Journal of Cereal Research
Volume 15 (1): 31-40
Homepage: http://epubs.icar.org.in/ejournal/index.php/JWR

Reveiw Article

Imperata cylindrica mediated doubled haploidy in wheat (*Triticum aestivum* L.): quittances and breakthrough

Silky Gandhi[1], Harinder Kumar Chaudhary[2], Dharam Pal[1], Satish Kumar[3], Charan Singh[3], Santosh Kumar Bishnoi[3] and Madhu Patial[1*]

[1] ICAR-IARI, Regional Station, Tutikandi Centre, Shimla (H.P), India
[2] CSKHPKV, Palampur
[3] ICAR-IIWBR, Karnal, India

Article history:
Received: 12 Feb., 2023
Revised: 11 Apr., 2023
Accepted: 22 Apr., 2023

Citation:
Gandhi S, HK Chaudhary, D Pal, S Kumar, C Singh, SK Bishnoi and M Patial. 2023. *Imperata cylindrica* mediated doubled haploidy in wheat (*Triticum aestivum* L.): quittances and breakthrough. *Journal of Cereal Research* ***15 (1):*** *31-40. http://doi.org/10.25174/2582-2675/2023/133100*

****Corresponding author:***
E-mail: mcaquarian@gmail.com

Abstract

Wheat crop has a major role in current food system and global food security. Beyond doubt, wheat production has increased substantially, however, there has been increase in world's population also, thereby, leading to increased food supply demand. To fulfil this demand, science today needs more efficient and new technologies for breeding wheat with faster pace. Conventional wheat breeding is time consuming as it requires the selfing from F_1 generation onwards to attain homozygosity. However, with the novel biotechnological tools there has been speeding up of the wheat breeding programmes. Doubled haploidy (DH) is one of the cutting-edge technologies which has been accepted widely for the improved wheat breeding programmes. Using DH technology, it is possible to attain the 100% homozygosity in a single generation and time to get complete homogenous breeding lines reduces to 1-2 years. Androgenesis (anther culture and microspore culture) and wheat × maize wide hybridization are two major methods to develop the double haploids in wheat. However, they have some limitations and need other techniques for DH induction. Among the different wide hybridization methods for DH development, *Imperata cylindrica* **mediated chromosome elimination is known as most economical and efficient. Application of this method with the conventional breeding is game changing tool in wheat breeding programmes, and complement the conventional breeding to develop the improved lines in shortest time.**

Keywords: double haploid, *Imperata cylindrica*, anther culture, wheat.

1. Introduction

Wheat, an essential staple food grain is considered as the leading source of calories and plant derived protein (Figueroa *et al.*, 2018). Additionally, wheat is source of B-group vitamins, minerals, and dietary fibres. Since ages, wheat has been used in the guise of chapatis, biscuits, breads, confectionary products, due to the elasticity of gluten. Considered as a good source of animal feed, wheat is also being used for ethanol production, brewing of wheat beer, raw material for cosmetics, and wheat protein as the substitutes of meat, and, to develop the wheat straw composites. Its germ and bran are source for dietary fibres and prevents digestive disorders, thereby, the whole wheat bread is known to be have health benefits and prevention of diet-related diseases (Yadav, 2011). Due to nutritive profile and relatively easy storage, harvesting, transportation, and processing, wheat, became the leading grain used for human consumption (Kulp, 2000). Globally, during 2022-23 wheat production is expected to a record of 779.6 million metric tonnes up slightly from 2021-22. India contributes up to 12% of the wheat production and is the second largest producer of wheat next to China followed by USA, France and Canada (Singh *et al*., 2007) Punjab, Uttar Pradesh (UP.

Global wheat demand is soaring in recent years because of umpteen factors; change in eating habits, population trends, and socio-economic conditions. Adding to this, climate change, a serious concern of the present time, could strongly affect the wheat crop. Therefore, continuous improvement in wheat is vital to meet the increasing demands of the world population. Doubtlessly, conventional breeding tools like hybridization, mutation breeding and shuttle breeding have resulted in the development of many high yielding wheat varieties but, are time consuming. Biotechnological techniques like double haploidy can be an important tool for the same (Patial *et al*., 2019). Doubled haploidy is production of haploids from hybrids progenies with the chromosome doubling, with favourable gene combinations (Bentolila *et al.,* 1992). Haploids are considered as sporophytes which contains gametic chromosome (n) numbers. Einkorn, emmer, and dinkel wheat consist of n= 7, n = 2x = 14, and n = 3x = 21 chromosomes having the constitution of A, AB, and ABD, respectively (Patial *et al*., 2019). Doubled haploidy (DH) technique can produce genetically stable pure lines in a single year while traditional methods need at least eight or nine generations and these homozygotic lines gives more realistic pictures of agronomic performance, due to which selection becomes more efficient.

With the advancement in science, new technological windows like marker assisted selection, genomics, CRISPR/Cas9, proteomics have emerged to develop improved wheat varieties for changing climate. Of these, recent advances, and achievements in doubled haploidy are known to augment conventional breeding by development of homozygous lines in shortest time- frame along with additional advantages (Table 1).

Table 1: Comparison of conventional and doubled haploidy technique

Sr. no	Factor	Doubled haploidy	Conventional breeding
1	Time	2-3 years	7-8 years or more
2	Homozygosity	100 percent	No
3	Heterosis fixation	Yes	No
4	Recessive mutant identification	Very easy	Tough
5	Expenditure	High	Low

2. Doubled Haploidy technology: Advantages and applications

Doubled haploidy technique is used to generate pure lines for basic research and development of commercial cultivars. These lines are genetically homozygous, produced when spontaneous or induced chromosome duplication of haploid cells occurs (Inagaki, 1997). This technology is one of the biggest achievements in plant breeding because of production of completely homozygous plant within a year (Hooghvorst and Nogues, 2021). Doubled haploidy technology plays an important role in studying the genetic control of traits in wheat, in marker assisted selection, genomics and genetic engineering (Eliby *et al*., 2022). Doubled haploidy breeding accelerates in crop improvement by providing instant homozygosity leading to fixation of desirable characters of recombinants as well as reduction in number of generations required to achieve success. Researchers have applied different methods for production of DHs in wheat which includes anther culture, ovule culture, chromosome elimination following wide hybridization, haploid inducer gene/s and chemical treatments (Patial *et al*., 2019, Singh *et al.,* 2013) and used DH lines for wheat improvement against different biotic and abiotic stresses (Table 2). All these methods have varying rate of accuracy and efficacy. Among all the DH induction techniques, anther culture and wide hybridization of wheat with barley, maize and *Imperata cylindrica* have been widely used in breeding programmes in wheat and reported to be the most effective for DH production due to their high efficacy.

Table 2: Double haploid population developed for wheat improvement against biotic and abiotic stresses

Method	Cross	DH lines (Name/No.)	Biotic/ Abiotic Stress	Resistance gene	Reference
Anther culture	CPAN1676	10	Drought tolerance	*HVA1*	(Chauhan and Khurana, 2011)
Anther culture	T39 × Ozon and genotype T36 × Hondia	-	Leaf rust	-	(Weigt *et al.*, 2016) an introduction of leaf rust resistance genes into highyielding wheat genotypes is essential. One of such genes is Lr19 (leaf rust resistance gene
Anther culture	Line-115 × Gemmeiza-7 Line-115 × Giza-164 Gemmeiza-7 × Giza-164 Giza-164 × Giza-168	(4) Line-115 × Gemmeiza-7 (4)Line-115 × Giza-164 (5) Gemmeiza-7 × Giza-164 (2)Giza-164 × Giza-168	Salt tolerance	SL, CAT, and SDW	(Al-Ashkar *et al.*, 2019)100, and 200 mM NaCl
Wheat × maize	Trigo BR 35 and IAC 13-Lorena	-	Leaf rust	-	(Brammer *et al.*, 2004)which, under the high inoculum pressure of the southern region, has been resistant to leaf rust for more than 12 years, and the susceptible cultivar IAC 13-Lorena. Haplodiploidization via in vitro gimnogenesis was done by somatic elimination of the pollen donor genome after maize pollination of the F1 plants. The advantages and usefulness of double haploids (DH
Wheat × **maize**	(Kharchia*TW161) F1* Seneca 60	47 and 20	Salt tolerance		(Mahmood and Baenziger, 2008)
Wheat × maize	Kariega 9 cv. Avocet S	254	Stripe, leaf and stem rust	-	(Prins *et al.*, 2011)
Wheat × maize	CI13227 × Lakin	181	Leaf rust	-	(Lu *et al.*, 2017)a new doubled-haploid (DH
Wheat × maize	Angas ph1b, ISR991.1 and ISR1049.5	-	Stem rust	-	(Singh *et al.*, 2019)but there are very few reports of rust resistance being transferred from this species to wheat. Here, we report wheat-Th. bessarabicum–derived stem rust and stripe rust–resistant doubled haploid (DH.

Wheat × maize	K1 – KBP0916 × Jantarka 2 – KBP0916 × STH9014, K3 – SMH8892 × KBH4942 K4 – KBP0916 × POB32408 K5 – D 323/07 × Patras K6 – D 414/07-4 × KWS Ozon.	65 (K1-K4) 30 (K5,K6)	Eye spot	*Pch1*	(Wisniewska *et al.,* 2019)
Bulbosum	Westonia×6/Sr24 and wheat- Agropyron recombinant WA1	-	Stem rust	-	(Mago *et al*., 2011)
I.cylinderica	(HS542) and winter (China 84-40022)	-	Stripe and leaf rust	-	(Patial *et al*., 2021)

3. Anther Culture Technology

The first discovery of haploid plants in *Datura stramonium* by Blakeslee *et al.* (1922), attracted the attention of many geneticists, physiologists and also plant embryologists. Till 1960, haploids were reported in seventy-one species which belonged to 39 genera and 14 family, but the research was limited because of low frequency production of haploids (Kimber and Riley, 1963). However, reports have been published on haploids plants induction *in-vitro* by anther culture technique majorly, in 247 species of angiosperms from 88 genera representing 34 families (Maheshwari *et al*., 1982). Double haploid lines developed by the anther culture technique proved to be an efficient method for fixing the genes of rust resistance and desirable double haploids can be developed by this method. DH lines were generated with this method by crossing 'Ghods' and '9106' (Moieni and Vallavieille-Pope, 1997). Kharoba is the first wheat cultivar developed with the technique of double haploidy (anther culture) derived from the cross between 'Altar 84'/*Aegilops squarrosa*221//'Pastor'/3/k134(6)/'Veery'//'Bobwhite'/'Pavon'/4/'Tilila', in the Morocco, which is resistant to the many disease (Elhaddoury *et al*., 2012).

Anther culture provides many benefits in the breeding programmes, majorly the production of homozygous lines within a single generation. Inbred lines can be produced *via* combining the rapid propagation along with the selection. Methods such as MAS (marker assisted selection), genetic transformation and QTL analysis can be combined with the *in-vitro* anther culture to accomplish several desired goals of research in breeding. It does accelerate the breeding of new varieties and aids in the quick selection of recessive alleles (Lantos and Pauk, 2020; Chauhan and Khurana, 2011).

Despite so much of progress made in the anther culture technique since 1973, it has some limitations such as: highly genotype specific (Almouslem *et al.,* 1998; Garcia-Lamas *et al.,* 2004) and low frequency in haploids. The DH produced due to anther culture have genetic alterations which are very much unpredictable because of gametoclonal variation along with a major limitation of high rate albinism (Jauhar *et al*., 2009). Anther culture is influenced by: genotype (Andersen *et al*., 1987), donor plant growth conditions (Orshinsky and Sadasivaiah, 1997), the developmental stage of microspores (Haggag *et al.,* 1996), pre-culture treatments, and media components (Lazaridou *et al*., 2005). Earlier work reported lower frequency of green plants induction by using anther culture technique i.e. 0.7% (Ouyang *et al*., 1973). However, there has been improvement in the frequency of microspore embryogenesis in wheat (Chu *et al*., 1990). Liu *et al*., (2002) has reported a DH method by using the microspores involving multiple steps such as spikes pre-treated with inducer, isolation of microspore, and complex media culture, but this process is a complex needing standardization at each step. However, anther culture technique has been successfully opted in wheat breeding and scientist are working to enhance efficiency of the technique.

The other methods of haploid production i.e., unfertilized ovaries and ovules are also reportedly used by researchers. Different methods of DH induction have opened new avenue and have used them in wheat breeding programmes (Table 3).

Table 3: Different techniques of doubled haploid production

Sr. no	Method	Cross	Reference
1.	Anther culture	'Ghods' and '9106'	(Moieni and Vallavieille-Pope, 1997)
2.	Anther culture	'Altar 84'/Aegilops squarrosa 221//'Pastor'/3/k134(6)/'Veery'//'Bobwhite' /'Pavon'/4/'Tilila',	(Elhaddoury *et al.*, 2012).
3.	*Hordeum bulbosum*	T6-1 and Petkus rye	(Mago *et al.*, 2011).
4.	Wheat×maize	AC Cadillac'/Carberry × 'RL5405' (Sr33)/'Carberry'	(Zhang *et al.*, 2019)
5.	Wheat×maize	'Sabalan*2' × 'Kal-Blo "s"	(Moradi *et al.*, 2009)three F1 wheat hybrids were crossed using pollens of three maize hybrids. Out of 1071 pollinated florets, success in seed set ranged from 63.1% to 93.3% (mean 78%
6.	Wheat×maize	'MV17' and 'Falat'	(Moradi *et al.*, 2009)three F1 wheat hybrids were crossed using pollens of three maize hybrids. Out of 1071 pollinated florets, success in seed set ranged from 63.1% to 93.3% (mean 78%
7.	Wheat×maize	Trigo BR 35 and IAC 13-Lorena	(Brammer *et al.*, 2004)which, under the high inoculum pressure of the southern region, has been resistant to leaf rust for more than 12 years, and the susceptible cultivar IAC 13-Lorena. Haplodiploidization via in vitro gimnogenesis was done by somatic elimination of the pollen donor genome after maize pollination of the F1 plants. The advantages and usefulness of double haploids (DH.
8.	*I.cylinderica*	(HS 542/China 84-40,022)	(Patial *et al.*, 2021)

4. Wide hybridization involving Chromosome elimination techniques

Different wide hybridization methods have been developed for the haploid production in wheat involving interspecific and inter-generic hybridization. The wide hybridization methods reported: wheat x *I. cylinderica* (Chaudhary *et al.,* 2005), wheat x pearl millet (Ahmad and Comeau, 1990), wheat x teosinte (Suenaga *et al.*, 1998), wheat x maize (Laurie and Bennett, 1988), wheat x barley (Barclay, 1975) are found be successful and effective in DH production in wheat. These methods require chromosome elimination, embryo rescue and chromosome duplication for developing DH plants.

Barclay (1975) initiated chromosome elimination technique in wheat and recovered wheat haploids in crosses between the wheat variety Chinese spring and *Hordeum bulbosum*. The technique was genotype-specific due to the presence of dominant crossability inhibitor genes *Kr1* and *Kr2* genes, thereby, making the chromosome elimination technique to be more efficient and of practical value for some genotypes only. The *Kr* genes act by inhibiting foreign pollen tube growth at the base of the style and in the transmitting tract of the wheat ovary (Jalani and Moss, 1980; Snape *et al.*, 1986).

The production of haploid plants from wheat x maize introgression was first reported by Laurie and Bennett

(1988). Using this wheat × maize method of DH induction, Chaudhary *et al.* (2013) was successful in developing the DH 114 (Him Pratham) which is the first doubled haploid wheat in the hill regions of north- western Himalayas of India. Successful production of doubled haploid lines of wheat through pollination of wheat F_1 plants with maize pollens has been reported in cultivar 'MV17' and 'Falat', and also 'Sabalan*2' x 'Kal-Blo "s" using KSC 108, KSC 301, and KSC 704 as maize pollinators (Moradi *et al*., 2009)three F1 wheat hybrids were crossed using pollens of three maize hybrids. Out of 1071 pollinated florets, success in seed set ranged from 63.1% to 93.3% (mean 78%. Production of doubled haploid line has been successfully done with wheat-rye recombinant T6-1 and Petkus rye and between the a backcross line Westonia x 6/Sr24 and wheat- *Agropyron* recombinant WA1 (Mago *et al*., 2011). Another study reported development of 68 double haploids by wheat x maize hybridization method from the hybrid 'AC Cadillac'/Carberry x 'RL5405' (Sr33)/'Carberry', where the source of pollen was the corn variety, 'Quickie' (Zhang *et al*., 2019).

Zygotes arising in wheat x maize cross contain one complete haploid chromosome set from each parent, but the maize chromosomes have poorly defined centromeres and shows little affinity for spindle micro tubules. Therefore during the first few cell divisions, maize chromosomes are lost and haploid plantlets are produced (Laurie and Bennett, 1988). The endosperm of such seed is either absent or highly abnormal (Laurie and Bennett, 1987)including rye and Hordeum bulbosum, with Kr1 having the greater effect. However, a cytological study of wheat ovaries fixed 48 h after pollination showed that the wheat genotypes 'Highbury' (kr1, Kr2 and the embryos soon degenerate, if they are left to develop on the wheat plant, so it needs to be cultured. This method of producing the double haploids resulted in production of haploids from many commercial wheat cultivars and hybrids for obtaining homozygous recombinant lines resistant to Russian wheat aphid (Kisana *et al*., 1993). Therefore, wheat x maize system of doubled haploid production, which is genotypic non-specific, used by the researches for doubled haploidy induction in wheat. Although the wheat × maize system of haploid production is quite successful, yet, maize crop needs to be grown in the greenhouse to coincide flowering with wheat which increases the haploid production cost (Patial *et al.,* 2019). Therefore, search for new DH induction technique led to identification of *Imperata cylindrica*, an efficient grass for DH induction in wheat.

5. *Imperata cylindrica* mediated doubled haploidy: Advantages and Progress

The *Imperata cylindrica* (2n=20), is and efficient pollen source and better performing than maize. It is genotypic non-specific. *Imperata cylindrica,* also known as "cogon grass" is a member of *Gramineae* family. It is a winter season perennial grass which grows wild and is available in the surrounding of the wheat fields and flowers at the same time as wheat. *I. cylindrica* imparts potential advantages in double haploid breeding in wheat (Chaudhary, 2008; Patial *et al.,* 2021).

a) *I. cylindrica* does not requires growing in the greenhouse, repetitive sowing and raising the pollen parent due of its perennial nature.
b) Haploid embryo formation and regeneration in *I. cylindrica* mediated technique is more than the maize mediated system.
c) *I. cylindrica* can be widely used for DH induction in all wheat genotypes due to its pollen's insensitiveness to cross ability inhibitor genes.
d) *I. cylindrica* is the most economical and efficient technique for DH induction in wheat due to its genotypic non-specificity, simplicity, time saving and also the absence of somaclonal variation imparting low regeneration cost for doubled haploid production in wheat (Patial *et al*., 2016).

Chaudhary *et al*., (2019), for the first time reported the system of DH induction in wheat *via I. cylindrica* approach of double haploidy breeding and the production of DH in crosses between triticale × wheat and wheat × rye hybrids. The variability in the haploid induction parameters was observed for the embryo formation and regeneration. Sharma *et al.* (2019) also reported the induction of DH via this approach and highlighted the enhanced DH induction, efficiency, time saving and less costly approach of DH induction in wheat. Komeda *et al*., (2007) reported the successful fertilization of parental gametes by the cytological observations and showed complete elimination from nuclei at first cell division.

Kishore *et al.* (2011) reported the backcrosses between the F_1 hybrids of spring wheat genotype (HS 375 and

C 306) and winter wheat genotypes (Saptdhara and Tyari) with the Indian rye. By using the pollens of maize and *Imperata cylindrica*, intergeneric hybridization was executed in wheat-rye derived backcrosses to study the relative efficiency of maize and *I. cylindrica* chromosome elimination approaches in haploid plants induction. The seed formation in both pollen sources was same. However, the relative frequency of embryo carrying seeds in *I. cylindrica* ranged from 8% to 30% whereas in case of maize, no embryo carrying seed was obtained. Therefore, the comparison of haploid induction in wheat x rye backcross derivatives showed that wheat x *I. cylindrica* technique was superior and cost effective, with synchronized flowering with wheat flowering season as compare to the wheat × maize system.

Patial *et al*. (2015) from intergeneric hybridization between F_1's of winter and spring wheat with *Imperata cylindrica* produced haploid wheat plants by complete chromosome elimination of *I. cylindrica* chromosome. DH plants were generated from most of the crosses. In another study Patial *et al*. (2017) used two spring and three winter wheat genotypes which were crossed in Line x Tester manner forming six F_1's and three spring and one winter wheat genotypes for two three-way F_1's. The wheat F_1's and three- way F_1's were crossed with *I. cylindrica* resulted in mean frequency of pseudoseed formation 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. Thereafter, Patial *et al*., (2021) further standardized the technique of *I. cylindrica* mediated chromosome elimination technique for increasing the efficiency of doubled haploid production in wheat. By using standardised protocol Patial *et al*., (2022) further reported the development of first genetic stock by *I. cylindrica*-mediated elimination technique. The work highlighted the development of the wheat doubled haploid genetic stock resistant to yellow and brown rust *via* intergeneric hybridization with *I. cylindrica*. The doubled haploid genetic stock was developed by crossing wheat F_1 with *I. cylindrica.* Then the DH-1 was tested against eight races of yellow and eighteen races of brown rust at seedling stage and mix races of yellow and brown rust in adult plant stages. DH-1 showed resistance to all yellow and brown pathotypes except for the *77-5* race of brown rust at seeding stage. Kapoor *et al*., (2021) used maize and *I. cylindrica* as pollen source for induction of haploids in wheat through chromosome elimination method. He reported *I. cylindrica* with higher frequency in induction with (18.39%) compared with the maize with (4.08%). Hence, the development of doubled haploids by *I. cylindrica* mediated chromosome elimination technique is an effective tool for the development of wheat DH lines in shortest timeframe (Patial *et al*., 2021).

Conclusion

Double haploid technique is advantageous in shortening the time period to release a variety along with their use in various genetic studies. Double haploid lines have applications in basic and applied researches. The technique offers many advantages such as time saving, space and labour, small selection population sizes, elimination of deleterious mutations, useful in the development of transgenics, additional variation in the form of gametoclonal variations and elimination of dominant alleles controlling undesirable traits (Patial, 2016). The technique aids in the commercial breeding to develop the mapping population using efficient haploid induction procedures (Niu *et al*., 2014). *I. cylindrica* has several advantages over the other methods as it is a winter season plant and coincides well with the wheat flowering in the natural environment, its nearby availability to wheat fields, and its efficiency to produce more DH populations. Hence, *I. cylindrica* is an efficient method for accelerating the wheat breeding programs via double haploidy approach.

Acknowledgments

The authors acknowledge ICAR-IARI, New Delhi for inhouse project on rust resistance barley breeding at ICAR-IARI, Regional Station, Tutikandi Centre, Shimla (H.P).

Author contributions

The review was written and enriched by MP, SG and SKB. All authors read, edited, and approved the final manuscript.

Conflict of interest: No

Declaration

The authors declare no conflict of interest.

6. References

1. Ahmad C and A Comeau. Wheat x pearl millet hybridization: consequence and potential. *Euphytica* 1990; ***50***:181-190.
2. Al-Ashkar, I Alderfasi, A El-Hendawy, S Al-Suhaibani, N El-Kafafi and Seleiman, MF. Detecting salt tolerance in doubled haploid wheat lines. *Agronomy* 2019; **9**:211.
3. Almouslem AB, VR Bommineni, PP Jauhar, TS Peterson and MB Rao. Haploid durum wheat production via hybridization with maize. *Crop Sci.* 1998; 38:108-1087.
4. Andersen SB, IK Due and A Olesen. The response of anther culture in a genetically wide material of winter wheat (*Triticum aestivum L.*). *Plant Breed.* 1987; ***99***: 181-186.
5. Barclay IR. High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. *Nature* 1975; ***256***: 410-411.
6. Bentolila S, T Hardy, C Guitton and G Freyssinet. Comparative genetic analyses of F_2 plants and anther culture derived plants of maize. *Genome* 1992;***35***(4):575-82.
7. Blakeslee AF, J Belling , ME Farnham and AD Bergner. A Haploid mutant in the jimson weed, "*Datura stramonium.*" *Science* 1922; ***55***: 646-647.
8. Brammer SP, MIB de Moraes Fernandes, AL Barcellos and SCK Milach. Genetic analysis of adult-plant resistance to leaf rust in a double haploid wheat (*Triticum aestivum* L. em Thell) population. *Genet. Mol. Biol.* 2004; ***27***: 432-436.
9. Chaudhary HK, GS Sethi, S Singh, A Pratap and S Sharma. Efficient haploid induction in wheat by using pollen of *Imperata cylindrica. Plant Breed.* 2005; ***124***: 96-98.
10. Chaudhary HK, PK Sharma, NV Manoj and K Singh. New frontiers in chromosome elimination-mediated doubled haploidy breeding: Focus on speed breeding in bread and durum wheat. Indian J. Genet. *Plant Breed.* 2019; ***79***: 254-263.
11. Chaudhary HK, T Tayeng, V Kaila and SA Rather. Enhancing the efficiency of wide hybridization mediated chromosome engineering for high precision crop improvement with special reference to wheat 9 Imperata cylindrica system. *The Nucl.* 2013; ***56***(1):7-14
12. Chaudhary HK. Dynamics of wheat *Imperata cylindrica*: a new chromosome elimination mediated system for efficient haploid induction in wheat. In: Appels R, Eastwood R, Lagudah E, Langridge P, Mackay M, McIntyre L, Sharp P, 11th international wheat genetics symposium, University of Sydney Press, Australia 2008; ***2***: 647-650
13. Chauhan H and P Khurana. Use of doubled haploid technology for development of stable drought tolerant bread wheat (*Triticum aestivum L.*) transgenics. *Plant Biotechnol. J.* 2011; ***9***: 408-417.
14. Chu CC, RD Hill and L Brule-Babel. High frequency of pollen embryoid formation and plant regeneration in *Triticum aestivum L.* on monosaccharide containing media. *Plant Sci.* 1990; ***66***: 255-262.
15. Elhaddoury J, S Lhaloui, SM Udupa, B Moatassim, R Taiq, M Rabeh, M Kamlaoui and M Hammadi. Registration of 'Kharoba': A Bread Wheat Cultivar Developed through Doubled Haploid Breeding. *J. Plant Regist.* 2012; ***6***: 169-173.
16. Eliby S, S Bekkuzhina, O Kishchenko, G Iskakova, G Kylyshbayeva, S Jatayev, K Soole, P Langridge, N Borisjuk and Y Shavrukov. Developments and prospects for doubled haploid wheat. *Biotechnol. Adv.* 2022; ***60***: 108007.
17. Figueroa M, KE Hammond-Kosack and PS Solomon. A review of wheat diseases-a field perspective. *Mol. Plant Pathol.* 2018; ***19***: 1523-1536.
18. Garcia-Lamas C, MC Ramirez and J Ballesteros. Effect of pollinator on haploid production in durum wheat crossed with maize and pearl millet. *Plant Breed.* 2004;***123***: 201-203.
19. Haggag ME and MA El Hennawy. Anther culture of Egyptian wheat cultivars. Annals of Agricultural Science, Ain Shams Univ. 1996.
20. Hooghvorst I and S Nogues. Chromosome doubling methods in doubled haploid and haploid inducer-mediated genome-editing systems in major crops. *Plant Cell Rep.* 2021;***40***: 255-270.
21. Inagaki M. Technical advances in wheat haploid production using ultra-wide crosses. *JIRCAS J.* 1997; ***4***:51-62.

22. Jalani BS and JP Moss. The site of action of the crossability genes (Kr1, Kr2) between triticum and secale. I. Pollen germination, pollen tube growth, and number of pollen tubes. *Euphytica* 1980; ***29***: 571–579.
23. Jauhar PP, SS Xu and PS Baenziger. Haploidy in cultivated wheats: induction and utility in basic and applied research. *Crop Sci.* 2009; ***49***: 737-755.
24. Kapoor C, HK Chaudhary, P Sharma, A Relan, NV Manoj, K Singh and VK Sood. *In vivo* haploid induction potential of Himalayan maize (*Zea mays*) and cogon grass (*Imperata cylindrica*) gene pools in different segregational cycles of intra and inter-generic crosses of wheat. *Plant Genet. Resour.* 2021; ***19***: 522-529.
25. Kimber G and R Riley Haploid angiosperms. *Bot. Rev.* 1963;***29***: 480-531.
26. Kisana NS, KK Nkongolo, JS Quick and DL Johnson. Production of doubled haploids by anther Culture and wheat x maize method in a wheat breeding programme. *Plant Breed.* 1993; ***110***:96-102.
27. Kishore N, HK Chaudhary, RK Chahota, V Kumar, SP Sood, S Jeberson and T Tayeng. Relative efficiency of the maize- and *Imperata cylindrica*-mediated chromosome elimination approaches for induction of haploids of wheat-rye derivatives. *Plant Breed.* 2011;***130***: 192-194.
28. Komeda N, HK Chaudhary, G Suzuki and Y Mukai. Cytological evidence for chromosome elimination in wheat x *Imperata cylindrica* hybrids. *Genes Genet Syst* 2007; ***82***:241-248.
29. Kulp K. Handbook of Cereal Science and Technology, Revised and Expanded. Handb. *Cereal Sci. Technol.* Revis. 2000. Expand.
30. Lantos C and J Pauk. Factors influencing the efficiency of wheat anther culture. Acta Biol. *Cracoviensia Ser. Bot.* 2020; ***62***: 7-16.
31. Laurie DA and MD Bennett. The production of haploid wheat plants from wheat x maize crosses. *Theor. Appl. Genet.* 1988; ***76***: 393-397.
32. Laurie DA and MD Bennett. The effect of the crossability loci Kr1 and Kr2 on fertilization frequency in hexaploid wheat x maize crosses. *Theor. Appl. Genet.* 1987; ***73***: 403-409.
33. Lazaridou TB, AS Lithourgidis, ST Kotzamanidis and DG Roupakias. Anther Culture Response of Barley Genotypes to Cold Pretreatments and Culture Media. *Russ. J. Plant Physiol.* 2005; ***52***: 696-699.
34. Liu W, MY Zheng, EA Polle and CF Konzak. Highly efficient doubled-haploid production in wheat (Triticum aestivum L.) via Induced microspore embryogenesis. *Crop Sci.* 2002; ***42***: 686-692.
35. Lu Y, RL Bowden, G Zhang, X Xu, AK Fritz and G Bai. Quantitative Trait Loci for Slow-Rusting Resistance to Leaf Rust in Doubled-Haploid Wheat Population CI13227 × Lakin. *Phytopathology* 2017; ***107***: 1372-1380.
36. Mago R, GJ Lawrence and JG Ellis. The application of DNA marker and doubled-haploid technology for stacking multiple stem rust resistance genes in wheat. *Mol. Breed.* 2011; ***27***: 329-335.
37. Maheshwari SC, A Rashid and AK Tyagi. Special paper: haploids from pollen grains-retrospect and prospect. *Am.* J. *Bot.* 1982; ***69***: 865-879.
38. Mahmood A and PS Baenziger. Creation of salt tolerant wheat doubled haploid lines from wheat x maize crosses. *Cereal Res. Commun.* 2008; ***36***: 361-371.
39. Moeini A, C De vallavieille-pope and A Sarrafi. Potential use of doubled haploid lines for the screening of resistance to yellow rust (*Puccinia striiformis*) in hexaploid wheat. *Plant Breeding* 1997;***116*** (6): 595-597.
40. Moradi P, A Haghnazari, R Bozorgipour and B Sharma. Development of yellow rust resistant doubled haploid lines of wheat through wheat × maize crosses. *Int. J. Plant Prod.* 2009; **3**: 77-88.
41. Niu Z, A Jiang, W Abu Hammad, A Oladzadabbasabadi, SS Xu, M Mergoum and EM Elias. Review of doubled haploid production in durum and common wheat through wheat × maize hybridization. *Plant Breed.* 2014; ***133***: 313-320.
42. Orshinsky BR and RS Sadasivaiah. Effect of plant growth conditions, plating density and genotype on the anther culture response of soft white spring wheat hybrids. *Plant Cell Rep.* 1997;***16***:758–762.
43. Ouyang T, H Hu, CC Chuang and CC Tseng. Induction of pollen plants from anthers of *Triticum aestivum* L. cultured in vitro. *Sci Sin.* 1973; ***16***:79-95.

44. Patial M, HK Chaudhary, N Sharma, OP Gangwar, N Kishore, D Pal, KK Pramanick, SC Bhardwaj and R Chauhan. Developing genetic stock for yellow and brown rust resistance in *Triticum aestivum* L. *via Imperata cylindrica*-mediated doubled haploidy technique. *Cereal Res. Commun.* 2021;***6***:1-10.

45. Patial M, HK Chaudhary, N Sharma, OP Gangwar, N Kishore, D Pal, KK Pramanick, SC Bhardwaj and R Chauhan. Developing genetic stock for yellow and brown rust resistance in *Triticum aestivum L.* via *Imperata cylindrica*-mediated doubled haploidy technique. *Cereal Res. Commun.* 2022; ***50***: 439-448.

46. Patial M, D Pal, HK Chaudhary, J Kumar and KV Prabhu. 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. *Indian J. Genet. Plant Breed.* 2017; ***77***: 316-320.

47. Patial M, D Pal, HK Chaudhary, J Kumar and A Thakur. Exploring the potential of *Imperata Cylindrica*, a wild grass for development of doubled haploids in wheat. *Int. J. Trop. Agric.* 2016; ***34***: 1211-1213.

48. Patial M, D Pal, J Kumar and H Chaudhary. Doubled haploids production in wheat (*Triticum aestivum L.*) via *Imperata cylindrica* mediated chromosome elimination approach. *Int. J. Trop. Agric.* 2015; ***33***: 3333-3335.

49. Patial M, D Pal, A Thakur, RS Bana and S Patial. Doubled haploidy techniques in wheat (*Triticum aestivum L.*): An Overview. *Proc. Natl. Acad. Sci. India Sect. B - Biol. Sci.* 2019; ***89***: 27-41.

50. Prins R, ZA Pretorius, CM Bender and A Lehmensiek. QTL mapping of stripe, leaf and stem rust resistance genes in a Kariega × Avocet S doubled haploid wheat population. *Mol. Breed.* 2011; ***27***:259-270.

51. Sharma P, HK Chaudhary, NV Manoj, K Singh, A Relan and VK Sood. Haploid induction in *Triticale* × **Wheat and Wheat × Rye Derivatives Following** *Imperata cylindrica*-mediated chromosome elimination approach. *Cereal Res. Commun.* 2019; ***47***: 701-713.

52. Singh AK, P Zhang, C Dong, J Li, R Trethowan and P Sharp. Molecular cytogenetic characterization of stem rust and stripe rust resistance in wheat-*Thinopyrum bessarabicum*–derived doubled haploid lines. *Mol. Breed.* 2019; ***39*** (9).

53. Singh G, MK Singh, MS Saharan, C Singh, BS Tyagi and I Sharma. Genetic analysis and traits association in two double haploid populations of bread wheat (*Triticum aestivum* L.). *Journal of Cereal Research* 2013; ***4***(1).

54. Singh H, AK Singh, HL Kushwaha and A Singh. Energy consumption pattern of wheat production in India. *Energy* 2007; ***32***: 1848-1854.

55. Snape JW, E Simpson and BBParker. Criteria for the selection and use of dihaploid systems in cereal breeding programmes. In: Horn W, Jensen CJ, Odenbach W, Schieder O (eds) Genetic manipulation in plant breeding. Walter de Gruyter, Berlin. 1986. pp 217–229

56. Suenaga K, AR Morshedi and NL Darvey. Evaluation of teosinte lines as pollen parents for wheat haploid production. *Cereal Res. Commun.* 1998; ***26***: 119-125.

57. Weigt D, A Kiel, J Nawracala, A Tomkowiak, D Kurasiak-Popowska, I Siatkowski and B Lugowska. Obtaining doubled haploid lines of the Lr19 gene using anther cultures of winter wheat genotypes. *Biotechnologia* 2016; ***97***: 285-293.

58. Wisniewska H, M Majka, M Kwiatek, M Gawłowska, M Surma, T Adamski, Z Kaczmarek, T Drzazga, B Lugowska and M Korbas et al. Production of wheat-doubled haploids resistant to eyespot supported by marker-assisted selection. Electron. J. *Biotechnol.* 2019; ***37***: 11-17.

59. Yadav P. Nutritional Contents and Medicinal Properties of Wheat: A Review. Life Sci. *Med.* 2011; LMSR-22.

60. Zhang B, D Chi, C Hiebert, T Fetch, B McCallum, A Xue, W Cao, R Depauw and G Fedak. Pyramiding stem rust resistance genes to race TTKSK (Ug99) in wheat. *Can. J. Plant Patho.* 2019; ***41***: 443-449.