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New frontiers in chromosome elimination-mediated doubled haploidy breeding: Focus on speed breeding in bread and durum wheat

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Abstract

Chromosome elimination – a dynamic process occurring during wide hybridization in wheat when crossed with various Gramineae genera, has opened new horizon in accelerating the genetic upgradation endeavours in wheat with enhanced precision and efficiency. Since the invention of *Hordeum bulbosum* approach, some other potential systems leading to the chromosome elimination *viz*., wheat x *Zea mays* and wheat x *Imperata cylindrica* have further enhanced the opportunities to increase the doubled haploid production efficiency in wheat. *Imperata cylindrica* - mediated chromosome elimination approach- an innovation of this Lab has recorded a striking success not only in bread wheat, but also succeeded in inducing haploids appreciably in wheat x rye and triticale x wheat derivatives as well as in the durum wheat. GISH- a novel tool of the molecular cytogenetic approach was used to identify, detect and track the elimination of the *I. cylindrica* chromosomes in wheat x *I. cylindrica* hybrids at different stages of the mitotic division which enunciated quick elimination of the chromosomes in the first division and attributed to higher recovery of the haploid embryos. The out come of the improved technique has been the release of a doubled haploid wheat variety Him Pratham. The innovative protocols developed can enhance the DH production efficiency in wheat and accelerate improvement.

Keywords: Chromosome elimination, doubled haploidy, *Imperata cylindrica*, bread wheat durum wheat

Introduction

Wheat is one of the most important staple crops consumed throughout the world feeding almost 80 per cent of the population with approximately 218.54 million hectares area under cultivation producing 771.17 million tonnes globally (Anonymous 2017). With the ever increasing human population and change in diet, the demand for wheat is bound to increase considerably. Due to the availability of circumscribed arable land, further increase in wheat production must be achieved by increasing productivity to land already in use. Upgradation programme of wheat requires development of high yielding and nutritionally superior varieties to cater the demand of product-specific domestic and international market. Continued success in plant breeding can only be realized if considerable amount of new variability is available for selection. Broadening the genetic variability will make crop production more sustainable under various biotic and abiotic stresses which are posing major threat in current and upcoming scenario. Wild relatives maintain a wide range of allelic diversity for traits related to their fitness such as disease & insect resistance and enhanced tolerance to abiotic stresses. Improvement through conventional breeding approaches is quite lengthy, tedious and requires longer duration to reach homozygosity. This breeding method may take up to 10-12 years to develop a single wheat cultivar. However, through the adoption of doubled haploid breeding, the time frame (Baenziger et al. 2001) to reach homozygosity can markedly be reduced (Fig. 1). This alternative technique to conventional breeding (Garcia-llamas et al. 2004) substantially serves as a method of fixing recombinant gametes directly into fertile homozygous lines, reduces the time requirement for attaining the absolute homozygosity and also increases the selection efficiency in crop breeding by manifolds. Doubled haploids are valuable tool in advanced breeding programmes to accelerate production of homozygous lines in a single step without the need of time consuming selfing while conventional

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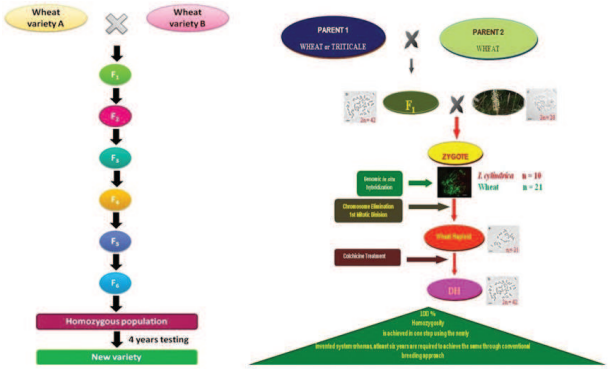

Fig. 1. Diagramatically representing comparison of conventional and DH breeding techniques (Chaudhary et al. 2013b)

breeding takes at least six generations to achieve homozygosity. Haploid plants can be produced by several techniques such as androgenesis, gynogenesis, parthenogenesis, pseudogamy and chromosome elimination through wide hybridization.

Haploid induction techniques

Since the use of haploidy in plant breeding, many methods have been developed across the world for efficient haploid induction, which were limited by the low number of haploids recovered.The techniques for haploid induction can be broadly classified as gametic embryogenesis and wide hybridization. Recent advances in biotechnological tools like tissue culture and related disciplines with the achievement of haploid embryo formation from *in vitro* culture of Datura anthers (Guha and Maheshwari 1964), which was followed by successful *in vitro* haploid production in tobacco (Nitsch 1969). Subsequently, wheat haploids have also been produced by anther culture (Ouyang et al. 1973), isolated microspore culture (Wei 1982) and by using wide hybridization with *Hordeum bulbosum*, *Zea mays* (Barclay 1975; Laurie and Bennett 1986, 1988; Inagaki 1995) and *Imperata cylindrica* (Chaudhary et al. 2005). However, anther culture is genotype dependent (Wehr and Zeller 1990; Chaudhary et al. 2003; Pratap et al. 2006; Khiabani et al. 2008; Grauda et al. 2010; El-Hennawy et al. 2011) with high frequency of albino plants with a mean of 30% of all regenerated plants (Abd El-Maksoud and Bedo 1992; Abd El-Maksoud et al. 1993). Gynogenic induction using un-pollinated flower parts has been successful in several species, such as onion, sugar beet, cucumber, squash, gerbera, sunflower, wheat, barley etc. (Bohanec 2009; Chen et al. 2011) but its application in breeding is mainly restricted to onion and sugar beet. Although gynogenetic regenerants show higher genetic stability and a lower rate of albino plants compared to androgenetic ones, still gynogenesis is least preferred technique (Forster et al. 2007) for haploid induction due to its very low efficiency and is used mainly in plants in which other induction techniques have failed. Another practically possible approach of haploid induction that may overcome the drawbacks of androgenesis and gynogenesis includes chromosome elimination-mediated doubled haploidy breeding using wide hybridization among crop species, which may be either interspecific or intergeneric.

Chromosome elimination-mediated doubled haploidy breeding

Wide hybridization being an important cytogenetic and plant breeding technique has attracted majority of scientific community for sustainable improvement of wheat. Wide hybridization (interspecific and intergeneric) is a potential tool to introduce alien variation and to transfer desirable traits from wild species into cultivated species. However, during such type of hybridization endeavours various reproductive barriers are encountered which are classified as pre-fertilization and post-fertilization barriers. Pre-fertilization barriers include failure of zygote formation due to pollen-stigma incompatibility and failure of pollen tube to reach the ovary, whereas post-fertilization barriers comprise of failure of zygote development and uniparental or preferential chromosome elimination. Uniparental chromosome elimination may result from differential behaviour of two parental genomes. The chromosome elimination has been explained on the basis of difference in timing of essential mitotic processes attributable to asynchronous cell cycling (Gupta et al. 1969), asynchrony in nucleoprotein synthesis leading to a loss of the most retarded chromosomes (Laurie and Bennett 1989) and formation of multipolar spindles (Subrahmanyam and Kasha 1973). Other hypotheses that have been suggested are the genome elimination by nuclear extrusions (Gernand et al. 2006) and spatial separation of genomes during interphase (Linde-Laursen and von Bothmer 1999). In addition, degradation of alien chromosomes by host-specific nucleases (Davies 1974), parent-specific inactivation of centromeres (Mochida et al. 2004), incompatibility of paternal centromere and maternal kinetochore proteins (Komeda et al. 2007), role of centromere specific histone mutants (Maruthachalam and Chan 2010) and uniparental nondisjunction of anaphase chromosomes (Ishii et al. 2010) may lead to uniparental chromosome

elimination during wide hybridization. The actual cellular mechanism involved in the process of uniparental chromosome elimination remains poorly understood. Chromosome elimination of one parental or uniparental genome acts as barrier for gene introgresion from alien species into crop plants but the process helps in production of large numbers of doubledhaploid (DH) plants for breeding and mapping (Devaux and Pickering 2005). Wide hybridization is most practised technique for efficient production of doubled haploids. Various genera were used for generation of haploids with wheat and among them *Hordeum bulbosum*, *Zea mays*, *Tripsacum dactyloides*, *Pennisetum glaucum*, *Coixlacryma-jobi* and *Imperata cylindrica* have been found to respond better than others.

Hordeum bulbosum approach

In cereals, the first report on wide hybridization following chromosome elimination was *H. vulgare* × *H. bulbosum*, commonly known as 'bulbosum method' (Stephan 1969; Kasha and Kao 1970; Lange 1971). In this cross, haploid embryos were reported to be formed as a result of elimination of the chromosomes of the wild relative, *H. bulbosum* (2n=2x=14) during early embryogenesis from the cells of developing embryos. Kasha and Kao (1970) presented evidence to show that these haploids are produced by the elimination of *H. bulbosum* chromosomes, not by parthenogenesis. After 12-14 days of pollination, embryos were excised from the developing caryopsis and cultured *in vitro* due to the poor development of endosperm. This bulbosum approach was the first method used in breeding programmes to produce large number of haploids across most genotypes.

Triticum aestivum × *H. bulbosum system*

Barclay (1975) was the pioneer to use the bulbosum technique in wheat for the haploid induction. When 'Chinese Spring' variety of *T. aestivum* (2n = 6x = 42) was crossed with *H. bulbosum* (2n = 2x = 14), haploid wheat plantlets were obtained as a result of elimination of *H. bulbosum* chromosomes from the interspecifc hybrid during its early embryogenesis (Barclay 1975; Zenketler and Straub 1979). However, this method was not successful with other wheat varieties for haploid induction due to the effect of dominant crossability inhibitor alleles *Kr1*, *Kr2*, *Kr3* and *Kr4* located on 5B, 5A, 5D and 1A chromosome arms (Riley and Chapman 1967; Krolow 1970; Sitch et al. 1985; Zheng et al. 1992) which prevent the entry of *H. Bulbosum* pollen tube into the ovary of wheat. The 'Chinese Spring' variety of bread wheat was successfully hybridized to *H. bulbosum* and haploid embryos were produced due to the presence of recessive crossability alleles *viz*., *kr1 and kr2*. Factors found on chromosomes 3A, 3B and 3D were also responsible for crossability between 'Chinese Spring' wheat and *H. bulbosum* (Miller et al. 1983). The sensitivity of the *H. bulbosum* pollen to the crossability inhibitor genes limited its application and was utilized to limited extent in breeding programmes.

Wheat × maize system

The first report on embryos induction from the crosses between hexaploid wheat and maize was presented by Zenkteler and Nitzsche (1984). Later, their results were cytologically confirmed by Laurie and Bennett (1986). According to the studies, a karyotypically unstable hybrid zygote with 21 wheat chromosomes and 10 maize chromosomes was produced as a result of this cross (Laurie and Bennett 1988). Possibly during cell divisions, due to the progressive loss of centromere activity of maize chromosomes and lack of their attachment to the spindle microtubules, the maize chromosomes failed to move towards the spindle poles. As a result haploid wheat embryos were formed from this intergeneric hybridization due to elimination of maize chromosomes from the hybrid zygote during first few cycles of cell division (Laurie and Bennett 1989). The maize was reported to be insensitive to the action of dominant genes *Kr1* and *Kr2*, located on the long arms of chromosome 5B and 5A, respectively (Sitch et al. 1985). Due to the insensitivity of maize pollen to the crossability inhibitor genes, this method could be applied to a wide range of wheat as well as maize genotypes including those recalcitrant to androgenesis (Cherkaoui et al. 2000; Chaudhary et al. 2002; Singh et al. 2004; Pratapet al. 2006).

The maize-mediated system of haploid induction was found significantly better than androgenesis with respect to embryo formation and haploid plantlet regeneration both in wheat and triticale (Pratap et al. 2006). This system is fast, economically viable, easy in application and more efficient than others due to low level of genotype specificity (Cherkaoui et al. 2000).The advantage of wheat x maize hybridization technique over anther culture and the bulbosum technique in respect of reduced genotypic specificity, absence of albinism and ease of application make it more efficient for the production of haploids in common wheat (Wang et al. 1991). The same system was quite efficiently utilized in the development of the first doubled- haploid wheat variety of India - Him Pratham

(Chaudhary et al. 2013b) by Dr.Harinder Kumar Chaudhary, CSK HP Agricultural University, Palampur, Himachal Pradesh, India (Fig. 2).

Fig. 2. DH 114 (Him Pratham) - first doubled haploid wheat variety of India generated through chromosome elimination-mediated system of DH breeding (Chaudhary et al. 2013b)

The advantages of wheat x maize system over anther culture and bulbosum technique make this haploid induction method more acceptable by the breeders worldwide in various wheat improvement programmes. However, for the synchronization of the flowering times of maize and wheat, the experiments can only be practiced under greenhouse conditions.This is one of the major limitations of this haploid induction technique and keeping this in view, various efforts have been made by breeders throughout the world to look for substitute pollen source whose flowering must synchronise with wheat under natural conditions. Some of the alternative pollen sources for haploid induction in wheat include pearl millet (Ahmad and Comeau 1990; Inagaki and Mujeeb-Kazi 1995; Ohkawa et al. 1992), *Tripsacum dactyloides* (Riera- Lizarazu and Mujeeb-Kazi1992) and Job's tears (Mochida and Tsujimoto 2001). At last, an alternative potential pollen source, *Imperata cylindrica*, a perennial weedy grass has been reported by Chaudhary et al. 2005 as the efficient and significantly superior over maize-mediated system for doubled haploid production in wheat.

Wheat × Imperata cylindrica system

Imperata cylindrica (2n= 20) also known as cogon grass or kunai grass, is a species of grass placed in family Poaceae, sub-family Panicoideae and tribe Andropogoneae. It is a perennial rhizomatous grass native to east and south-east Asia, India, Australia and eastern and southern Africa. It coincides well for flowering with that of wheat under natural conditions and is available in almost all parts of the world wherever wheat is cultivated. This has emerged as an efficient alternative pollen source for doubled haploid production in wheat and found to be significantly superior over maize- mediated haploid induction (Chaudhary et al. 2005). Intergeneric crosses between *I. cylindrica* and wheat followed by elimination of *I. cylindrica* genome has emerged as the system of choice for inducing haploids in bread wheat (Chaudhary et al. 2005; 2013a and 2013b; Chaudhary 2008a, 2008b, 2009, 2010a, 2010b, 2012, 2013a and 2013b, Tayeng et al. 2012) as well as in durum wheat (Mahato and Chaudhary 2015). And, it is more effective, efficient, fast, genotype independent, having synchronization in flowering and above all the simple technique for haploid induction when compared with wheat x maize-mediated haploid development technique (Chaudhary 2010a).

Haploid induction following wheat x *I. cylindrica* - mediated chromosome elimination technique and innovative protocols

Of the Poaceae genera *viz*., *Zea mays, Sorghum bicolour, Pennisetum americanum, Setaria italica, Festuca arundinaceae, I. cylindrica, Cynodon dactylon, Lolium temulentum* and *Phalaris minor* tested for haploid plant production, *I. cylindrica* produced more embryos and haploids over others (Chaudhary et al. 2005; Chaudhary 2008a and b). This potential pollen source is genotype non-specific and is able to induce haploids in any genotype of wheat, triticale or their derivatives. Keeping in view the genetic influence of paternal and maternal parents in haploid induction and genetic diversity of *I. cylindrica* over the geographical locations (Rather et al. 2017), the haploid induction efficiency of wheat × *I. cylindrica* can be enhanced by using the more responsive wheat and *I. cylindrica* genotypes for hybridization (Rather et al. 2014). Pratap et al. (2005) further explored maize and *I. cylindrica* for effective haploid induction in triticale x wheat crosses.Chaudhary (2008a and b) reported that *I. cylindrica* performed significantly better than maize for all the haploid induction parameters in wheat and triticale and their derivatives.All the crosses resulted in embryo formation and green haploid plantlet regeneration but *I. cylindrica* outperformed maize for all the haploid induction parameters. Kishore et al. (2011) used maize and *I. cylindrica* for haploid induction in spring and winter wheat × Himalayan rye derivatives and observed that the *I. cylindrica* produced

appreciable number of haploid embryos whereas maize produced none. It was concluded from the study that this approach could be applied to triticale x wheat derivatives efficiently (Fig. 3). Badiyal et al. (2014)

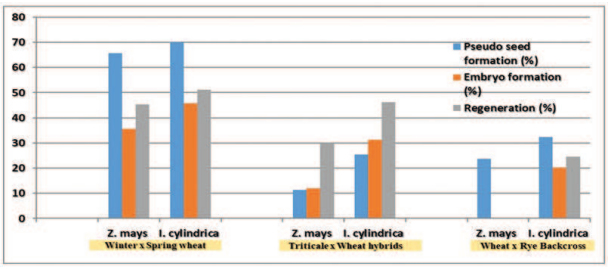

Fig. 3. Relative efficiency of various haploid induction parameters utilizing maize- and*I. cylindrica*-mediated systems (Chaudhary 2008a and b; Kishore et al. 2011)

observed high haploid embryo formation frequency in triticale x wheat derivatives ranging from 3.5% to 33.15% whereas regeneration frequency was found to be between 6.51% and 71.25% following *I. cylindrica* mediated haploid induction.

The relative efficiency for induction of haploids in durum wheat through maize- and *I. cylindrica*-mediated chromosome elimination was studied by Mahato and Chaudhary, 2015 using two genotypes of maize and *I. cylindrica* as pollen sources and seven durum wheat genotypes utilized as maternal parent. The response of *I. cylindrica* was highest in all the haploid induction parameters, and hence it appeared to be the most efficient source for induction of haploids in *T. durum* (Fig. 4).

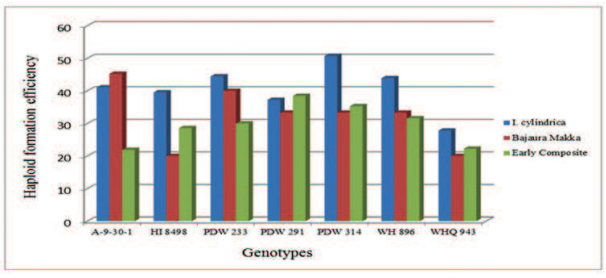

Fig. 4. Comparative performance of maize and *I. cylindrica* pollen sources on haploid formation efficiency in durum wheat (Mahato and Chaudhary 2015)

Pollen preservation potential of *Imperata cylindrica*

To extend the period for hybridization amongst wheat and *I. cylindrica*, pollen preservation of *I. cylindrica* can be a boon to the breeders. Rather et al. (2017) carried out the pollen preservation of *I. cylindrica* under varied preservation regimes (–80°C, –20°C and 4°C) and concluded that the pollen preserved at -20°C can be quite efficiently used to undertake the wheat × *I. cylindrica* hybridization at places where the pollen source is not available. Moreover, the *I. cylindrica* pollen preserved at –20°C can be used to extend the doubled haploidy breeding programmes by at least one month.

The efficiency of haploid induction system critically depends on the functional viability and relative longevity of the pollen of the parent. Maize pollen has been observed to lose its viability completely within 50 minutes (Luna et al. 2001; Aylor 2004; Muui et al. 2007). Considering this aspect, a field study was conducted for comparative assessment of the viability of freshly harvested pollen of maize and *I. cylindrica*. *I. cylindrica* pollen, being viable for significantly longer period than maize can hasten the haploid induction endeavours (Mayel et al. 2015).

In vivo manipulation of colchicine for enhancing DH frequency

Tayeng et al. (2012) gave an alternative of *in vitro* colchicine application for efficient doubling of chromosomes and presented the first report concerning *in vivo* manipulation of colchicine for enhancement of doubled haploid formation frequency. For determining the most efficient dose of colchicine at *in vivo*, different doses ranging from 100 to 10000 ppm with and without 2, 4-D application were injected to the uppermost internodes of *Imperata cylindrica*-pollinated wheat plants at various intervals. A colchicine dose of 2000 ppm was concluded to be the most efficient and economically viable for DH production at *in vivo* level, if injected after 48 hrs of pollination (Fig. 5). Doubling

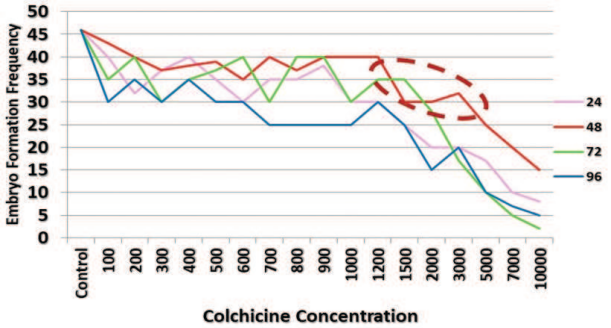

Fig. 5. Effect of *in vivo* application of different concentration of colchicine on embryo formation frequency and doubling of chromosomes in wheat after varied time intervalof pollination with *I. cylindrica* (Tayeng et al. 2012)

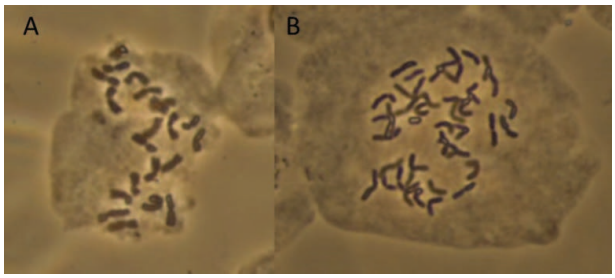

Fig. 6. Cytology of regenerated plants with and without colchicine A) Without colchicine B) *in vivo* application of colchicine (2000 ppm at 48 hours after pollination with *I. cylindrica*). (Tayeng et al. 2012)

of the chromosomes was further confirmed through cytology from the roots of the regenerated plantlets after the colchicine application. Hence, *in vivo* application of colchicine can improve the DH formation frequency and save time and energy required for the efficient recovery of doubled haploids.

Asynchrony for easy and economical haploid induction in wheat

Anthesis in case of wheat spikes takes place bi-directionally and it initiates from the upper middle portion of the spike. Chaudhary et al. (2013a and b) revealed that the asynchronous behaviour of anthesis within wheat spikes can be efficiently exploited for haploid induction by pollinating the non-emasculated spikes with *I. cylindrica* pollen. Morphological marker can be utilized for differentiating selfed and hybrid seeds. Hybrid seeds do not contain endosperm whereas selfed seeds contain endosperm which could be observed using incandescent bulbs (Fig. 7).

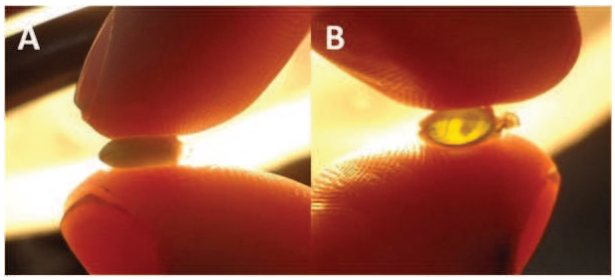

Fig. 7. A) Selfed seeds B) Hybrid seed containing embryo without endosperm (Chaudhary et al. 2013b)

Molecular cytogenetic techniques assisted (MCTA) assessment of chromosome elimination of Imperata cylindrica *under wheat background*

For a successful gene transfer during introgression breeding, monitoring the alien chromatin is critical. Cytogenetic tools have facilitated this approach by identifying new introgressed genes from alien species in wheat. The application of newer biochemical and molecular biological tools to cytogenetics has led to the development of chromosome banding (Gill and Kimber 1974) and florescence *insitu* hybridization (FISH) techniques (Rayburn and Gill 1985) in wheat, which have revolutionized the crop improvement programmes. Further, FISH complements such efforts by supplementing information on size of genomic insert and its possible integration sites that are valuable not only in understanding the amenability of genomic insertion but also in its acceptability and expression. The advantage of genomic *in situ* hybridization (GISH) is recognition of all alien chromosome segments contained in nucleus and is therefore the method of choice when interspecific crosses and derived introgressed lines are analyzed to reveal alien chromosomes and translocations.Application of GISH facilitated in ascertaining that the haploid plants developed as a result of wheat × *I. cylindrica* crosses have not originated parthenogenetically but has hybrid origin. GISH has also assisted in understanding the timeline and events during elimination of *I. cylindrica* chromosomes. Cytological investigation of the wheat x *I. cylindrica* chromosome elimination system has shown that there is no endosperm formation and elimination of chromosomes of *I. cylindrica* takes place in first zygotic division in seed development thus allowing production of embryo-carrying seeds (Komeda et al. 2007) (Fig. 8). The combination of *I. cylindrica*-mediated doubled haploid production and molecular cytogenetic techniques like GISH and FISH can accelerate the alien introgression mediated wheat breeding programmes in various farming systems (Chaudhary 2007).

Maize versus *Imperata cylindrica*-mediated chromosome elimination approach

Being a winter season perennial grass and growing wild in nature, the flowering of *I. cylindrica* coincides well with wheat flowering *I. cylindrica*- mediated chromosome elimination approach more efficient and economical than maize which is *rabi* crop and need green house facilities. Additionally, in case of *I. cylindrica* mediated approach, there is no need of raising the pollen parent as it occurs every year due to its perennial nature. Both maize and *I. cylindrica* pollen is insensitive to crossability inhibitor genes (Chaudhary et al. 2005; Laurie and Bennett 1989). *I. cylindrica* system is superior to the wheat x maize

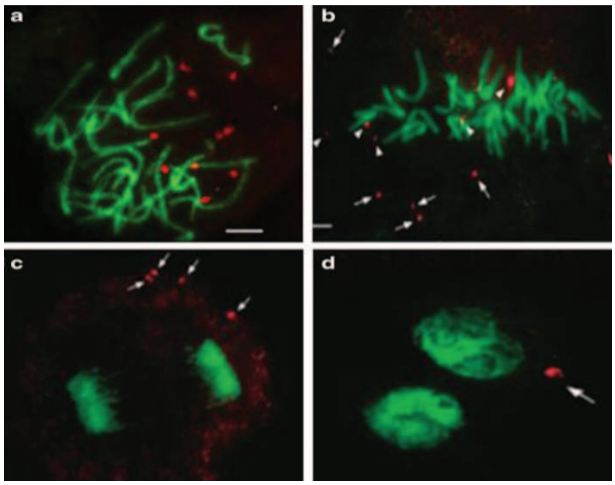

Fig. 8. Cytological evidence of elimination of *I. cylindrica* chromosomes in wheat × *I. cylindrica* hybrids during mitosis by utilizing Genomic *in situ* hybridization approach (Green: Wheat chromosomes, Red: *I. cylindrica* chromosomes). a. Interspecific hybrid, b. Abnormal movement of *Imperata* chromosomes, c. Anaphase cell exhibiting *I. cylindrica* chromosomes elimination, d. Extruding *Imperata* micronucleus in Interphase cells after 3-4 days of pollination (Komeda et al. 2007)

system in various aspects *viz.,* higher haploid embryo formation and regeneration frequency in diverse intervarietal and intergeneric crosses and their derivatives (Chaudhary et al. 2005; Kishore et al. 2011), in induction of haploid plants in the wheat-rye backcross where maize was found to be unsuccessful (Kishore et al. 2011). Another drawback of maize-mediated system is that in most of wheat × maize derived embryos, elimination of maize chromosomes occurs during the first three cell-division cycles (Laurie and Bennett 1989) that may lead to formation of disrupt endosperm. Whereas, in case of *I. cylindrica*, there is no endosperm formation and the elimination of chromosomes of *I. cylindrica* takes place in the first zygotic division as shown in Fig. 8. (Komeda et al. 2007).

Conclusion

Gene introgression utilizing wide hybridization has been extensively used in various crop improvement endeavours across the world for generating diversity. Since conventional plant breeding takes more time to obtain stable and homozygous population, modern approaches like doubled haploidy (DH) breeding is adopted toreduce the time span, labour and cost of such endeavours. Doubled haploid technology in wheat has progressed many folds since its discovery and recently improvised with the aid of biotechnological tools. The major advantages of the technology has been the fixing of heterotic effect instantly amongst the hybrids, small population size for selection, elimination of deleterious mutations and weak plant types, useful in development of transgenics, additional variation in the form of gametoclonal variation and elimination of dominant alleles controlling undesirable traits.Wide hybridization followed by uniparental chromosome elimination like *bulbosum* approach and wheat × maize has revolutionized the genetic improvement programmes in wheat. Wheat × *I. cylindrica*, the newly invented system of chromosome elimination, has shown promising results in producing haploids in hexaploid wheat, tetraploid wheat, triticale, triticale × wheat and wheat x rye derivatives. *I. cylindrica*, the perennial grass has the benefit of synchronous flowering with that of wheat and provides the pollen throughout the season. These attributes make this approach very reasonable and widely applicable for effective haploid induction in wheat. Keeping in consideration the importance of chromosome elimination mediated approaches of DH breeding, plant breeders can look into opportunities for focusing on the development of such genotype non-specific and efficient haploid induction systems in other crops.

Declaration

The authors declare no conflict of interest.

References

Abd El-Maksoud M. M. and Bedo Z. 1992. Half-diallel analysis of different characters in wheat anther culture. Acta Agr. Hung., **41**: 235-42.

Abd El-Maksoud M. M., Karsai I. and Bedo Z. 1993. Agronomic traits of wheat lines developed by the doubled haploid, single seed descent and pedigree methods after three cycles of selection. Acta Agr. Hung., **42**: 377-82.

Ahmad F. and Comeau A. 1990. Wheat × pearl millet hybridization: Consequence and potential. Euphytica, **50**: 181-190.

Anonymous. 2017. Food and Agriculture Organization of the United Nations http://www. FAO stat.fao.org.com

Aylor D. E. 2004. Survival of maize (*Zea mays*) pollen exposed in the atmosphere. Agric. For. Meteorol., **123**: 125-33.

Badiyal A., Chaudhary H. K., Jamwal N. S., Hussain W., Mahato A. and Bhatt A. K. 2014. Interactive genotypic

influence of triticale and wheat on their crossability and haploid induction under varied agroclimatic regimes. Cereal Res. Commun., **42**: 700-09.

Baenziger P. S., Moonkim K. and Halilogue K. 2001.Wheat in vitro breeding. *In*: The world wheat book: A history of wheat breeding (Eds. AP Bonjean and WJ Angus), Lavoisier Publishers, Paris: 979-1000.

Barclay I. R. 1975. High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature, **256**: 410-411.

Bohanec B. 2009. Doubled haploids via gynogenesis. *In*: Advances in haploid production in higher plants (Eds. A Touraev, B Forster and M Jain). Springer, Heidelberg: 35-46.

Chaudhary H. K. 2007. Dynamics of doubled haploidy breeding and molecular cytogenetic approaches *vis-à-vis* genetic upgradation of bread wheat for organic and low input farming systems in north-western Himalayas. Proc. Eucarpia Symp. on Organic and Sustainable, Low-input Agriculture with Genotype x Environment Interactions. 7-9 October, 2007, Wageningen, Netherlands: 54.

Chaudhary H. K. 2008a. Dynamics of wheat × *Imperata cylindrica* - a new chromosome elimination mediated system for efficient haploid induction in wheat. Proc. 11th Intern. Wheat Genetics Symp., 24-29 August, 2008, University of Sydney Press, Brisbane, Australia, **2**: 647-650.

Chaudhary H. K. 2008b. Dynamics of doubled haploidy breeding and molecular cytogenetic approaches in bread wheat - Focus on north-west Himalayan regions. Proc. Advances in Chromosome Science (Eds. K Taniguchi and X Zhang), 1-4 December, 2008. The Society of Chromosome Research, Japan, **3**: 67-69.

Chaudhary H. K. 2009. New frontiers in chromosome engineering: Genetic upgradation of bread wheat for varied agro-climatic situations in north-west Himalayas. Proc. National Seminar on Designing Crops for the Changing Climate (Eds. SMS Tomar et al. 2009), 30-31 October, 2009, Ranchi, Jharkhand: 51-52.

Chaudhary H. K. 2010a. Chromosome elimination process- a boon or bane for alien introgression in wheat. Proc. 4th Asian Chromosome Colloquium (Eds. X Zhang et al.), 10-14 October, 2010, Beijing, China: 110-111.

Chaudhary H. K. 2010b. New frontiers in DH Breeding: Dynamics of wheat x *Imperata cylindrica* system of chromosome elimination- mediated approach of DH production for striking success in alien introgression endeavours in bread wheat. Proc. Eucarpia Cereal Section Meeting: Innovations in Cereal Breeding, 6-8 April, 2010, Cambridge, England: 73.

Chaudhary H. K. 2012. New frontiers in chromosome engineering for enhanced and high precision crop improvement. Proc. National Seminar on Plant Cytogenetics, 3-7 January, 2012, Patiala, Punjab: 35-36.

Chaudhary H. K. 2013a. New frontiers in chromosome elimination mediated doubled haploidy breeding for accelerated and high precision genetic upgradation in wheat. Proc. Intern. Triticeae Mapping Initiative and Plant & Animal Genome XXI Conference, 12-16 January, 2013, San Diego, USA: 211.

Chaudhary H. K. 2013b. Dynamics of chromosome engineering for accelerated and high precision crop improvement. Proc. Indian Science Congress, 3-7 January, 2013, Kolkata: 24.

Chaudhary H. K., Dhaliwal I., Singh S. and Sethi G. S. 2003.Genetics of androgenesis in winter and spring wheat genotypes. Euphytica, **132**: 311-319.

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

Chaudhary H. K., Singh S. And Sethi G. S. 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., Tayeng T., Kaila V. and Rather S. A. 2013a. Use of asynchrony in flowering for easy and economical polyhaploid induction in wheat following *Imperata cylindrica*-mediated chromosome elimination approach. Plant Breed., **132**: 155-58.

Chaudhary H. K., Tayeng T., Kaila V. and Rather S. A. 2013b. Enhancing the efficiency of wide hybridization mediated chromosome engineering for high precision crop improvement with special reference to wheat × *Imperata cylindrica* system. Nucleus, **56**: 7-14.

Chen J. F., Cui L., Malik A. A. and Mbira K. G. 2011. *In vitro* haploid and dihaploid production via unfertilized ovule culture. Plant Cell Tiss. Org. Cult., **104**: 311-19.

Cherkaoui S., Lamsaouri O., Chlyah A. and Chlyah H. 2000. Durum wheat × maize crosses for haploid wheat production: influence of parental genotypes and various experimental factors. Plant Breed., **119**: 31-36.

Davies D. R. 1974.Chromosome elimination in inter-specific hybrids. Hered., **32**: 267-70.

Devaux P. and Pickering R. 2005. Haploids in the improvement of Poaceae. *In*: Biotechnology in Agriculture and Forestry: Haploids in Crop Improvement II. Berlin, Heidelberg, Springer: 215-242.

El-Hennawy M. A., Abdalla A. F., Shafey S. A. and Al-Ashkar I. M. 2011. Production of doubled haploid

wheat lines (*Triticum aestivum* L.) using anther culture technique. Ann. Agric. Sci., **56**: 63-72.

Forster B. P., Herberle-Bors E., Kasha K. J. and Touraev A. 2007.The resurgence of haploids in higher plants. Trends Plant. Sci., **12**: 368-375.

Garcia-llamas C., Ramirez M. C. and Ballesteros J. 2004. Effect of pollinator on haploid production in durum wheat crossed with maize and pearl millet. Plant Breed., **123**: 201-203.

Gernand D., Rutten T., Pickering R. And Houben A. 2006.Elimination of chromosomes in *Hordeum vulgare* × *H. bulbosum* crosses at mitosis and interphase involves micronucleus formation and progressive heterochromatinization. Cytogenet. Genome Res., **114**: 169-74.

Gill B. S. and Kimber G. 1974. Giemsa C-banding and the evolution of wheat. Proc. National Academy of Sciences, **71**: 4086-4090.

Grauda D., Lepse N., Strazdina V., Kokina I., Lapina L., Mikelsone1 A., Lubinskis L. andRashal I. 2010. Obtaining of doubled haploid lines by anther culture method for the Latvian wheat breeding. Agron. Res., **8**: 545-552.

Guha S. and Maheshwari S. C. 1964. *In vitro* production of embryos from anthers of Datura. Nature, **204**: 497-98.

Gupta S. B. 1969. Duration ofmitotic cycle and regulation of DNA replication in *Nicotiana plumbaginifolia* and a hybrid derivative of *N. tabacum* showing chromosome instability. Can. J. Genet.Cytol., **11**: 133-42.

Inagaki M. N. and Mujeeb-Kazi A. 1995.Comparison of polyhaploid production frequencies in crosses of hexaploid wheat with maize, pearl millet and sorghum. Breed Sci., **45**: 157-161.

Ishii T., Ueda T., Tanaka H. and Tsujimoto H. 2010.Chromosome elimination by wide hybridization between triticeae or oat plant and pearl millet: pearl millet chromosome dynamics in hybrid embryo cells. Chromosome Res., **18**: 821-31.

Kasha K. J. and Kao K. N. 1970.High frequency haploid production in barley (*Hordeum vulgare* L.). Nature, **225:** 874-876.

Khiabani B. N., Vedadi C., Rahmani E. and Shalmani M. 2008. Response of some Iranian wheat genotypes to anther culture system. Indian J. Biotechnol., **7**: 531-535.

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

Komeda N., Chaudhary H. K. and Mukai Y. 2007. Cytological evidence for chromosome elimination inwheat × *Imperata cylindrica* hybrids. Genes Genet. Syst., **82**: 241-248.

Krolow K. D. 1970. Investigations on compatibility between wheat and rye. Z. Pflanzenzuchtung, **64**: 44-72.

Lange W. 1971. Crosses between *Hordeum vulgare* L. and *H. Bulbosum* L. I. Production, morphology and meiosis of hybrids, haploids and dihaploids. Euphytica, **20**: 14-29.

Laurie D. A. and Bennett M. D. 1986. Wheat × maize hybridization. Can. J. Genet. Cytol., **28**: 313-316.

Laurie D. A. and Bennett M. D. 1988. The production of haploid wheat plants from wheat × maize crosses. Theor. Appl. Genet., **76**: 393-397.

Laurie D. A. and Bennett M. D. 1989. The timing of chromosome elimination in hexaploid wheat × maize crosses. Genome, **32**: 953-61.

Linde-Laursen I. and von Bothmer R. 1999. Orderly arrangement of the chromosomes within barley genomes of chromosome-eliminating *Hordeum lechleri* × barley hybrids. Genome, **42**: 225-36.

Luna S., Figueroa V. J., Baltazar M. B., Gomez M. R., Townsend L. R. and Schoper J. B. 2001. Maize pollen longevity and distance isolation requirements for effective pollen control. Crop Sci., **41**: 1551-57.

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

Maruthachalam R. and Chan S. W. L. 2010. Haploid plants produced by centromere-mediated genome elimination. Nature, **464**: 615-619.

Mayel A., Chaudhary H. K., Badiyal A. and Jamwal N. S. 2015. Comparative pollination efficiency of freshly harvested pollen of *Imperata cylindrica* and *Zea mays* for haploid induction in bread wheat. Cereal Res. Commun., **44**: 1-10.

Miller T. E., Reader S. M. and Gale M. D. 1983. The effect of homoeologous group 3 chromosomes on chromosome pairing and crossability in *Triticum aestivum*. Can. J. Genet. Cytol., **25**: 634-641.

Mochida K. and Tsujimoto H. 2001. Production of wheat doubled haploids by pollination with Job's Tears (*Coixlachry-majobi* L.). J. Hered., **92**: 81-83.

Mochida K., Tsujimoto H. and Sasakuma T. 2004. Confocal analysis of chromosome behaviour in wheat × maize zygotes. Genome, **47**: 199-205.

Muui C. W., Muasya R. M., Rao N. and Anjichi V. E. 2007. Pollen longevity in ecologically different zones of Western Kenya. Afr. Crop Sci. J., **15**: 43-49.

Nitsch J. P. and Nitsch C. 1969. Haploid plants from pollen grains. Science, **163**: 85-87.

Ohkawa Y., Suenaga K. and Ogawa T. 1992. Production of haploid wheat plants through pollination of sorghum pollen. Jpn. J. Breed., **42**: 891-894.

Ouyang Y. W., Hu C. C., Chuang C. C. and Tseng C. C. 1973. Induction of pollen plants from anthers of *Triticum aestivum* L. cultured *in vitro*. Sci. Sin., **16**: 79-95.

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

Pratap A., Sethi G. S. and Chaudhary H. K. 2006. Relative efficiency of anther culture and chromosome elimination technique for haploid induction in triticale × wheat and triticale × triticale hybrids. Euphytica, **150**: 339-345.

Rather S. A., Chaudhary H. K. and Kaila V. 2014. Proportional contribution and potential of maternal and paternal genotypes for polyhaploid induction in wheat × *Imperata cylindrica* chromosome elimination approach. Cereal Res. Commun., **42**: 19-26.

Rather S. A., Chaudhary H. K. and Kaila V. 2017. Pollen preservation potential of *Imperata cylindrica*- an efficient source for doubled haploid production in wheat. Cereal Res. Commun., **45**: 525-534.

Rayburn A. L. and Gill B. S. 1985. Use of biotin-labeled probes to map specific DNA sequences on wheat chromosomes. J. Hered., **76**: 78-81.

Riera-Lizarazu O. and Mujeeb-Kazi A. 1992. Polyhaploid production in the Triticeae: wheat × tripsacum crosses. Crop Sci., **33**: 973-976.

Riley R. and Chapman V. 1967. The inheritance in wheat of crossability with rye. Genet. Res., **9**: 259-267.

Singh S., Sethi G. S. and Chaudhary H. K. 2004. Differential responsiveness of winter and spring wheat genotypes to maize-mediated production of haploids. Cereal Res. Commun., **32**: 201-207.

Sitch L. A., Snape J. W. and Firman S. J. 1985. Intra chromosomal mapping of crossability genes in wheat (*Triticum aestivum*). Theor. Appl. Genet., **70**: 309-314.

Stephan S. 1969. Haploid barley from crosses of *Hordeum bulbosum* (2*x*) × *Hordeum vulgare* (2*x*). Can. J. Genet.Cytol., **11**: 602-608.

Subrahmanyam N. C. and Kasha K. J. 1973. Selective chromosomal elimination during haploid formation in barley following interspecific hybridization. Chromosoma, **42**: 111-25.

Tayeng T., Chaudhary H. K. and Kishore N. 2012. Enhancing doubled haploidproduction efficiency in wheat (*Triticum aestivum* L. em. Thell) by *in vivo* colchicine manipulation in *Imperata cylindrica* mediated chromosome elimination approach. Plant Breed., **131**: 574-578.

Wang J. L., Sun J. S., Lu T. G., Fang R., Cui H. R., Cheng S. Z. and Yang C. 1991. Fertilization and embryo development in wheat × maize crosses. Acta Bot. Sin., **33**: 674-679.

Wehr B. F. and Zeller F. J. 1990. *In vitro* microspore reaction of different German wheat cultivars. Theor. Appl. Genet., **79**: 77-80.

Wei Z. M. 1982. Pollen callus culture in *Triticum aestivum*. Theor. Appl. Genet., **67**: 71-73.

Zenketler M. and Straub J. 1979.Cytoembryological study on the process of fertilization and the development of haploid embryo of *Triticum aestivum* (2n = 42) after crossing with *Hordeum bulbosum* (2n = 14). Z. Pflanzenzuchtung, **82**: 36-44.

Zenkteler M. and Nitzsche W. 1984. Wide hybridization experiments in cereals. Theor. Appl. Genet., **68**: 311-316.

Zheng Y. L., Luo M. C., Yen C. and Yang J. L. 1992. Chromosome location of a new crossability gene in common wheat. Wheat Inf. Serv., **75**: 36-40.