Chapter 6
Distant Hybridisation and Doubled-Haploidy Breeding

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Abstract The combination of genomes from diverse genetic backgrounds through wide hybridisation has become very important during the present days of global climate change. However, in some cases it is not possible to recover hybrids with genomes from both the parental species. The elimination of whole chromosome complement of one of the parents from the wide hybrids, that is, uniparental chromosome elimination, has acted as a boon to the crop breeders for rapid genetic upgradation of the crop varieties. This chapter depicts various chromosome elimination approaches of doubled-haploidy breeding in barley, wheat, oats, triticale and potato. The chapter also presents the possible mechanisms of chromosome elimination including its advantages to the other DH breeding systems in crop plants. It also covers various investigations undertaken throughout the world and the efficiency of various chromosome elimination systems in induction of haploids.

Keywords Wheat • Potato • Wheat X maize • Wide hybridisation • Chromosome elimination • Haploid

6.1 Introduction

Crop improvement involves genetic manipulation of plants in a predetermined way, which often utilises the transfer of genes from one source or genetic background to another. When a plant breeder has determined the direction in which a crop is to be improved, the next crucial step is to find a source of the appropriate gene(s) for making the desired change(s). Once an appropriate source (germplasm) has been

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found, the next step is to transfer the gene(s) to the parent to be improved. In flowering species, the conventional method of gene transfer is by crossing or sexual hybridisation. This procedure causes genes from the two parents to be assembled into a new genetic matrix. It follows that if parents are not genetically compatible, gene transfer by sexual means cannot occur at all. Artificial sexual hybridisation is the most common conventional method of generating a segregating population for selection in breeding of flowering species. In some breeding programmes, the hybrid (F_1) is the final product. However, in most situations, the F_1 is selfed to generate recombinants (F_2) as a result of recombination of the parental genomes or a segregating population, in which selection is practiced. The tools of modern biotechnology now enable and assist the breeders to transfer genes by circumventing the sexual process, that is, without crossing. More significantly, gene transfer can transcend natural reproductive or genetic barriers. Transfers can occur between unrelated plants and even between different species.

The first choice of parents for use in a breeding programme are cultivars and experimental materials with the traits of interest. Most of the time, plant breeders make elite×elite crosses as they are highly adapted and improved materials. Even though genetic gains from such crosses may not always be dramatic, they are nonetheless significant enough to warrant the practice. After exhausting the variability in the elite germplasm as well as in the cultivated species, the breeder may look elsewhere, based on the gene pool concept of Harlan and de Wet (1971). Hybridisation is a strong evolutionary force which can potentially reshape the genetic composition of populations and create novel genotypes that facilitate adaptation to new environments (Stebbins 1950). Crosses involving materials outside the cultivated species are collectively described as wide crosses. When the wide cross involves another species, it is called an interspecific cross. When it involves a plant from another genus, it is called an intergeneric cross. Intra- and interspecific hybridisation are common means of extending the range of variation beyond that displayed by the parental species.

The primary purpose of wide crosses is to improve a species for economic production by transferring one or a few genes, or segment of chromosomes or whole chromosomes, across interspecific or intergeneric boundaries. The genes may condition a specific disease or pest resistance or may be a product quality trait. Combining genomes from diverse backgrounds may trigger a complementary gene action or even introduce a few genes that could produce previously unobserved phenotypes that may be superior to the parental expression of both qualitative and quantitative traits. Wide crosses often produce sterile hybrids. The genome of such hybrids can be doubled to create a new fertile alloploid species, such as triticale. Cytogenetic studies following a wide cross may be used to understand the phylogenic relationships between the parents of a cross.

Interspecific hybridisation provides information on phylogenetic relationships between any two species giving clues with regard to evolutionary patterns. Often generation of such information is based on cross compatibility, chromosome association and pollen fertility. Such information also helps in developing breeding strategies for introgression of genes from related species into economically useful species. As it creates genetic variation, it has great potential for plant improvement (Goodman et al. 1987; Choudhary et al. 2000; Sain et al. 2002). For certain crops, plant breeders in the

twentieth century have increasingly used interspecific hybridisation for gene transfer from a non-cultivated plant species to a crop variety in a related species. Goodman et al. (1987) presented a list of species in which gene transfers have been successful.

Wild relatives may be sources of useful traits for the improvement of crops. From plant breeding point of view, it is desirable to document the possibility of transferring traits to a crop plant from its wild relatives through conventional sexual hybridisation. Sexual exchanges between species as sources of genetic variability to improve crops have been made possible during the last century by the discovery of efficient ways to circumvent the natural barriers to genetic exchange (Goodman et al. 1987). However, inherent problems of specific introgression such as hybrid instability, infertility, non-Mendelian segregations and low levels of intergenomic crossing-over can constitute important limitations (Stebbins 1950). Moreover, features associated with polyploidy or ploidy dissimilarity between species may result in additional constraints for interspecific gene flow (Rieseberg et al. 2000). After a hybrid plant has been successfully recovered, differences in the number or the compatibility of parental chromosomes may cause sterility. Cytogenetic manipulations have been instrumental in obtaining stable gene transfers. Sterility may result from incomplete or unstable pairing of chromosomes during cell division. For a desired gene from the donor to be incorporated into a chromosome of the crop variety, recombination must take place. If the two species are closely related, natural pairing and recombination may occur (Goodman et al. 1987). High pairing affinity contributes so that once the barriers separating the species are overcome, the gene pools of the two genera are interchangeable (Zwierzykowski et al. 1999). Until recently, the results of interspecific hybridisation could only be studied in a fairly indirect manner. One method was to analyse the phenotype of hybrids, such as the symmetry of morphological characters or the viability of pollen or seed. Alternatively, meiosis in hybrids could be studied by light microscopy and the degree of differentiation between hybridising taxa estimated by analyses of chromosome pairing behaviour and meiotic abnormalities (Rieseberg et al. 2000). Although both of these approaches have been extremely valuable, they can only provide glimpses into the complex interactions of alien genes and genomes following genetic recombination. Cytological analyses are usually performed to evaluate the meiotic process in experimental hybrids. Species with close genetic affinity produce hybrids with regular chromosome pairing, while the hybrids of those more distantly related species have meiotic irregularities and are sterile (Marfil et al. 2006). In diploid interspecific hybrids, the meiotic analysis of chromosome association in the F_1 generation shows the genetic homology between the respective pairs of chromosomes. However, in interspecific tetraploid or hexaploid hybrids, chromosome pairing is affected by the number and similarity among genomes.

Interspecific hybrids have the potential to capture hybrid vigour as well as combine traits that do not occur within a single species (Volker and Orme 1988). Because a breeder always wants to add new type of characteristics to the current cultivars, interspecific hybridisation is indispensable to combine diverse gene pools. Thus interspecific or intergeneric hybrids have the enormous potential to extend not only their qualitative but also quantitative traits such as the type of flower, plant phenotypes and other single dominant traits from parent species with an environmental adaptation. While natural hybrids can exist between species whose flowering times overlap, pre- and post-fertilisation barriers hinder the frequency of these hybrids.

Reproductive barriers to wide or distant hybridisation can be divided into two broad groups—premating and postmating. The premating barriers include failure of zygote formation due to fertilisation barriers like pollen–stigma incompatibility and failure of pollen tube to reach the ovary, whereas the postmating barriers comprise failure of zygote development and uniparental chromosome elimination. The uniparental chromosome elimination acts a bane for the transfer of desirable traits from the wild species into the genetic background of target species, that is, cultivated species. But at the same time, it may act as a boon when whole chromosome complement of the wild species is eliminated resulting in the development of haploid plants of the recipient species. The doubled haploids, produced by doubling the chromosome number of the haploids, have been quite efficiently used by the breeders for achieving absolute homozygosity in just 2 years, thereby saving 5 years of varietal development programmes (Fig. 6.1a, b). Moreover, they have helped us in the quick development of mapping populations. The haploids are also useful in the development of transgenics.

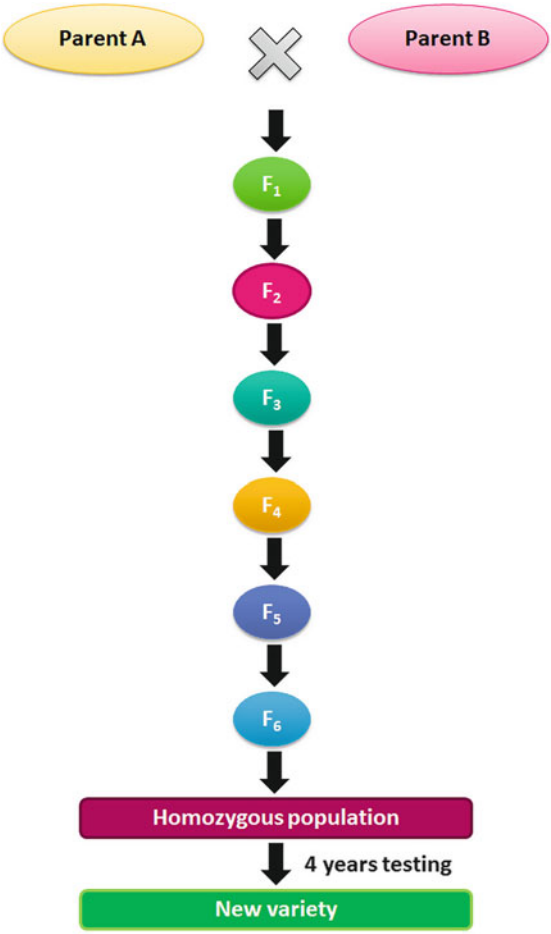

Fig. 6.1 (a and b) Flow chart exhibiting comparison of the conventional and DH breeding approaches

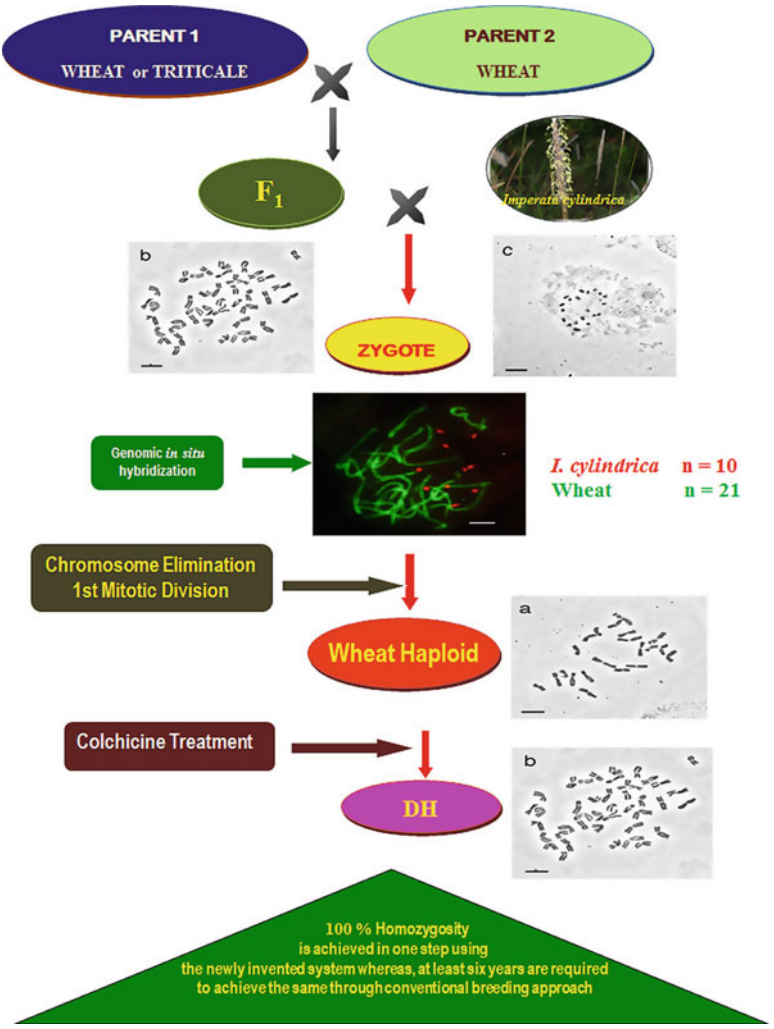

Fig. 6.1 (continued)

The doubled haploidy (DH) breeding following chromosome elimination approach has been exploited in various crops like barley, wheat, oats, triticale, rye and potato, where the other techniques of haploid induction like anther, pollen/microspore culture and ovule culture were not so efficient. In order to apply the DH systems successfully to a breeding programme, any technique should fulfil the following three criteria: (1) DH line(s) should be produced efficiently from all the genotypes, (2) DH should represent a random sample of the parental gametes and (3) DH should be genetically normal and stable (Snape et al. 1986).

Wide crossing between species has been shown to be a very effective and successful method for haploid induction in several species. It exploits haploidy from the

female gametic line and involves both interspecific and intergeneric pollinations. In some interspecific and intergeneric crosses of the Poaceae and Panicoideae, fertilisation is followed by paternal chromosome elimination from the hybrid embryo. In these crosses, the endosperm is either not formed or poorly developed; thus, such embryos do not mature in the caryopsis, and embryo rescue and in vitro culture are necessary. The production of doubled haploids through chromosome elimination occurring during wide crossing is most common in cereals (Laurie et al. 1990).

Several explanations have been proposed to account for uniparental chromosome elimination, viz., difference in timing of essential mitotic processes attributable to asynchronous cell cycling (Gupta 1969) and asynchrony in nucleoprotein synthesis leading to a loss of the most retarded chromosomes (Bennett et al. 1976; Laurie and Bennett 1989). Other hypotheses that have been put forward are the formation of multipolar spindles (Subrahmanyam and Kasha 1973), spatial separation of genomes during interphase (Finch 1983 and Linde-Laursen and von Bothmer 1999) and genome elimination by nuclear extrusions (Gernand et al. 2005, 2006). In addition, degradation of alien chromosomes by host-specific nucleases (Davies 1974), uniparental nondisjunction of anaphase chromosomes (Ishii et al. 2010) and parent-specific inactivation of centromeres (Finch 1983; Jin et al. 2004; Mochida et al. 2004) have been suggested. The actual cellular mechanism involved in the process of uniparental chromosome elimination remains poorly understood.

6.2 Doubled Haploid Through Distant Hybridisation

Various distant hybridisation-mediated doubled haploid techniques are discussed hereunder:

6.2.1 Hordeum vulgare × H. bulbosum

The first method in cereals based on wide crossing following chromosome elimination was *H. vulgare* × *H. bulbosum*, commonly known as 'bulbosum method' (Stephan 1969; Kasha and Kao 1970; Lange 1971). During early embryogenesis, chromosomes of the wild relative are preferentially eliminated from the cells of developing embryos leading to the formation of the haploid embryos. The endosperm is frequently formed, but its development is usually disturbed; hence, at 12–14 days of pollination, the embryos are excised from developing caryopsis and cultured in vitro. The bulbosum method was the first haploid induction method to produce large number of haploids across most genotypes and this method quickly entered into breeding programmes. Kasha and Kao (1970) presented evidence to show that these haploids are not caused by parthenogenesis but by the elimination of *H. bulbosum* chromosomes. This elimination is under genetic control (Ho and Kasha 1975). Haploids of *H. vulgare* are also obtained when it is used as a male parent in the wide hybridisation programme. This method represents a considerable

advanced approach in the production of barley haploids and it has a number of advantages over anther culture. In particular, haploids can be produced from any cultivar of barley, whereas with anther culture, success is dependent on the genotype.

The parent-specific inactivation of centromeres during the mitosis-dependent process of chromosome elimination in *H. vulgare* × *H. bulbosum* hybrids was confirmed by Sanie et al. (2011). They reported that the loss of centromeric histone H3 (CENH3) from centromeres precedes uniparental chromosome elimination in interspecific barley hybrids. Gernand et al. (2006) studied the mechanism underlying selective elimination of the paternal chromosomes during the development of *H. vulgare* × *H. bulbosum* hybrid embryos that is restricted to an early stage of development. In almost all embryos, most of the *H. bulbosum* chromatin undergoes a fast rate of elimination within 9 days after pollination. According to them, elimination of chromosomes in *H. vulgare* × *H. bulbosum* crosses occurs during mitosis and interphase involves micronucleus formation and progressive heterochromatinisation. The rate of chromosome elimination differs significantly between hybrids, while within each hybrid, differences in mean chromosome number were recorded between and within individual tillers. An increase in temperature from 25 to 30 °C caused a significant increase in the rate of elimination of *H. bulbosum* chromosomes (Humphreys 1978). A high efficiency of *H. bulbosum*-mediated haploid production in barley was achieved using a floret culture technique in which florets pollinated with *H. bulbosum* are cultured on modified N_6 medium containing 0.5 mg/l kinetin and 1.2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) (Chen and Hayes 1989). Toojinda et al. (2000) used bulbosum approach for mapping qualitative and quantitative disease resistance genes in a doubled-haploid population of barley (*H. vulgare*).

Keeping in view the increased efficiency of bulbosum technique of haploid induction in barley as compared to anther culture, the method was extended to wheat where the androgenesis-mediated haploid induction response was very poor and genotype specific.

6.2.2 *Triticum aestivum* × *H. bulbosum*

Haploid wheat plantlets were obtained when 'Chinese Spring' variety of *T. aestivum* (2n = 6x = 42) was crossed with *H. bulbosum* (2n = 2x = 14). This happened 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 just like anther culture 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 possesses recessive crossability alleles, that is, *kr1 and kr2*. Jalani and Moss (1980) reported that the crossability genes have little effect on pollen germination and on the time taken for the pollen tubes to reach the micropyle. The number of pollen tubes reaching the micropyle is, however, affected by the *Kr*

genes, as high crossable genotypes have more pollen tubes than the low crossable ones. Factors affecting crossability between 'Chinese Spring' wheat and *H. bulbosum* were also found on chromosomes 3A, 3B and 3D (Miller et al. 1983). This system was hence useful to a limited extent due to the sensitivity of the *H. bulbosum* pollen to the crossability inhibitor genes.

6.2.3 Wheat × Maize

Zenkteler and Nitzsche (1984) reported for the first time that embryos were frequently formed when hexaploid wheat was pollinated with maize. Later, their results were confirmed by Laurie and Bennett (1986). They cytologically demonstrated that the maize pollen normally germinated and grew into the wheat embryo sac where the wheat egg was fertilised by the maize pollen. A hybrid zygote with 21 wheat chromosomes and 10 maize chromosomes was produced (Laurie and Bennett 1988). The hybrid zygotes were karyotypically unstable, and the maize chromosomes failed to move towards the spindle poles during cell divisions. Possibly, their centromeres failed to attach to the spindle microtubules due to progressive loss of centromere activity. Resultantly, maize chromosomes were eliminated after three to four mitotic cell divisions forming wheat haploid embryo with n = 21 chromosomes (Laurie and Bennett 1989).

Some earlier studies showed that wheat × maize system has more efficiency of embryo formation as compared to other techniques. For haploid embryo production a system of wheat × maize crossing is widely used due to higher production of haploid embryos as compared to other grass species pollination systems (Inagaki and Tahir 1990; Kisana et al. 1993; Inagaki and Mujeeb-Kazi 1995). 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 maize chromosome elimination system in wheat is insensitive to crossability inhibitor genes (Laurie and Bennett 1989) and it enables the production of large number of haploids from any genotype including those recalcitrant to androgenesis (Inagaki et al. 1998; David et al. 1999; Cherkaoui et al. 2000; Chaudhary et al. 2002; Singh et al. 2004; Pratap et al. 2006). Several other investigations of haploid wheat production through wide crossing have since been reported (Laurie and Bennett 1989; Laurie and Reymondie 1991; Matzk and Mahn 1994; Suenaga 1994; Morshedi and Darvey 1995). It appears that a wide range of wheat and maize genotypes can be used to produce haploid wheats, although there is evidence to suggest that the efficiency of production is variable (Suenaga 1994). Haploid production efficiency is affected by the proportion of pollinated florets which develop haploid embryos. Yields of haploid embryos have been reported to be as high as 53 % (Morshedi and Darvey 1995) and as low as 1 % (Suenaga and Nakajima 1989) depending upon a wide range of variables. Factors that affect the yield of haploid embryos include genotypic differences between individual wheat and maize lines (lnagaki and Tahir 1990; Suenaga 1994; Chaudhary et al. 2002; Sharma et al. 2005;

Pratap and Chaudhary 2007; Dhiman et al. 2012), the timing and use of exogenous growth substances to stimulate ovule development (Suenaga and Nakajima 1989) and environmental factors (especially temperature) during and after pollination.

Laurie and Bennett (1989) reported that all maize chromosomes were lost during the first three cell division cycles in most embryos. All embryos with four or more cells had micronuclei, showing that embryo development was dependent on fertilisation. The only primary endosperm metaphase obtained in the experiment had 42 wheat and 10 maize chromosomes, and the presence of micronuclei in most developing endosperms showed that at least 85 % were of hybrid origin.

Zhang et al. (1996) comparatively analysed the embryogenesis in wheat × maize hybrids and self-pollinated wheat plants using paraffin sectioning. They reported that development of embryo is not accompanied by the formation of an endosperm and the endosperm nuclei remain free in the cytoplasm, fail to advance into the cellular stage and degenerate later.

Pratap et al. (2005) evaluated the comparative efficiency of anther culture and maize-mediated system of haploid induction in wheat and triticale genotypes. They reported that haploid plantlet formation was significantly higher through maize-mediated approach as compared to androgenesis in both wheat and triticale genotypes. Auxin analogues play a key role in the induction and maintenance of haploid wheat embryos. Pratap and Chaudhary (2012) investigated the comparative effect of auxins on induction of polyhaploids in triticale × wheat through wheat × maize system.

Wang et al. (1991) studied the frequency of fertilisation and embryo formation in wheat × maize crosses. Hybrid embryos and endosperms obtained from wheat × maize hybridisation were karyotypically unstable and were characterised by rapid elimination of the maize chromosomes to produce haploid wheat embryos. Hence, the reduced genotypic specificity, absence of albinism and ease of application make the wheat × maize hybridisation technique more efficient than the anther culture and the *bulbosum* technique for the production of haploids in common wheat. Accordingly, Inagaki and Tahir (1991), Sun et al. (1992) and Kasha et al. (1995) advocated the use of this technique for breeding purpose by raising a large number of wheat haploids.

Suenaga and Nakajima (1993) evaluated 110 wheat DH lines derived from wheat × maize crosses and found that 15 DH lines were variable for two traits like extreme dwarfism, low seed fertility, alteration of spike type and strips. Analysis of variance within and between DH lines showed the presence of heterogeneity/heterozygosity in the DH lines/plants. Limited studies have been conducted on this line. They inferred that most of the variations detected in the DH lines were due to the effect of colchicine treatment. Similarly Kammholz et al. (1998) also found that expected normal segregation pattern for six glutenin loci across the seven crosses indicated that wheat × maize system is stable across the generations and may meet the third criterion proposed by Snape et al. (1986) for practical wheat breeding programmes. Moreover, Lefebvre and Devaux (1996) also reported normal segregation for 1BL–1RS chromosome through wheat × maize system of cross but which deviates from 1:1 in the haploid progenies produced by anther culture. The wheat × maize system was quite efficiently utilised in the development of the first doubled-haploid wheat variety of India (Him Pratham) (Fig. 6.2) by Dr. Harinder

Fig. 6.2 First doubled-haploid wheat variety of India: DH 114 (Him Pratham) developed through chromosome elimination-mediated approach

Kumar Chaudhary of CSK HP Agricultural University, Palampur, Himachal Pradesh, India (Chaudhary 2013).

Inagaki et al. (1997) crossed hexaploid triticale as well as triticale substitution lines with maize. Hexaploid triticales produced embryos at low frequencies (0.0–5.4 %), whereas higher frequencies were obtained in substitution lines with 2D and 4D chromosomes. This gives an indication that the D-genome chromosomes in triticale genetic background have the effect of increasing the frequency of polyploidy production in triticale × maize crosses. However, maize-mediated system was not able to induce any haploids in wheat × rye derivatives (Kishore et al. 2011).

Durum wheat (2n = 4x = 28) or macaroni wheat is the only tetraploid species of wheat of commercial importance that is widely cultivated today. The ploidy level is not a barrier in the production of haploid embryos through wheat × maize system, and haploids were produced in durum wheat using maize as the pollen source (Ahmad and Chowdhry 2005). Haploid seedlings were recovered from *Triticum turgidum* ssp. *turgidum* cv 'Rampton Rivet' pollinated with maize following in vivo treatment of ovaries with 2,4-D for 2 weeks and subsequent embryo culture. The recovery of haploid seedlings from *T. turgidum* ssp. *durum* cv. 'Wakona' pollinated with maize necessitated the addition of $AgNO_3$ to the 2,4-D treatment (O'Donoughue and Bennett 1994). Almouslem et al. (1998) also reported haploid durum wheat production via hybridisation with maize. Ballesteros et al. (2003) analysed the influence of the relative humidity of the environment, when culturing detached tillers during the production of haploid plants in durum wheat by the maize method and they found that low relative humidity increases haploid induction in durum wheat × maize crosses.

The high haploid induction efficiency and genotype non-specificity of wheat × maize system in comparison to anther culture and bulbosum technique

make the system more practicable. However, the flowering times of maize and wheat can be matched under field conditions in subtropical and tropical climates only, while in other areas experiments are run under glasshouse conditions. Keeping this in view, various efforts have been made throughout the world to search for alternative pollen source for haploid induction in wheat 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-Kazi 1992) and Job's tears (Mochida and Tsujimoto 2001). More recently, *Imperata cylindrica*, a perennial weedy grass has been reported as the most efficient pollen source for the induction of haploids in wheat, wheat × rye and triticale (Chaudhary et al. 2005; Pratap et al. 2005).

6.2.4 *Wheat × Tripsacum dactyloides*

To extend the crossing cycle duration, Riera-Lizarazu and Mujeeb-Kazi (1992) performed intergeneric crosses of *T. aestivum*, *T. turgidum* L. and *T. turgidum* × *Aegilops squarrosa* L. (*T. tauschii*) synthetic hexaploids ($2n = 6x = 42$; AABBDD) with *Tripsacum dactyloides* ($2n = 2x = 36$) as a pollen donor which resulted in progenies that were polyhaploids of the Triticeae parents, presumably due to elimination of the *Tripsacum dactyloides* chromosomes during early embryo development. Embryo recovery frequencies were 20.6 % for *T. aestivum* cultivars, 26.8 % for *T. turgidum* cultivars and 23.5 % for the synthetic hexaploids. Plant regeneration ranged between 66.7 and 78.5 % over the three maternal crossing groups. As with maize, polyhaploid production in the Triticeae with *Tripsacum* is dependent upon a post-pollination treatment with 2,4-D to promote embryo development and shows no strong genotypic specificity. Limited meiotic analyses for the *T. aestivum* cultivars and synthetic hexaploids gave metaphase I associations characteristic of non-allosyndetic chromosomal pairing. Pollinations with *Tripsacum*, together with maize pollinations, offer an extended crossing cycle and in addition extend the range of alien species for producing polyhaploids in the Triticeae.

6.2.5 *Wheat × Pearl Millet*

Pearl millet (*Pennisetum glaucum*) is the most widely grown type of millet. It has been grown in Africa and the Indian subcontinent since prehistoric times. Pearl millet is well adapted to growing areas characterised by drought, low soil fertility and high temperature. Haploid wheat plants were obtained when crossed with pearl millet. The wheat plants retained a single pearl millet chromosome at tillering stage, but this chromosome was eliminated from pollen mother cells prior to and also during gamete formation (Ahmad and Comeau 1990). Laurie (1989) undertook wheat × pearl millet crosses to determine whether fertilisation occurred and any

resulting hybrids were karyotypically stable. Crosses between the hexaploid wheat genotype 'Chinese Spring' (*kr1*, *kr2*) and the pearl millet genotype 'Tift 23BE' yielded fertilisation in 28.6 % of the 220 florets pollinated. Chromosome counts from zygotes at metaphase confirmed the hybrid origin of the embryos. Three had the expected F_1 combination of 21 wheat and 7 pearl millet chromosomes, and a fourth had 21 wheat and 14 pearl millet chromosomes. The expected F_1 chromosome complement was also found in primary endosperm mitosis. The hybrid embryos were karyotypically unstable and probably lost all the pearl millet chromosomes in the first four cell division cycles. Similar results were obtained using two other wheat genotypes. Crosses between the hexaploid wheat genotype 'Highbury', which differs from 'Chinese Spring' in having alleles for reduced crossability with rye and *H. bulbosum* at the *Kr1* and *Kr2* loci, and 'Tift 23BE' registered fertilisation in 32 % of analysed florets. This was not significantly different from the frequency found in 'Chinese Spring', indicating that 'Tift 23BE' was insensitive to the action of the *Kr* genes. Crosses between the tetraploid wheat genotype 'Kubanka' and 'Tift 23BE' showed fertilisation in 48 % of florets.

Inagaki and Hash (1998) produced haploids in bread wheat, durum wheat and hexaploid triticale when crossed with pearl millet. The crossability of bread wheat was found to be higher as compared with maize. Inagaki and Mujeeb-Kazi (1995) compared the frequencies of haploid induction in wheat when crossed with maize, pearl millet and sorghum and they observed that maize-mediated haploid induction frequency was higher as compared to the other two which were found to be genotype specific. Deimling et al. (1994) obtained six embryos from which two doubled-haploid lines resulted after pollination of 48,000 emasculated flowers. One embryo was induced by pearl millet and others with maize. Overall, pearl millet could not show its superiority over the maize system in any case of haploid induction and the system was genotype specific.

6.2.6 Wheat × Job's Tears

Job's tears (*Coix lacryma-jobi*) is a tall grain-bearing tropical plant of the family Poaceae native to Southeast Asia but elsewhere cultivated in gardens as an annual. It has been naturalised in the southern United States and the New World tropics. In its native environment, it is grown in higher areas where rice and corn do not grow well. Job's tears are also commonly sold as Chinese pearl barley in Asian supermarkets, although *C. lacryma-jobi* is not closely related to barley (*Hordeum vulgare*). Job's tears is a perennial plant which forms several stalks and its pollen can be collected throughout the year when the plant is maintained in a controlled environment. Mochida and Tsujimoto (2001) produced wheat (*Triticum aestivum* L.) haploids by crossing with Job's tears (*Coix lachryma-jobi* L.) as the pollen parent. Pollination was followed by 2,4-D treatment, detached tiller culture and embryo culture, as described for maize pollination. The frequency of embryo formation was similar to that obtained by crossing wheat with maize pollen.

Fig. 6.3 Spike of *Imperata cylindrica*, the efficient pollen source for haploid induction in wheat

6.2.7 *Wheat × Imperata cylindrica*

Considering the above chromosome elimination-mediated haploid induction systems, no alternative pollen source was reported to overcome the problems of wheat × maize system viz., non-synchronisation of flowering with wheat naturally and poor performance in producing haploids from triticale × wheat and wheat × rye derivatives (Kishore et al. 2011). These constraints made it imperative to search for some other pollen source. Among all the *Gramineae* genera viz., *Zea mays*, *Sorghum bicolor*, *Pennisetum americanum*, *Setaria italica*, *Festuca arundinacea*, *Imperata 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; Pratap et al. 2005). Cogon grass (*I. cylindrica*) (Fig. 6.3) is a wild weedy perennial grass (2n = 2x = 20), does not require repeated sowings and its flowering coincides well with that of wheat and triticale under natural conditions. Furthermore, it is available under natural conditions in almost all parts of the world wherever wheat is cultivated. The *I. cylindrica*-mediated chromosome elimination approach of doubled-haploidy breeding is genotype non-specific for hybridisation with any variety of wheat, triticale or their derivatives.

I. cylindrica has been reported to perform significantly better than maize for all the haploid induction parameters in wheat and triticale and their derivatives (Chaudhary 2008a, b, 2012, 2013). Cytological investigation of the wheat × *I. cylindrica* chromosome elimination system has shown that there is no endosperm formation and the elimination of chromosomes of *I. cylindrica* takes place in the first zygotic division

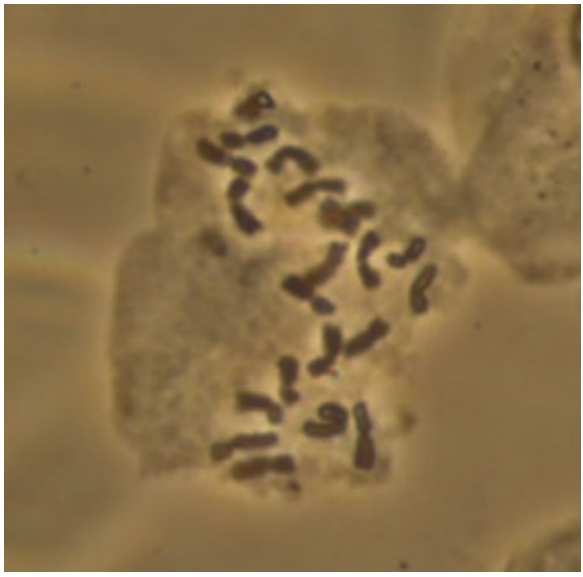

Fig. 6.4 Cytological confirmation of wheat haploid (n = 21) produced from wheat × *Imperata cylindrica* hybridisation (source: Tayeng et al. 2012)

in seed development, thus allowing the production of embryo-carrying seeds (Komeda et al. 2007). Recently, Tayeng et al. (2012) reported that the in vivo application of colchicine (2,000 ppm) enhances the doubled-haploid production efficiency in wheat × *I. cylindrica*-mediated chromosome elimination approach of doubled-haploidy breeding. The haploid chromosome set of wheat (n = 21) obtained after wheat × *I. cylindrica* hybridisation is shown in Fig. 6.4. According to Kaila et al. (2012), the chromosome elimination in wheat × *I. cylindrica* system is being triggered by the B and D genome of wheat. Similar to wheat × maize system, the mean response of wheat and *I. cylindrica* to haploid induction varies from genotype to genotype (Rather et al. 2013). The morphological marker, that is, absence of endosperm in haploid embryo-carrying seeds developed from wheat × *I. cylindrica* hybridisation, can be used quite efficiently to exploit the asynchronous behaviour of anthesis within wheat spikes (Chaudhary et al. 2013) for undertaking this wide hybridisation without emasculation. This endeavour has saved considerable time and energy required otherwise for emasculation in wheat × *I. cylindrica* hybridisation.

6.2.8 Triticale × Imperata cylindrica

Triticale is a hybrid of wheat (*Triticum*) and rye (*Secale*) first bred in laboratories during the late nineteenth century. Commercially available triticale is almost a second-generation hybrid, i.e. a cross between two kinds of primary (first cross) triticales. As a rule, triticale combines the yield potential and grain quality of wheat

with the disease and environmental stress tolerance (including stresses related to soil conditions) of rye.

As far as haploid induction following chromosome elimination approach of doubled-haploidy breeding is concerned, Kishore et al. (2011) carried out intergeneric hybridisation using pollen of maize and *I. cylindrica* in wheat–rye-derived backcross (BC_1F_1 and BC_1F_2) generations to study the relative efficiency of the two chromosome elimination systems. The relative efficiency of embryo-carrying seeds ranged from 8 to 30 % with *I. cylindrica*, whereas with maize, no embryo-carrying seeds were obtained. Wedzony et al. (1998) reported the production of haploid embryos in triticale by means of maize-mediated system. However, *I. cylindrica*-mediated system outperformed the maize system in triticale × wheat derivatives in respect of embryo formation and embryo regeneration frequency (Pratap et al. 2005).

6.2.9 Oat × maize

The common oat (*Avena sativa*) is a cereal mostly grown for its seed. Oats are suitable for human consumption as oatmeal and rolled oats; however, these are commonly used as livestock feed. Riera-Lizarazu et al. (1996) crossed hexaploid oat (2n = 6x = 42) and maize (2n = 2x = 20) and recovered 90 progenies through embryo rescue. Fifty-two plants (58 %) produced from oat × maize hybridisation were oat haploids (2n = 3x = 21) following maize chromosome elimination. Twenty-eight plants (31 %) were found to be stable partial hybrids with 1–4 maize chromosomes in addition to a haploid set of 21 oat chromosomes (2n = 21 + 1 to 2n = 21 + 4). Ten of the 90 plants produced were found to be apparent chromosomal chimeras, where some tissues in a given plant contained maize chromosomes while other tissues did not, or else different tissues contained a variable number of maize chromosomes. Jing-San and Tie-Gang (1995) crossed naked oat with maize and obtained haploid plants of naked oat.

Factors influencing the rate of caryopsis and haploid embryo production including genotype, post-pollination plant growth regulator application and temperature were investigated (Sidhu et al. 2006). The four growth regulators tested showed significant differences in their capacity to induce caryopsis formation with dicamba producing the highest numbers of caryopses, followed by picloram, 2,4-D and gibberellic acid (GA_3). No significant differences were observed between these growth regulators for their effect on embryo production. The concentration of dicamba was also important and was found to influence caryopsis but not embryo production, with 50 and 100 mg/l dicamba producing significantly more caryopses than 25 or 5 mg/l. Temperature had a significant impact on both caryopsis and embryo production with the magnitude and direction of response depending on genotype. Rates of haploid embryo production observed were between 0.8 and 6.7 % of the pollinated florets. The proportion of haploids, which survived and were successfully doubled with colchicine following transfer to soil, was between 72 and 81 %.

Rines (2002) produced haploids of cultivated oat from wide hybridisation with Panicoideae species, particularly maize. Haploid oat production by the maize wide

cross method appears to be less genotype restricted than haploid production by anther culture. However, the plant recovery frequencies reported to the tune of 1–2 % of maize-pollinated florets are low like those for oat haploid production by anther culture and not yet adequate for routine use in breeding. The oat × maize hybridisation results in novel features in respect of types and reproductive behaviour of plants recovered. These include maize chromosome retention in a portion of the recovered oat plants and partial self-fertility in oat haploid plants. These differences in products can be detrimental in routine production of doubled haploids for breeding, but on the other side the sea normalities have led to the recovery of valuable materials for genetic and genomic studies in both oat and maize. This report details protocols currently in use for recovery and for molecular and cytological characterisation of doubled-haploid oat plants, both with and without added maize chromosomes, from oat × maize hybridisation and describes features of derived plants that make them novel and valuable. Keeping in view the low frequency of haploid recovery through maize-mediated system, there is a need to search some other efficient pollen source so as to enhance the haploid induction efficiency in oat.

6.2.10 Solanum tuberosum × S. phureja

Doubled haploids can be produced from tetraploid genotypes of *S. tuberosum* (cultivated potato) by pollination with the diploid potato species, *S. phureja* (Mendiburu et al. 1974; De Maine 2003). In about 0.5 % of pollinated ovules, both male sperm cells of *S. phureja* take part in the formation of functional endosperm. The best pollinator lines of *S. phureja* were bred for a dominant purple spot embryo marker; thus, seeds containing haploid embryos can be easily distinguished from hybrid *S. tuberosum* × *S. phureja* seeds. Methods of more effective chromosome number duplication were developed more recently, and production of potato can now be obtained by androgenetic methods with a better efficiency (Jacobsen and Ramanna 1994; Rokka et al. 1996; Rokka 2003). Moreover, androgenesis is applicable to a much wider range of *Solanum* species in comparison to crosses with *S. phureja* (Jacobsen and Ramanna 1994; Aziz et al. 1999; Rokka 2003).

Montelongo-Escobedo and Rowe (1969) reported that the superior pollinator in potato haploidy breeding following chromosome elimination approach may be the one that produces a high frequency of restitution sperm nuclei. Dihaploid potatoes can be used for breeding purposes, including alien germplasm introgression or selection at the diploid level, but such plants are not homozygous. Haploids have a significant role in potato breeding programmes, since they enable interspecific hybridisation which would not be otherwise possible due to differences in ploidy levels and endosperm balance numbers. The gene pool of potato can be broadened, and certain valuable traits, such as disease resistance characters from the wild solanaceous species, can be more efficiently introgressed into cultivated potato (Rokka 2009).

6.3 Conclusion

Distant hybridisation has been quite extensively used in various crop improvement programmes as it results in the creation of genetic variation and broadening of the genetic base of the crop plants which helps them to adapt to changing climatic conditions. Among the various barriers involved in the transfer of desirable genes from wild species into the genetic background of modern-day crop varieties, chromosome elimination has been studied to a great extent by the researchers in various crops, especially cereals. The uniparental chromosome elimination in distant or wide hybrids leading to the development of haploids has speeded up the genetic improvement programmes in different crop species as it helps us to achieve the absolute homozygosity in 2 years, thereby saving 5 years of varietal development programmes. Moreover, it assists in the quick development of mapping populations used for molecular studies at various levels. The chromosome elimination-mediated approaches of doubled-haploidy breeding have been used quite efficiently in crops like barley, wheat, oats, triticale and potato, whereas other approaches, viz., androgenesis and gynogenesis, were not so efficient and practicable. The application of any doubled haploid technique to breeding programmes should be able to produce DH lines from all the genotypes, and the DHs should be genetically stable (Snape et al. 1986). The bulbosum technique of haploid induction was found genotype specific just like anther or ovule culture. The wheat × maize system was genotype non-specific, but it failed to produce haploids in wheat × rye derivatives (Kishore et al. 2011). Wheat × *I. cylindrica*, the newly invented system of chromosome elimination, has showed a great promise in producing haploids from wheat, triticale and wheat × rye derivatives (Chaudhary et al. 2005; Chaudhary 2013). Keeping in view that most of the studies in respect of doubled-haploidy breeding following uniparental chromosome elimination have been reported in cereals, the plant breeders should look forward for such types of genotype-non-specific and efficient haploid induction systems in other crops.

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