I J T A
© Serials Publications
National Academy of Agricultural Science (NAAS) Rating : 3. 03

Doubled Haploids Production in Wheat (*Triticum aestivum* L.) *ViaImperata Cylindrica* Mediated Chromosome Elimination Approach

Madhu Patial[1]*, Dharam Pal[1], Jagdish Kumar[1] and H.K. Chaudhary[2]

ABSTRACT: *Haploids and doubled haploids (DH) have been produced in wheat using chromosomal elimination following wide hybridization. Since 1975, when Barclay recovered wheat haploids in crosses between the wheat variety Chinese Spring and Hordeum bulbosumthis technique has been constantly improved and modified. The development and improvement of chromosomal elimination technique in wheat has allowed DH populations to be obtained on a large scale. Today DH lines are used in genetic studies, such as gene mapping, location of quantitative trait locus, marker/trait association studies, and genomics and to analyze the impact of the environment on the yield and quality of the seeds. Currently, DH technology has become more efficient and hence can be widely applied in wheat breeding. Additionally, DH technology is used to shorten the process of breeding new varieties. In the present study, intergeneric hybridization between F_1's of wheat with Imperata cylindrica (a wild species) was used for production of DH wheat plants. Wheat doubled haploids were formed by complete chromosome elimination of I. cylindrica chromosome followed by colchicines treatment and haploids regenerant were obtained from most of the crosses.*

Keywords: *Chromosome elimination,doubled haploids,Imperata cylindrica, Triticum aestivum.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important food crop of humanity and forms the central pillar of food security and economy of many countries. With the massive population burst, the demand for food is increasing. Looking into the future, global wheat requirements are expected to increase from the current 685 million tons (from 225 million hectares in 2009) with an average productivity of 3.04 t/ha [1] to about 900 million tons in the year 2020 with an average productivity of 4 t/ha to meet demand [2]. Thus, wheat not only has a key role to play in current food security, but also in future global food security and poverty reduction. However, the genetic gain in productivity is not keeping up pace with the growing mouths. Fulfilling this demand is very challenging in the face of increasing population lining with the climate change, increasing drought, heat stress and emergence of new virulent diseases and pests. Offsetting these challenges requires designing of an effective wheat breeding strategy supplemented with new technologies and tools.

The development of new varieties requires creation and introduction of genetic variation, inbreeding coupled with selection, and extensive evaluation of breeding materials at multiple locations to identify adapted and stable genotypes with desirable traits. Hence, the conventional breeding is a long process which requires atleast eight to nine years for varietal development. Recent advances in plant tissue culture and its related disciplines opened an avenue that greatly facilitated the varietal breeding scheme. Double haploidy breeding is one of the technique which offers a rapid mode for production of truly homozygous line and helps to expedite crop breeding programs for homogeneity. A number of plant species are being look for DH production and*Triticum aestivum* L. X *Imperata*

[1] Indian Council of Agricultural Research-Indian Agricultural Research Institute, Regional Station, Shimla, Himachal Pradesh-171004, India
* Corresponding authors. *E-mail* : *mcaquarain@gmail.com*
[2] Cytogenetics and Tissue culture Lab, Department of Plant Breeding and Genetics, CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh-176062, India

cylindrica L. interspecific hybridization (first reported by Chaudhary *et al*. [3] and Pratap *et al*. [4]) has come out to be the most efficient and promising technique. Therefore, present work was initiated to produce wheat DH populations *viaImperata cylindrica* L. interspecific hybridization.

MATERIALS AND METHODS

Plant Material and Crossing

The wheat F_1's (Table 1) were produced at Indian Council of Agricultural Research-Indian Agricultural Research Institute, Regional Station, Shimla, during Kharif 2013-14 which were grown at CSKHPKV, Palampur for DH production. These F_1s 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 with brush. The uppermost internodes of wheat spikes pollinated with pollen of *I. cylindrica* were injected with 100 ppm of 2, 4-D solution 24 hours after pollination and the injections were repeated for two consecutive days to improve pseudoseed formation.

Table 1
F1's used in the present study

S. No.	*F_1's*
1.	Zander33 X FLW-3
2.	Zander33 XFLW-13
3.	Zander33 XHS507
4.	Zander33 XHS542
5.	Zander33 XVL829
6.	Zander33 XHD2967
7.	Jing-dong X FLW-3
8.	Jing-dong XFLW-13
9.	Jing-dong XHS507
10.	Jing-dong XHS542
11.	Jing-dong XVL829
12.	Jing-dong XHD2967
13.	China 84 X FLW-3
14.	China 84 X FLW-13
15.	China 84 XHS507
16.	China 84 XHS542
17.	China 84 XVL829
18.	China 84 XHD2967

Embryo Rescue and Doubled Haploid Production

Embryos obtained from the cross lacked endosperm and so the embryo were harvested 18-20 days after pollination and seeds were examined for the presence of embryo, using the technique of Bains *et al.* [5]. The embryo obtained were excised under aseptic conditions and cultured on a nutrient medium comprising of standard MS medium supplemented with 0.5 mg/l kinetin, 20mg/L each of L-arginine, Lcysteine 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 [6] and Chaudhary *et al*. [7].

The roots of haploid plants 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 for hardening and were later transferred to the pots.

RESULTS AND DISCUSSION

Impereta cylindrica is an important source for substituting/supplementing maize mediated doubled haploid production of wheat as its pollens are abundantly available during wheat crossing period the same has also been reported by Chaudhary *et al.* [3]. In the present study also the abundant pollen alongwith synchronized flowering was observed thereby, highlighted the technique to be superior over maize mediated system. Also, no endosperm formation was there when wheat F_1s and three way F_1s were crossed with *I. cylindrica* proving its superiority over wheat x maize system for haploid induction. Pratap *et al*. [4]and Mahato and Chaudhary [8]have also reported the superiority of the system over other system of DH breeding in wheat.

Different crosses showed variable frequencies of haploid induction parameters which could be due to several reasons ranging from genetic to environmental as has also been reported by Amrani *et al*. [9] and Tadesee *et al.* [10]. High pseudoseed formation is attributed to auxin (2,4-D) application resulting in ovary growth (Suenaga[11]). High frequencies of pseudoseed formation (84.03-95.97%) in different crosses of wheat F_1's with *I. cylindrica* highlights the wide potential of the present approach over others due to its genotypic non-specific nature (Inagaki and Tahir, [12]).

The Doubled haploid plants were obtained from all the crosses except Zander 33X FLW-3, Zander 33 XFLW-13 Zander 33 XHS5 42, Jing-dong XHS 542 China 84 XHS 542 and China 84 XVL 829, which

highlighted the potential scope for production of wheat DH *via I. cylindrica* mediated chromosome elimination techniqueand genotypic non-specific nature of *I. cylindrica*.

REFERENCES

FAO. (2011), Statistical database [internet]. FAO 2011. www.fao.org.

Ortiz R, Trethowan RM, Ortiz Ferrara G, Iwanaga M, Dodds JH, Crouch JH, Crossa J, Braun HJ. (2007), High yield potential, shuttle breeding and a new international wheat improvement strategy. Euphytica **157**: 365–384

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.

Pratap A, Sethi GS, Chaudhary HK. (2005), Relative efficiency of different Gramineae genera for haploid induction in triticale and triticale × wheat hybrids through the chromosome elimination technique. Plant Breeding **124**: 147–153.

Bains, NS, MangatGS, Singh K, NandaGS. (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 H K, Singh S, Sethi GS. (2002), Interactive influence of wheat and maize genotypes on haploid induction in winter x spring wheat hybrids. Journal of Genetics & Breeding **56**: 259-266.

Mahato A, ChaudharyH 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 Breedingb **134**: 379–383.

Amrani, N, Sarrafi A, AlbertG. (1993), Genetic variability for haploid production in crosses between tetraploid and hexaploid wheats with maize. Plant Breeding **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): 15484-1549.

Suenaga, K. (1994), Doubled haploid system using intergeneric crosses between wheat (*Triticum aestivum*) and maize (*Zea mays*). National Institute ofAgro biological Resources (Japan) No. **9**: 83-139

Inagaki M, Tahir M. (1990), Comparison of haploid production frequencies in wheat varities crossed with *Hordeum bulbosum* L. and maize. Japanese Journal of Breeding **40**: 209-216.

❍❍❍