Review of doubled haploid production in durum and common wheat through wheat × maize hybridization

Zhixia Niu1, Aixiang Jiang2, Wesam Abu Hammad3, Atena Oladzadabrasabadi3, Steven S. Xu1, Mohamed Mergoum3 and Elias M. Elias3,4

1United States Department of Agriculture, Agricultural Research Service, Cereal Crops Research Unit, Fargo, ND 58102, USA; 2Biostatistics Department, Vanderbilt University, Nashville, TN 37232, USA; 3Department of Plant Sciences, North Dakota State University, Fargo, ND 58108, USA; 4Corresponding author, E-mail: elias.elias@ndsu.edu

Received May 15, 2013/Accepted December 21, 2013
Communicated by L. Hartl

Abstract

Production of doubled haploids (DHs) is an important methodology to speed the process of breeding and development of mapping populations in crops. The procedure for DH production includes two major steps: haploid induction and chromosome doubling. In recent years, wide hybridization between wheat and maize has become a popular approach for haploid production in wheat. In this method, the maize chromosomes are completely eliminated during the early development of the hybrid seeds after wheat spikes were pollinated with maize pollen. Numerous wheat cultivars and mapping populations have been developed using wheat–maize hybridization. In this study, we review the procedures of DH production of durum and common wheat via wide hybridization with maize, the factors which affect the efficiency of DH production, and the mechanism of selective elimination of the maize genome during the early development of the hybrid embryos. We also report a highly efficient protocol for DH production in durum and common wheat, which was established based on the optimal conditions for each of the factors that affect the efficiency of DH production.

Key words: doubled haploid production – wide hybridization – chromosome elimination – chromosome doubling – common wheat – durum wheat

Doubled haploids (DHs) in plants have complete homozygosity, which can be achieved in one generation from hybrid plants. Thus, using the DH method could speed cultivar and population development in crop improvement, genetic manipulation, and plant genome and gene mapping. The procedure for DH production includes two major steps: haploid induction and chromosome doubling. Chromosome doubling of haploid plants has been routinely and successfully performed using colchicine. However, the success and efficiency of haploid induction varies in different crop species. The successful utilization of the DH method in most crops to breed commercial cultivars and to develop mapping populations relies highly on an efficient protocol for inducing haploids.

Haploids in higher plants can occur spontaneously. They can also be artificially induced in vivo or in vitro by androgenesis and gynogenesis. The first report of the natural occurrence of sporophytic haploids was in the weed species Datura stramonium L. (Blakeslee et al. 1922). Later, the development of protocols for the production of haploid plants and the techniques of chromosome doubling led to the release of two DH cultivars in 1972 and 1980, respectively: ‘Maris Haplona’ in rapeseed (Brassica napus L.) using microspore culture and ‘Mingo’ in barley (Hordeum vulgare L.) using genome elimination (Thompson 1972, Ho and Jones 1980). In the late 1970s, DH technology developed slowly because of its labour and time-consuming process (Forster et al. 2007). But in recent years, technological innovation and the high demand of the end-use applications of DH lines, such as used for marker identification and gene mapping, drew attention to the development of DHs in higher plants (Forster et al. 2007).

Durum wheat (Triticum turgidum L. subsp. durum, 2n = 4x = 28 = AABB genomes) and common wheat (Triticum aestivum L., 2n = 6x = 42 = AABBD genomes) are two important cereal crops for human consumption. They are both self-pollinated allopolyploids. Previously, the development of durum and common wheat cultivars was mainly implemented using conventional breeding methods, including backcross selection, pedigree selection, bulk selection and single-seed descent (SSD; Mergoum et al. 2009). The development of populations used for gene discovery and mapping quantitative trait loci (QTL) also mainly relied on the conventional SSD method. These conventional breeding methods require several generations (at least five) of self-pollination to achieve homozygous lines. In contrast, the DH breeding method allows wheat breeders to develop ‘completely homozygous lines in one generation from early generation (F1 or F2)’ (Mergoum et al. 2009). When the induction of haploids via wheat × maize (Zea mays L.) hybridization became successful, the DH method became a popular approach for breeding common wheat cultivars and for developing mapping populations in both durum and common wheat. In the past several years, numerous wheat cultivars and mapping populations have been developed worldwide using the DH method. In this study, we review the methodologies of the induction of haploids in wheat and durum wheat, the factors which affect the efficiency of DH development via wide hybridization between durum/common wheat and maize and the mechanism of uniparental chromosome elimination during the hybrid embryo development. We also outline an efficient protocol for DH development in durum and common wheat.
Methodologies of the Development of Haploids in Wheat and Durum Wheat

There are two basic approaches for developing haploids in higher plants: androgenesis (anther or microspore culture) and gynogenesis (ovary or megaspore culture). Production of haploids via anther culture is an in vitro process in which microspore cells with haploid genomes develop to an embryo-like structure on culture medium. The embryo-like structure further develops to a haploid plantlet (Jaehr et al. 2009). In contrast, haploid induction through ovary or megasporop culture is a megaspore embryogenesis in which unfertilized eggs or other embryo sac cells develop into haploid plantlets by a parthenogenic development process (Yang and Zhou 1982). Production of haploids via anther culture was first reported in Datura innoxia Mill. in 1964 (Guha and Maheshwari 1964). The authors reported when anthers of Datura were cultured on Nitsch’s medium containing 15% coconut milk, large number of embryo-like structures were produced. Later in 1966, they reported that the embryo-like structures were generated from the pollen grains. Since then, anther culture has been widely and successfully utilized for large-scale production of haploids in many crop species because an anther contains a large number of microspores and can be easily collected (see review by Jaehr et al. 2009). Haploid induction from ovary culture was first reported in barley in 1976 (San Noeum 1976). Although haploids have been successfully developed from the in vitro culture of unfertilized ovules and ovaries in more than 20 angiosperm species since 1976 (Bu et al. 2004), ovary or megasporop culture has not been widely used for large-scale production of haploids in most plant species mainly due to the small number of megaspores in the plant and the difficulty in isolating of the female haploid cells from a plant (Krisof and Imre 1996).

In common wheat, haploid plants can be developed through both androgenesis and gynogenesis. As in other crops, however, typical gynogenesis through ovary or megasporop culture has not been used for cultivar and population development. Only androgenesis by anther culture has been successfully used for practical breeding. Many common wheat cultivars around the world were developed through anther culture such as ‘Florin’ (De Buysier et al. 1987), ‘McKenzie’ (Graf et al. 2003) and ‘AC Andrew’ (Sadashivala et al. 2004). Anther or microspore culture in wheat has some obstacles for haploid production, such as high rates of chromosome loss. Meanwhile, many authors have investigated for their ability to induce wheat haploids through wide hybridization. For pearl millet and sorghum, there was a strong wheat genotypic barrier for embryo formation (Inagaki and Muejeb-Kazi 1995, Maluszynski 2003) and cogongrass (Imperata cylindrica (L.) P. Beauv.) (Chaudhary et al. 2005), have been investigated for their ability to induce wheat haploids through wide hybridization. For pearl millet and sorghum, there was a strong wheat genotypic barrier for embryo formation (Inagaki and Muejeb-Kazi 1995, Maluszynski 2003). The wheat × cogongrass hybridization was reported to have an equal efficiency of haploid embryo formation as the wheat × maize hybridization system (Chaudhary et al. 2005). But, cogongrass is a noxious weed and is prohibited from being introduced in certain areas of North America. More recently, Ravi and Chan (2010) reported a centromere-mediated genome elimination procedure for producing haploids in Arabidopsis thaliana (L.) Heynh. by manipulating a centrome-specific histone CENH3. In this procedure, cenh3 null mutants used as either male or female were hybridized to normal plants, and the chromosomes entering the zygote from the mutant were eliminated during the hybrid embryo development (Ravi and Chan 2010). The centromere-mediated genome elimination directly produced hybrid seeds which contain chromosomes only from the normal parent used as either male or female, and this differs from the wide hybridization method which produces haploids having chromosomes only from the female parents. Because this procedure does not involve tissue culture and wide hybridization, it may provide a more efficient production of haploids in wheat and other crops. However, the procedure has not been used in wheat yet; even the feasibility for manipulation of the histone protein CENH3 in wheat has not been investigated. Therefore, the wheat × maize hybridization system is currently the most practical way to develop DHs in durum and common wheat (Laurie and Bennett 1988, Islam and Shepherd 1994, Pienaar and Lesch 1994, Inagaki et al. 1998, Campbell et al. 2000).
Factors Affecting Efficiency of DH Production in Wide Hybridization between Wheat/Durum Wheat and Maize

Although maize is relatively insensitive to the action of wheat Kr genes (Laurie and Bennett 1987, Ohkawa et al. 1992, Inagaki and Mujeeb-Kazi 1995, Li et al. 1996), many other factors affect the efficiency of DH production in the durum/common wheat × maize system. These factors include the wheat and maize genotypes; position of the spikelet in the flower; temperature; light intensity and photoperiod during the plant growth period; the type and concentration of plant growth regulators (PGRs) applied after pollination; the biochemical elements added to the rescue media, the concentration of colchicine used for chromosome doubling; and the plant growth stage, duration and temperature when the colchicine is applied (Laurie and Bennett 1989, Suenaga and Nakajima 1989, O’Donoughue and Bennett 1994, Pienaar and Lesch 1994, Inagaki and Bohorova 1995, Inagaki and Mujeeb-Kazi 1996, Morshedi et al. 1996, Pienaar et al. 1996, Wedzony and Van Lammeren 1996, Campbell et al. 1998).

Wheat and Maize Genotypes

The effects of wheat and maize genotypes on the frequency of haploid embryo formation and haploid plant production were investigated in numerous studies, but the results are still controversial. Suenaga and Nakajima (1989) reported that when four Japanese wheat cultivars were pollinated with five maize genotypes, the frequency of embryo formation was only affected by the maize genotypes, not by the wheat cultivars. But several other studies showed that wheat genotype significantly affects the percentages of the embryos per pollinated florets (Inagaki and Tahir 1990, Laurie and Reymondie 1991, Martins-Lopes et al. 2001, Niroula and Thapa 2009). Lefebvre and Devaux (1996) demonstrated that the haploid production efficiency was affected by both wheat and maize genotypes based on a study of 18 wheat F1 hybrids crossed with five maize genotypes using a replicated block design. They reported that the interaction between parental genotypes was significant for the number of haploid embryos/100 florets.

The efficiency of haploid induction in common wheat is usually higher than durum wheat when using wide hybridization with maize. High ploidy level and the D genome of common wheat may play an important role in DH production. Dogramaci-Alhuntepe and Jauchar (2001) pollinated durum wheat ‘Langdon’, 14 Langdon D-genome disomic substitution lines (Joppa and Williams 1988, Li et al. 2006) and Langdon ph1b mutant with maize pollen and found that the efficiency of haploid production varied among different substitution lines. The most efficient line was the 5D (5B) substitution, indicating that substituting chromosome 5D for 5B improves the efficiency of haploid production in durum wheat. Almouslem et al. (1998) reported clear genotypic differences of maternal durum genotypes used in the production of DH plants.

The Position of the Spikelet in the Flower and the Timing for Pollination

Wheat florets flower in order starting from the middle florets. It usually takes about 3 days for all the florets on a spike to finish flowering. The fertilization frequencies depend on the stage of the floret, and the best results occurred when the stigma was at the feathery stage (Laurie and Bennett 1989). The position of the spikelet significantly affected the ratio of embryo formation, and the highest values were obtained in the middle position of the spikes (Martins-Lopes et al. 2001), while another study demonstrated that the fertilization rate was not affected by spikelet position within a certain time of pollination (Bitsch et al. 1998). Pollination with maize at a time close to anthesis not only results in a better crossability, but also in a good embryo quality, which determines the germinating rate of the embryos.

Environmental Elements: Temperature, Light and Photoperiod

Both temperature and light intensity significantly influenced the frequency of haploid embryo recovery, with light intensity having a greater effect. Light intensity at 1000 μmol/m²/s radiance at 22/17°C (day/night) resulted in a high frequency of haploid embryos (Campbell et al. 1998). Light intensity may affect pollen tube growth, the predetermination step for successful fertilization (Campbell et al. 2001). The efficiency of DH production in the wheat × maize system is also affected by the time of year when the crosses are made, for example fall (August–December) vs. spring (January–April); the higher efficiency was usually obtained in the spring (Pienaar and Lesch 1994, Pienaar et al. 1996, Campbell et al. 2000). This could be a contribution of the longer photoperiod and stronger light intensity in the spring, which affects the female plant vigour, the fertilization ability of the egg cells, the pollen tube growth in the female plants and the viability of the hybrid seeds.

The environment influenced wheat embryo survival and pollen tube growth in a genotypically dependent manner (O’Donoughue and Bennett 1994, Campbell et al. 2001). Campbell et al. (2001) reported that when two wheat cultivars, ‘Karamu’ and ‘Kotuku’, were crossed with the same maize genotype at two irradiance levels (250 or 750 μmol/m²/s, PAR), pollen tube growth was significantly affected by light intensity in ‘Karamu’, but not in ‘Kotuku’. O’Donoughue and Bennett (1994) showed that the cultivar ‘Rampton Rivet’ had significantly better embryo recovery in a 20°C growth room than in an unheated glasshouse, whereas ‘Wakona’ and ‘Chinese Spring’ were unaffected.

Postpollination Treatment

There are two barriers to wheat DH production via maize hybridization. One is the low ratio of embryo/embryonless caryopsis produced, and the other is the absence of endosperm in the hybrid seeds (Zenkeleter and Nitzsche 1984, Laurie and Bennett 1986, 1987), which results in embryo death when they are left to develop in plants (Laurie and Bennett 1988). Postpollination treatments reported in the literature (Suenaga and Nakajima 1989, Laurie et al. 1990, Laurie and Reymondie 1991) include (i) immediately culturing the pollinated spikelets for 3 weeks (Laurie and Bennett 1988), (ii) successively applying 0.5 mg/l dichlorophenoxyacetic acid (2,4-D) to the pollinated spikes for 2–3 weeks, (iii) injecting or spraying 100 mg/l 2,4-D to the internode and/or to the spikelets of the pollinated spikes once or twice (Matzk and Mohr 1994), (iv) applying a solution of a combination of an auxin [picloram (4-amino-3,5,6-trichloropicolinic acid), 2,4-D, or 2,4,5-T (2,4,5-trichlorophenoxyacetic acid)] with 6-benzylaminopurine (6-BA) or with a combination of 2,4-D and gibberellic acid (GA3) in the florets at 24–30 h after pollination (Pienaar et al. 1997, Singh et al. 2001) and (v) applying a solution of dicamba (3,6-dichloro-o-anisic acid) or
Z. Niu, A. Jiang, W. Abu Hammad et al.

Embryo Rescue

The elements of the rescue medium affect embryo germination. Germination efficiency as affected by MS (Murashige and Skoog 1962), ½ MS (half strength MS) and B5 (Gamborg et al. 1968) media has been extensively tested (Suenaga and Nakajima 1989, Comeau et al. 1992, Cherkaoui et al. 2000, Dogramaci-Altun- tepe and Jauhar 2001). Cherkaoui et al. (2000) rescued excised embryos from 10 durum wheat cultivars crossed with eight maize genotypes on MS, ½ MS and B5 media and found that B5 and ½ MS media were more efficient than MS. The concentration of sucrose supplemented in the media is the major element affecting the germination of the rescued embryos. In our laboratory, we tested MS basal media supplemented with five different sucrose concentrations (0, 20, 50, 80 and 100.0 g/l) and found the maximum embryo germination rate was obtained at 50 g/l. We also tested embryo culture by the transplanted nurse endosperm method, in which the excised embryos were placed on the 20-day-old seed endosperm tissue and then cultured on the MS medium. This method was more efficient for the small embryos, but was more labour- and time-consuming compared with the regular embryo rescue method. Thus, it is more efficient to use both methods for the different size of embryos rescued, that is, the big embryos are directly cultured on MS medium, and the small embryos are cultured with transplanted nurse endosperm.

Another factor that is important in embryo rescue is knowing whether the seed has an embryo. To save time and energy, only seeds that are known to have embryos need to be rescued because, on average, only one-third of the seed carry embryos. Bains et al. (1998) developed a simple technique to distinguish between seed with and without embryos. They were able to detect 97.8% of the seed that contained embryos by placing immature seeds from wheat × maize crosses on a transparent surface illuminated from above.

Colchicine Treatment

A successful chromosome-doubling process is essential for the production of homozygous plants after haploid plants are derived from wheat or durum wheat hybridization with maize. Different doubling agents have been studied, such as caffeine (Thomas et al. 1997), nitrous oxide (Hansen et al. 1988), antimitotubule herbicides trifluralin or amiprophos-methyl (APM; Hansen and Andersen 1998); but the most commonly used chemical agent for chromosome doubling is colchicine, which disrupts mitosis by inhibiting formation of spindle fibres and disturbing normal polar chromosomal migration, resulting in chromosome doubling (Jensen 1974). Many factors affect the chromosome-doubling process, such as colchicine concentration, addition of other synthetic compounds, treatment temperature and length, the development stage of plants and growing conditions after colchicine treatment.

Colchicine treatment can be applied at different stages, from postpollination of the female plants to the tillering stages of the haploid plants (Jensen 1974, Thiebaut et al. 1979, Inagaki 1985, Sood et al. 2003). In cereal crops, such as wheat and barley, colchicine treatment is normally recommended at the 3- to 4-tiller stage for 5–8 h by submerging the whole root system in a colchicine solution containing 0.1% colchicine, 2% dimethyl sulfoxide (DMSO), 0.3 ml/l Tween 20 and 10 mg/l of gibberellic acid (GA3; Jensen 1974, Thiebaut et al. 1979, Inagaki 1985); and the doubling rate can reach 95.6% (Inagaki 1985). The colchicine treatment can be applied before the seedling stage of haploid plants by adding 0.5% colchicine to the rescue medium for 48 h. The colchicine solution (1% colchicine with 100 ppm 2,4-D) can also be injected into the uppermost internode of pollinated spikes at 48 and 72 h after pollination, and the chromosome-doubling rate varies from 33% to 100% (Sood et al. 2003). We found that the rate of embryo germination would be greatly reduced when adding colchicine to the rescue media or injecting colchicine to the internodes after pollination (unpublished data).

The higher colchicine concentration increases the doubling rate, but it also results in deformed plants, low survival rate and increased cost. The optimal colchicine treatment should have a high rate of embryo germination and a high plant survival rate, with a high rate of chromosome doubling. For wheat haploid chromosome doubling, we treated haploid plants at the 2- to 3-tiller stage with a solution containing 0.45 g/l colchicine, 20 ml/l DMSO, 100 mg/l GA3, 0.3 ml/l Tween 20 with a pH 5.5 for 6–8 h at 18–20°C in the dark. During the treatment, a gentle air flow into the colchicine solution supplied the roots with oxygen. After treatment, the plants were rinsed with running water overnight, transferred to soil and kept in a growth chamber at 14–16°C under 16/h day/night until the new tillers emerged. The plants were then moved to normal growing conditions in a greenhouse. The plant survival rate could reach 99% and the chromosome-doubling rate could reach 96–98% (unpublished data).

Mechanism of Uniparental Chromosome Elimination in the Wide Hybridization System

Various theories have been proposed to explain the mechanism of uniparental chromosome elimination in the wide hybridization system. However, there is no conclusive explanation for the actual process. Chromosome elimination could be caused by the difference in timing of mitotic processes (Gupta 1969), the genomic balance (Kasha and Kao 1970) and/or the failure of the chromosome to initiate or to complete either congregation at metaphase or migration to the poles at anaphase (Bennett et al. 1976). In the barley-H. bulbosum system, the genetic factors controlling genomic balance are located on barley chromosomes.
2H and 3H (Ho and Kasha 1975), and a gene controlling incompatibility between barley and H. bulbosum was located on barley chromosome 5H (Pickering 1983). Orton and Tai (1977) proposed that the spindle organizer mechanism played a role in chromosome elimination. They indicated that if the parental spindle organizers are genetically similar, normal chromosome behaviour will result; but, if the spindle organizers are genetically dissimilar, independent function occurs even within the same protoplast of an offspring. Each spindle organizer will then migrate and establish itself as a pole and attract its own chromosome. The net results will be multipolar cell division and the separation of different genomes into different daughter cells (Orton and Tai 1977). Sanie et al. (2011) studied the role of the centromere-specific histone H3 variant (CENH3) in the process of uniparental chromosome elimination in crosses of H. vulgare × Hordeum bulbosum. Four conclusions were drawn from their study: centromere inactivity of H. bulbosum chromosomes triggers uniparental chromosome elimination; centromeric loss of CENH3 protein causes centromere inactivity; not all CENH3 variants get incorporated into centromeres if multiple CENH3s are present; and ‘diploid barley species encode two CENH3 variants, the proteins of which are intermingled within centromeres throughout mitosis and meiosis’.

During hybrid embryo development in wheat × pearl millet crosses, all pearl millet chromosomes occupied peripheral interphase positions and were randomly eliminated between six and 23 days after pollination (Gernand et al. 2005). In hybrid embryos between wheat and maize, the maize chromosomes to be eliminated were peripherally located on the metaphase plates and lagged behind the wheat chromosomes at anaphase (Laurie and Bennett 1989). However, in the barley × H. bulbosum system, the H. bulbosum chromosomes did not directly undergo the distinct peripheral localization process, but they went through the nuclear extrusion and budding process (Kim et al. 2002). In the wheat × pearl millet system, Gernand et al. (2005) observed that the heterochromatinization and DNA fragmentation of micronucleated pearl millet chromatin were similar to the events of heterochromatinization and DNA fragmentation of micronucleated pearl millet chromatin were similar to the events of programmed cell death (Fukuda 2000). So, the plants could conserve the ability to distinguish host chromatin/DNA from foreign chromatin/DNA and eliminate the alien genome from the host genome. Specific chromatin topology and posttranslational histone modifications might be the key for the recognition and subsequent elimination of DNA, which might result in endonuclease activation and genome-specific fragmentation (Houben et al. 2011).

Outlines of a Highly Efficient Approach to Develop DHs in Wheat and Durum Wheat

Based on the factors affecting DH production via wheat × maize hybridization, we optimized the major factors to improve efficiency of DH production and developed a highly efficient protocol, which is outlined as follow:

1. Plant sweet corn (hybrid cultivar ‘Early Sunglow’) seed 10–14 days earlier than durum/common wheat F1 hybrids. Plant corn for 5–6 times with an interval of 5–7 days.
2. Plant durum/common wheat F1 seeds at the same time as the third corn planting.
3. During the durum/common wheat heading stage, emasculate the durum/common wheat spikes.
4. At 2–3 days after emasculations, collect fresh corn pollen (light yellow colour), and pollinate emasculated durum/common wheat spikes.
5. At 24 h after pollination, spray pollinated spikes with 2.4-D solution (213.05 mg/l, pH = 10.36).
6. At 14–16 days after treatment with 2.4-D, cut the spikes from the plants, and remove the seeds from the spikes for embryo rescue.
7. Sterilize the seeds with 70% ethanol for 1 min and then 20% Clorox (commercial solution) for 15 min, and rinse three times with sterilized distilled H2O.
8. Aseptically excise the embryos, and culture the big embryos directly on the MS basal medium with 50 g/l sucrose and 8% agar in Petri dishes. Culture the small embryos on the MS media with transplanted nursing endosperm. Keep the Petri dishes with embryos at room temperature (20–24°C) in the dark for 1–2 weeks.
9. After the embryos germinate, transfer the small seedlings to test tubes or jars containing ½ MS media with 30 g/l sucrose and 8 g/l agar. Keep them at 20–24°C, 16h (light/dark) h for about 2 weeks.
10. When the plants grow up to 5–6 cm, transfer them to 4-inch clay pots filled with soil and fertilize with slow-release fertilizer. Keep them in the growth chamber at 18–20°C 16h (day/night) h photoperiod.
11. When plants have grown to the 2–3 tiller stage, dig the plants from clay pots, wash the roots thoroughly and immerse the roots and crown parts of the plants in colchicine solution: colchicine (0.45 g/l) + DMSO (20 ml/l) + GA3 (100 mg/l) + Tween 80 (0.3 ml/l), pH = 5.5, at 20–22°C, dark for 8 h. During the treatment, provide a gentle air flow into the colchicine solution.
12. After the colchicine treatment, rinse the plants with running water overnight, and transfer them back to clay pots filled with soil and fertilized with slow-release fertilizer.
13. Keep the plants in a growth chamber (16h/day/night photoperiod at 14–16°C) for about 2 weeks until plants recover (new tillers grow out) and then move the plants to the greenhouse (16h/day/night photoperiod) at 20–24°C until maturity.

We observed that air flow during the colchicine treatment and the low temperature (14–16°C) condition after treatment greatly improve the survival of the colchicine-treated plants. Using this procedure, rates of survival and chromosome doubling of the treated haploid plants were over 90%. This protocol has been successfully used to develop mapping populations and adapted germplasm in our wheat germplasm enhancement programme and durum wheat breeding programme (Chu et al. 2008a, b, 2009, 2010a, b, 2011a, b, Gu et al. 2010). Twelve populations each consisting of over 120 DH lines have been developed to identify and map various genes for important agronomic traits such as gluten strength, seed dormancy (Gu et al. 2010), growth habit (Chu et al. 2011b) and resistance to various wheat diseases such as tan spot (Chu et al. 2008b, 2009, 2010a), leaf rust (Chu et al. 2009), Stagonospora nodorum blotch (Chu et al. 2010b, Friesen et al. 2012), Fusarium head blight (Chu et al. 2011a) and crown rust (Niu et al. 2013). In addition, we have provided this protocol to numerous wheat genetics and breeding research programmes.

Acknowledgements

Authors thank Stan S. Stancyk for the technical support during the development of the protocols and to Daryl L. Klindworth for revising the manuscript.
References


Inagaki, M. N., W. H. Pfeiffer, M. Mergoum, and A. Mujeeb-Kazi, 1996: Production of polyhaploids
Laurie, D. A., and M. D. Bennett, 1989: The timing of chromosome elimi-
Inagaki, M. N., and A. Mujeeb-Kazi, 1995: Comparison of polyhaploid
Lefebvre, D., and P. Devaux, 1996: Doubled haploids of wheat from
Kim, N. S., K. C. Armstrong, G. Fedak, K. Ho, and N. I. Park, 2002:
Kasha, K. J., and K. N. Kao, 1970: High frequency haploid production
Inagaki, M., and A. Mujeeb-Kazi, 1995: Factors affecting the frequencies of
Ho, K. M., and K. J. Kasha, 1975: Genetic control of chromosome elimi-
Kasha, K. J., and K. N. Kao, 1970: High frequency haploid production

Lein, A., 1943: Die geneticische Grundlage der Kreuzbarkeit zwischen
Li, D. W., J. W. Qio, P. Ouyang, Q. X. You, L. D. Dawli, Q. Jiwen, O.
Ping, and Y. Qingxiao, 1996: High frequency of fertilization and embryo
development for Langdon durum D-genome disomic substitution lines. Genome 49, 1545—1554.
Liu, W., Y. Zheng, E. Polle, and C. F. Konzak, 2002: Highly efficient
doubled-haploid production in wheat (*Triticum aestivum* L.) via
Maluszynski, M., 2003: Doubled Haploid Production in Crop Plants:
Martins-Lopes, P. F., H. Guedes-Pinto, O. Pinto-Carnide, and J. Snape, 2001: The effect of spikelet position on the success frequencies of
wheat haploid production using the maize cross system. Euphytica 121, 265—271.
Matiz, F., and A. Mahn, 1994: Improved techniques for haploid production
in wheat using chromosome elimination. Plant Breed. 113, 125—129.
S. Xu, and J. K. Ransonn, 2009: Spring wheat breeding. In: M. J. Ca-
Morshedi, A. R., N. L. Darvey, and K. Suenaga, 1996: The effects of
methods and the timing of 2,4-D application on seed set, embryo
formation and haploid production in wheat maize crosses. In: B. A. Rich-
ards, C. W. Writy, H. M. Rawson, G. J. Rebetzke, J. L. Davidson, and R. I. S. Brettell (eds), Proceedings of the 8th Assembly of the
Wheat Breeding Society of Australia. 100—103. Australian Wheat
Breeding Society, Canberra, ACT, Australia.
Murashige, T., and S. Skoog, 1962: A revised medium for rapid growth
and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473—497.
Niroula, R. K., and D. B. Thapa, 2009: Response of wheat genotypes to
Niu, Z., K. D. Puri, S. Chao, Y. Jin, B. B. Steffenson, S. X. Xu, and S.
Zhong, 2013: Genetic analysis and molecular mapping of crown rust
14052-013-2245-z.
O’Donoughue, L. S., and M. D. Bennett, 1994: Durum wheat haploid
Ohtawaya, Y., K. Suenaga, and T. Ogawa, 1992: Production of haploid
Orton, T. J., and W. Tai, 1977: Chromosome elimination in a complex
Pickering, R. A., 1983: The location of a gene for incompatibility involving
42, 206—209.
temperature on chromosome elimination during embryo development in crosses
involving *Hordeum spp.*, wheat (*Triticum aestivum* L.) and rye (*Secale
Newslett. 40, 40—42.
State University, Manhattan, KS.
Pienaar, R. D. V., M. Horn, and A. J. G. Lesch, 1997: A reliable proto-
85, 49.
Ravi, M., and S. W. L. Chan, 2010: Haploid plants produced by centro-

Riera-Lizarazu, O., A. Mujeeb-Kazi, and M. D. H. M. William, 1992: 
Maize (Zea mays L.) mediated polyhaploid production in some Triti-

Sadasivaiah, R. S., S. M. Perkovic, D. C. Pearson, B. Postman, and B. 
L. Beres, 2004: Registration of ‘AC Andrew’ wheat. Crop Sci. 44, 
696—697.

San Noeum, L. H., 1976: Haploides d’Hordeum vulgare L par culture in 

Sanie, M., R. Pickering, K. Kumke, S. Nasuda, and A. Houben, 2011: 
Loss of centromeric histone H3 (CENH3) from centromeres precedes 
uniparental chromosome elimination in interspecific barley hybrids. 
108. Available at: www.pnas.org/cgi/doi/10.1073/pnas.1103190108 
(last accessed on October 5, 2013).

Simmonds, J. S., 1989: Improved androgenesis of winter cultivars of 
Triticum aestivum L. in response to low temperature treatment of 

Singh, S., R. K. Behl, and M. S. Punia, 2001: Production of double 
haploids via maize pollination in wheat. Cereal Res. Commun. 29, 
289—296.

Sood, S., R. Dhawan, K. Singh, and N. S. Bains, 2003: Development of 
ovel chromosome doubling strategies for wheat × maize system of 
heat haploid production. Plant Breed. 122, 493—496.

Suenaga, K., and K. Nakajima, 1989: Efficient production of haploid 
wheat (Triticum aestivum) through crosses between Japanese wheat 
and maize (Zea mays). Plant Cell Rep. 8, 263—266.

Thiebaut, J., K. J. Kash, and A. Tsai, 1979: Influence of plant develop-
ment stage, temperature and plant hormones on chromosome doubling 

Thomas, J., Q. Chen, and N. Howes, 1997: Chromosome doubling of 
haploids of common wheat with caffeine. Genome 40, 552—558.

Thompson, K. F., 1972: Oil-seed rape. In Reports of the Plant Breeding 
Institute, 94—96. Cambridge University Press, Cambridge, UK.

Tuveissen, I. K. D., S. Pedersen, and S. B. Andersen, 1989: Nuclear 
genomes affecting albinism in wheat (Triticum aestivum L.) anther 

Wedzony, M., and A. A. M. Van Lammeren, 1996: Pollen tube growth 
and early embryogenesis in wheat maize crosses influenced by 2,4-D. 

development via unfertilized ovule culture in Doritis pulcherrima. 

plants from unpollinated ovaries and ovules. Theor. Appl. Genet. 63, 
97—104.

resources of wheat for genetic hybridization among Secale and Aegi-
lops. Int. Triticale Symp. 42—52.

Zenkteler, M., and W. Nitsche, 1984: Wide hybridization experiments 

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