional method* of stabilizing the genes and developing a pure line requires self-pollinating plants for eight or nine generations. Hence its supplementation with the recent biotechnological tools is imperative. Recent advances in biotechnology have opened vast avenues to facilitate the current Plant Breeding. Double haploid (DH) breeding is one of the techniques which not only helps in accelerating conventional plant breeding programmes and make early release of cultivars but also provides 100 per cent homozygous lines. Doubled haploidy breeding also offers unique advantages to breeders which include saving time, space and labour.

Among the vast wide hybridization techniques used, the DH production *via I. cylindrica* mediated chromosomal elimination technique (first reported by Chaudhary *et al* [3]) has been reported to be the most economical and efficient one due to its genotypic non specificity, simplicity, efficacy, lack of somaclonal variation and albino plants, time saving ability and higher regeneration rate coupled with low cost. Hence the present work to develop doubled haploids in wheat was done using *I. cylindrica* mediated chromosome elimination technique.

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## MATERIALS AND METHODS

### Three Winter Wheat Genotypes

ZANDER 33, Jingdong and China 84-40022 were crossed with FLW-3, FLW-13, HS507, HS 542, VL829 and HD2967. These $F_1$'s plants were hand emasculated 2 to 3 days before anthesis without cutting the florets so as to avoid injury. The upper and basal spiklets, and all but the primary and secondary florets of the remaining spiklets were removed. Next morning fresh pollen from *I. cylindrica* was collected in petriplate and applied to the feathery stigma of the emasculated florets with brush. The uppermost internodes of wheat spikes pollinated with pollen of *I. cylindrica* were injected with 100ppm of 2,4-D soliution 24 hours after pollination and the injections were repeated for two consecutive days to improve pseudoseed formation.

Embryos obtained from the cross lacked endosperm and so the embryo excised from caryopsis of wheat $F_1$s or three way $F_1$'s were harvested 18-20 days after pollination and seeds were examined for the presence of embryo, using the technique of Bains *et al.[4]*. Embryo carrying pseudoseeds were recognized by putting them against the light source and cultured in MS medium supplemented with 0.5 mg/l kinetin, 20mg/L each of L-arginine, L-cysteine and L-leucine, 400 mg/l glutamine, 30g/l sucrose and 8 g/l agar-agar. The cultured embryos were incubated in darkness at 20± 2°C for the first two weeks and then, when the first regeneration indications appeared, were transferred to a culture room under a 10/14 hours light/dark regime and light intensity of 3000 Lux until they developed sufficiently. The protocol for developing the haploids was used as per Laurie and Bennett [5] and Chaudhary *et al*. [6].

When haploid plants reached 4-5 tiller stage, the roots were treated with solution containing colchicine (0.1%) and 1.5% DMSO for six hours and then rinsed with distilled water. The plants were then transferred in pots to a growth chamber and allowed to grow at 16/12°C day/night temperature for one month. Observations were recorded with respect to total number of florets pollinated, number of pseudoseeds, number of embryo carrying pseudoseed and regeneration of embryos in each cross. The significance of difference for haploid induction parameters *viz*., pseudoseed formation, embryo formation and haploid regeneration frequencies was analyzed by simple t test.

## RESULTS AND DISCUSSION

In the present study, the abundant pollen alongwith synchronized flowering with wheat was observed in *Imperata cylindric*a so large number of florets were pollinated in each cross (upto 1012 in Zander 33 × FLW-3). Chaudhary [7, 8] has reported that *I. cylindrica* performed better than maize and leads to high precision genetic upgradation in wheat. Badiyal *et al*. [9] had also highlighted the potential of *I. cylindrica* mediated approach for haploid induction in *Triticale* × wheat cross. Mahato and Chaudhary [10] have also highlighted the relative efficiency of *I. cylindrica* over maize for haploid induction in *T. durum*. Patial *et al*. [11] had also highlighted the importance of *I. cylindrica* mediated chromosome elimination technique for getting high frequency of haploid plants in wheat.

For haploid induction, different crosses showed variable frequencies of DH induction parameters which could be due to several reasons ranging from genetic to environmental (Amrani *et al*. [12] and Tadesee *et al.* [13]). High pseudoseed formation is attributed to auxin (2, 4-D) application resulting in ovary growth (Suenaga [14]). High frequency of pseudoseed formation in all crosses of wheat $F_1$'s with *I. cylindrica* highlighted the wide potential of the present approach over others due to its genotypic non-specific nature (Suenaga [14]; Kishore *et al.* [15]). The variation in the frequencies of different haploid induction parameters observed in the present study may be attributed to various reasons. The extent of crossabiltiy of wheat genotypes depends on the dominant alleles of crossability genes, namely *Kr1*, *Kr2* and *Kr4* (Zheng *et al.* [16]) located on long arms of 5B, 5A 5D and 1A (Sitch *et al.* [17]) chromosomes, respectively and hence may be responsible for the same.

Intergeneric hybridization of different wheat crosses with *Imperata cylindrica* resulted in forty one haploid plants. These haploids were treated with colchicine for doubled haploid production. The plant mortality after colchicine was more and only seven DH plants were recovered.

The present study therefore suggests ample scope for exploitation of *Imperata cylindrica* for getting further yield breakthrough and instant homozygosity in early generation in wheat and the need for standardization of colchicine treatment of haploid plants for getting more DHs in wheat.

## *References*

Velu G, Singh RP. (2013), Phenotyping in wheat breeding. *In*: Phenotyping in Plant Breeding. Siva Kumar Panguluri, Are Ashok Kumar. Springer publication pp 41-72.

BSPA. (2015), Plant Breeding the essential platform for sustainable agriculture. http://www.bspb.co.uk/sg_userfiles/BSPB_%E2%80%93_Plant_Breeding_for_Sustainability.pdf.

Chaudhary HK, Sethi GS, Singh S, Pratap A, Sharma S. (2005), Efficient haploid induction in wheat by using pollen of *Imperata cylindrica.* Plant Breeding 124: 96-98.

Bains NS, Mangat GS, Singh K, Nanda GS. (1998), A simple technique for the identification of embryo-carrying seeds from wheat x maize crosses prior to dissection. Plant Breeding 117: 191-192.

Laurie DA, Bennett MD. (1987), Wide crosses involving maize (*Zea mays*). Annual Report of the Plant Breeding Institute, 1986-87, pp.66.

Chaudhary HK, Singh S, Sethi GS. (2002), Interactive influence of wheat and maize genotypes on haploid induction in winter x spring wheat hybrids. Journal of Genetics and Breeding 56: 259-266.

Chaudhary HK. (2013a), New frontiers in chromosome elimination mediated doubled haploidy breeding for accelerated and high precision genetic upgradation in wheat. Proc. Int Triticeae Mapping Initiative and Plant and Animal Genome XXI Conference, San Diego, USA, 12-16 January, 2013, pp. 26.

Chaudhary HK, Tayeng T, Kaila V, Rather, SA. (2013b), Enhancing the efficiency of wide hybridization mediated chromosome engineering for high precision crop improvement with special reference to wheat × *Imperata cylindrica* system. The Nucleus. 56(1):7-14.

Badiyal A, Chaudhary HK, Jamwal NS, Hussain W, Mahato A, Bhatt AK. (2014), Interactive genotypic influence of triticale and wheat on their crossability and haploid induction under varied agroclimatic regimes. *Cereal Research Communications*, 42: 700-709.

Mahato A, Chaudhary H K. (2015), Relative efficiency of maize and *Imperata cylindrica* for haploid induction in *Triticum durum* following chromosome elimination-mediated approach of doubled haploid breeding. Plant Breeding 134: 379-383.

Patial M, Pal D, Kumar J, Chaudhary HK. (2015), Doubled haploid production in wheat using *Imperata cylindrica* mediated chromosome elimination technique. World Academy of Science, Engineering and Technology Vol: 3, No: 2 2015. pp 1.

Amrani N, Sarrafi A, Albert G. (1993). Genetic variability for haploid production in crosses between tetraploid and hexaploid wheats with maize. Plant Breed. 110: 123-128.

Tadesee W, Inagaki M, Tawkaz S, Baum M, Ginkel M V. (2012), Recent advances and application of doubled haploids in wheat breeding. African Journal of Biotechnology 11(89): 1548-1549.

Suenaga K. (1994), Doubled haploid system using intergeneric crosses between wheat (*Triticum aestivum*) and maize (*Zea mays*). National Institute of Agrobiological Resources (Japan) 9: 83-139.

Kishore N, Chaudhary HK, Chahota RK, Kumar V, Sood SP, Jeberson S, Tayeng T. (2011), Relative efficiency of the maize and *Imperata cylindrica*-mediated chromosome elimination approaches for induction of haploids of wheat-rye derivatives. Plant Breeding 130: 192–194.

Zheng YL, Luo MC, Yen C, Yang, JL. (1992), Chromosome location of a new crossability gene in common wheat. Wheat Inf. Serv. 75:36–40.

Sitch LA, Snape JW, Firman, SJ. (1985), Intrachromosomal mapping of crossability genes in wheat (*Triticum aestivum*). Theor. Appl. Genet. 70: 309-314.