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Review

Recent advances and application of doubled haploids in wheat breeding

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Genetic improvement to develop varieties with high yield potential and resistance/tolerance to abiotic and biotic stresses with acceptable end use qualities is the most viable and environment friendly option to increase wheat yield in a sustainable fashion. *In vitro* haploid production followed by chromosome doubling greatly enhances the production of complete homozygous wheat lines in a single generation and increases the precision and efficiency of selection process in wheat breeding. It also enables the detection of linkage and gene interactions, estimate genetic variance and the number of genes for quantitative characteristics, produce genetic translocations, substitutions and chromosome addition lines, and facilitate genetic transformation and mutation studies. Wheat cultivars developed from doubled haploids using anther-culture and maize induction systems have been released for cultivation in both developed and developing countries. In this review, the origin and production of haploids, techniques in anther-culture and wheat x maize wide crosses, and their application in wheat breeding are summarized.

Key words: Doubled haploid, wheat breeding, wheat yield.

INTRODUCTION

Wheat is the most widely grown cereal crop in the world and one of the central pillars of global food security. About 651 million tons of wheat was produced on 217 million hectares in 2010 with productivity level of 3 t/ha^{-1} (FAO, 2012). After the quantum leap of the Green Revolution, wheat yields have been rising by only 1.1% per year, a level that falls far short of the demand of a population that is growing by 1.5% or more annually. According to some estimates, the global wheat production must increase at least by 1.6% annually to meet a projected yearly wheat demand of 760 million tons by 2020 (Rosegrat et al., 2001).

Genetic improvement to develop varieties with high yield potential and resistance/tolerance to abiotic and biotic stresses with acceptable end use qualities is the most viable and environment friendly option to increase wheat yield in a sustainable fashion. Such improvement of crops requires creation and introduction of genetic variation, inbreeding coupled with selection and extensive evaluation of breeding materials at multi-locations to identify adapted and stable genotypes with desirable agronomic traits. Variations could be created by sexual crosses (usually single, three-way, double or back crosses) and mutation breeding. Breeders have used different methods to fix and develop homozygous genotypes from such variations. Isolation of homozygous and homogeneous genotypes through conventional inbreeding methods (single seed descent, backcrossing and selfing in the field, plus in some cases, the use of off-season nurseries and shuttle breeding approaches) requires several cycles of inbreeding and selection, making it the most tedious, time consuming and expensive phase of any breeding program. Furthermore, in conventional plant breeding, truly homozygous lines are rare and most selection contains some heterozygous loci (Baenziger and Peterson, 1992; Baenziger and

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DePauw, 2009).

Recent advances in plant tissue culture and its related disciplines opened an avenue that greatly facilitated the haplodiplodization breeding scheme, which enables the extraction of instant homozygous lines/varieties from crop plants with any degree of heterogeneity in a single generation (Baenziger and DePauw, 2009; Wu et al., 2012). Haploid individuals are sporophytes with gametic chromosome number (Riley, 1974; Ouyang et al., 1973) and by doubling the haploid chromosome complements doubled haploids (DH) can instantly be produced. In this review, the origin and production of haploids, techniques in anther-culture and wheat x maize wide crosses, and their application in wheat breeding are summarized.

DOUBLED HAPLOIDS: ORIGIN AND PRODUCTION

All the cultivated wheat belongs to the genus *Triticum*, which in turn is divided into three major taxonomic groups: einkorn, emmer and dinkel by Schultz (1913). This classification was supported by the pioneering cytological study of Sakamura (1918), who found that Schultz's three wheat groups also differ in their chromosome number; the einkorns are diploids (2n = 2x = 14), the emmers are tetraploids (2n = 4x = 28) and the dinkels are hexaploids (2n = 6x = 42), all with the basic chromosome number x = 7. Soon after, based on cytogenetic analysis, Kihara (1924) designated the genome formulae for the cultivated einkorn (*Triticum monococcum* L., 2n = 2x = 14), emmer (*Triticum turgidum* L. 2n = 4x= 28) and dinkel (*Triticum aestivum*, 2n = 6x = 42) as AA, AABB and AABBDD, respectively.

Each group of wheat forms their own respective haploids. Haploids are sporophytes that contain gametic chromosome numbers (n). The haploids from einkorn, emmer and dinkel posses n = x = 7, n = 2x = 14, and n = 3x = 21chromosomes with genomic constitution of A, AB and ABD, respectively (Quisenberry and Reitz, 1967; Fehr, 1993; Folling and Olesen, 2002). Haploids can originate spontaneously in nature or as a result of various induction techniques. Spontaneous development of haploid plants has been known since 1922, when Blakeslee first described this phenomenon in *Datura stramonium* (Blakeslee et al., 1922). However, spontaneous occurrence is a rare event and therefore of limited practical value. Doubled haploid (DH) is a genotype produced when haploid cells undergo the process of chromosome doubling (Ouyang et al., 1973; Picard and De Buyser, 1973). Doubled haploid production requires induction of haploids and doubling of chromosomes (Snape, 1989; Raina, 1997).

Haploid induction techniques

The potential of haploidy for plant breeding arose in 1964 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* and *Zea mays* (Barclay, 1975; Laurie and Bennett, 1986, 1988; Inagaki, 1997). Anther culture and wheat x maize wide cross systems are the two most commonly used induction methods in wheat.

Anther culture

The first success in regeneration of wheat (*T. aestivun*) plants through anther-culture was achieved in the early 1970s (Ouyang et al., 1973; Picard and De Buyser, 1973; Liang et al., 1987; Baenziger et al., 1989). Anther-culture exploits the fact that a certain proportion of pollen grains *in situ* are embryogenic. These pollen grains can develop into embryos only when they are placed on artificial medium. In anther culture systems, stress is of critical importance for blocking gametophytic development and for triggering pollen embryogenesis in competent microspores (Bueno et al., 1997; Touraev et al., 1997). The process of anther-culture begins in the selection of primary wheat spikes which contain anthers with pollen at the mid-late uninucleate stage of development (Ouyang et al., 1973; Picard and De Buyser, 1973; Liu et al., 2002). The stage of pollen development is very important as minor deviations can lead to major decreases in yield. Anthers are then aseptically dissected and cultured on induction medium such as CHB3, N6 and ½ MS, and incubated in darkness at 26 - 28°C for 4 to 6 weeks after which calli are transferred to a solid plant regeneration medium and incubated in a culture room with 25°C and 16 h day length for about 30 days. Green plantlets are transferred to culture tubes containing 20 ml of modified plantlet regeneration medium. After one to two months of hardening, vigorous seedlings were transplanted into pots with 2:1:1 soil : sand : peat : moss mixture and kept in a plastic house (Tawkaz, 2011).

Anther culture is used in many cereal breeding programmes and is more cost-effective than intergeneric crosses in the production of DHs (Snape et al*.,* 1986; Pratap et al., 2006). However, anther culture is highly dependent on genotypes (Wehr and Zeller, 1990; Pratap et al., 2006, Khiabani et al., 2008; Grauda et al., 2010; El-Hennawy et al., 2011; Tawkaz, 2011). The procedure of anther-culture in wheat is illustrated in Figure 1. To counter the genetic effect, components of the system such as growth environment of donor plants, modification of the medium components, changing the physical state of the medium (liquid or solid), cold pretreatment of anthers before culturing, increasing the incubation temperature during the first few days of culture and

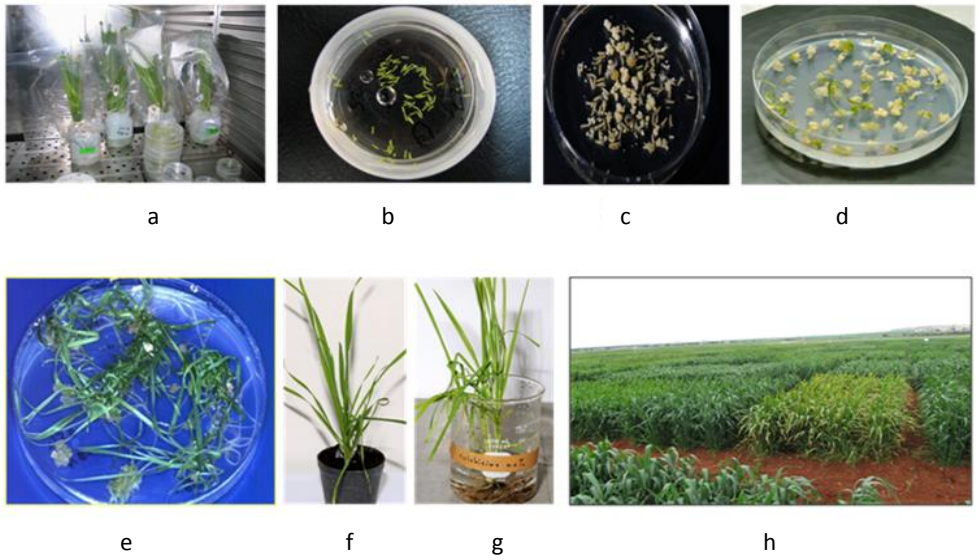

Figure 1. Procedures of anther-culture for doubled haploid wheat production: a = pre-treatment of the donor plants at 4°C; b = anthers in liquid induction medium; c = developing of embryos in liquid induction medium; d=embryo converting to green on solid regeneration medium; e= green plants in regeneration medium; f = haploid plants at acclimatization stage; g = haploid plants under colchicine treatment (0.2%); h = doubled haploid lines in the field showing uniformity within lines (Tawkaz, 2011).

subjecting anther explants to gamma irradiation have been investigated by different authors (Karimzadeh et al., 1995; Karsai et al., 1994; Xynias et al., 2001; Zamani et al., 2003; Shirdelmoghanloo et al., 2009; Tawkaz, 2011). Recently, Grauda et al. (2010) have reported increased DH production efficiency through the utilization of androgenic microspore culture (AMC) induction medium with copper. The positive influence of copper while obtaining DH plants in anther culture is expressed both in reducing the number of albino plants and in increased numbers of green plants-regenerants. These effects are related to improved survival of microspores during the different tissue culture stages and with the synchronization of the first microspore symmetric division (Wojnarowiez et al., 2002; Jacquard et al., 2009).

A further disadvantage of anther-culture in barley and wheat breeding is in the high rates of albino plants. In some bread wheat genotypes the frequency of albinism has been found to range from 20-50% with a mean of 30% of all regenerated plants (Abd El-Maksoud, 1992; Abd El-Maksoud et al., 1993; Tawkaz, 2011). Durum wheat (*T. turgidum* subsp. Durum), is very recalcitrant to anther-culture with low regeneration rate and very high frequency of albino plants (Cistué et al., 2006, 2009; Labbani et al., 2007). In an effort to improve this problem in durum wheat, Ayed et al. (2010) have applied different pre-treatments and found that cold treatment for five weeks (4°C) improved significantly the embryogenesis induction and green plant regeneration with green : albino plant ratio of about 75%. Mannitol pretreatment has also be found to be effective in improving the efficiency of anther-culture in durum wheat (Labbani et al., 2007; Ayed et al., 2010). Previously, Bueno et al. (1997) have reported the significant effect of starvation and heat shock on successful production of haploid plants in cork oak.

Wheat x maize wide cross method of haploid induction

Wide crosses have been utilized for the production of haploids for crop improvement and genetic studies (Baum et al., 1992). Bread wheat DH are produced by various intergeneric crosses viz., wheat x maize (Laurie and Reymondie, 1991; Inagaki and Mujeeb-Kazi, 1995; Inagaki, 2003) wheat x pearl millet (Inagaki and Mujeeb-Kazi, 1995; Amin et al., 2010), wheat x teosinte (Suenaga et al., 1997), wheat x barely (Barclay, 1975) and wheat x sorghum (Inagaki and Mujeeb-Kazi, 1995). The crossability of *T. aestivum* × *Hordeum bulbosum* depends on the wheat allelic composition for the Kr genes responsible for the incompatibility between these two species (Sitch and Snape, 1987). Production of haploids in wheat through wheat x maize crossing was reportedsuccessful without the development of albino plants (Sadasivaiah et al., 1999; Ushiyama et al., 2007) and insensitivity of maize to the action of crossability inhibitor (Kr) genes. Several reports demonstrated the success of doubled haploid plant production using maize pollen on hexaploid wheat (Suenga and Nakajima, 1989; Amrani et al., 1993; Niroula et al., 2007) but relatively few durum wheat genotypes show such crossability with

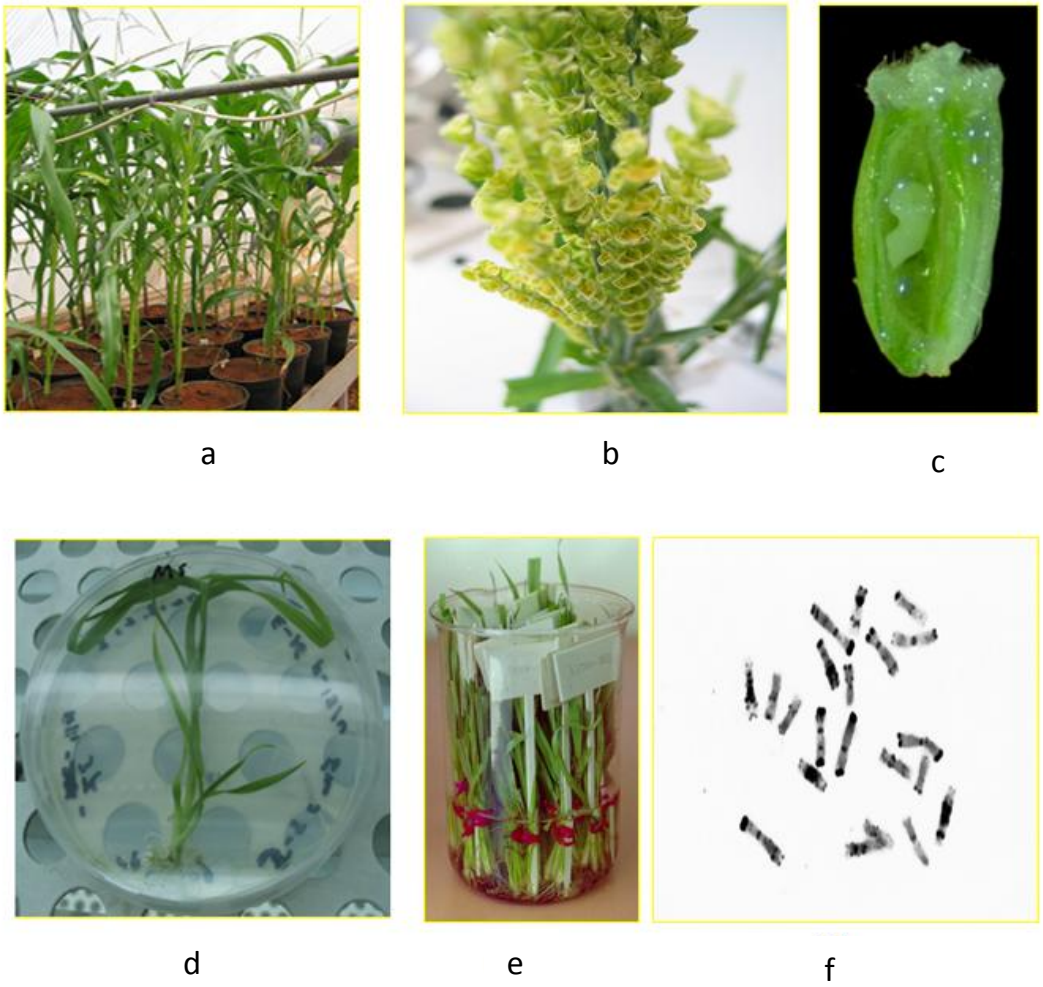

Figure 2. Procedures of wheat x maize wide cross method for doubled haploid wheat production: a = growing maize plants; b= pollinated spikes with maize pollen; c =obtained seed from crosses with maize; d = plant developing from rescued embryo; e = haploid plants under colchicine treatment (0.2%); f = chromosomes counting (Inagaki, 2003).

maize (Almouslem et al., 1998; David et al., 1999; Garcia-Llamas et al., 2004). Recently, Sourour et al. (2012) have reported the production of successful DH durum wheat genotypes using the wheat x maize system.

In wheat x maize system, the culture of wheat donor plants is carried out in climate chambers and in the greenhouse. One to two days before anthesis, the wheat florets are emasculated and two days later they are pollinated with fresh maize pollen. Cryopreserved (-80°C) maize pollen can also be successfully utilized for pollination of wheat florets. However, the efficiency of haploid formation was quite low as compared to fresh pollen (Inagaki, 1997). But this technique is quite advantageous where synchronization is a problem. Once the pollination is complete, the application of auxin is essential for the successful recovery of haploid wheat embryos (Suenga and Nakajima, 1989; Niroula et al., 2007). Recently, frequency of polyhaploid embryo formation has greatly improved through the manipulation of Dicamba alone or in combination with 2,4-dichlorophenoxyacetic acid (2,4-D) (Almouslem et al., 1998; O'Donoughue and Bennett, 1994; Garcia-Llamas et al., 2004; Ahmad and Chowdhry, 2005). Embryo rescue is essential to recover haploid plantlets from wheat x maize system (Sood et al., 2003; Suenga and Nakajima, 1989; Niroula et al., 2007; Zhang et al., 1996; Niroula and Bimb, 2009) after 14 to 20 days of post pollination. Excised embryos can be cultured on either full strength MS (Murshige and Skoog, 1962) or ½ MS or B5 basal medium (Gamborg et al., 1968) containing various modifications of organic supplements (Zenkler and Nitzsche, 1984; Zhang et al., 1996; Suenga et al., 1997; Campbell et al., 2000; Singh et al., 2004; Ayed et al., 2011) and can be grown *in vitro* for 3 to 5 weeks at 20 to 25°C and 16 h day length. Generally, seedlings are ready for transfer up to that period and need to be hardened for one week in growth chamber at the same environmental regime. Figure 2 illustrates the wheat x maize haploid

production system.

Chromosome doubling

Haploid plants are infertile as sexual fertility depends upon meiotic division of the diploid chromosome number. Spontaneous rates of chromosome doubling among plants derived from microspores of wheat are relatively low. In many experiments, only 15 to 20% of the plants obtained are capable of seed set by selfing without a treatment for chromosome doubling (Hansen et al., 1988; Navarro-Alvarez et al., 1994; Ouyang et al., 1994). To regain fertility, the number of wheat chromosomes need to be doubled following haploid embryo rescue and seedling formation. Colchicine is the most frequently used drug for chromosome doubling in plants (Ouyang et al., 1994; Soriano et al., 2007). The drug inhibits spindle function during mitosis and disturbs normal polar segregation of sister chromatids to form a restitution nucleus. Upon mitotic divisions of such affected cells, chromosome doubled chimera sectors are formed, which lead to partial fertility of the plant if they comprise sexual organs. Colchicine is traditionally administered to the young plants established in soil at the 3 to 5 tillering stages.

For the treatment of colchicine, the procedure proposed by Inagaki (1997) is very efficient. According to his method, roots of the haploid seedling are pruned leaving a zone of 2 to 3 cm and submerged in a 0.1% colchicine solution supplemented with 2% dimethyl sulfoxide and ca. 0.05% Tween 20 at 20°C for 5 h. After this treatment, the roots are washed free of residual colchicine and potted in peat soil. In a large scale production of doubled haploids, the colchicine treatment of individual plants after establishment in soil is expensive both in terms of chemical and labour costs. It causes high mortality rate and production of mixoploids or chimeric plants that leads to low seed production and therefore, an additional growth cycle for seed multiplication before evaluation in the field (Chen et al., 1994; Islam, 2010). Chromosome doubling techniques directly integrated into the haploid induction procedures may have a potential for more cost efficient chromosome doubling of haploids for future wheat breeding. Different methods for *in vitro* chromosome doubling of wheat have been proposed. Colchicine has been added directly to the anther culture induction medium at concentrations of about 0.2 g/l (500 _M). Anthers were transferred to colchicine free medium after 72 h, resulting in a frequency of fertile plants up to about 70% (Barnabas et al., 1991; Navarro-Alvarez et al., 1994). Alternatively, Ouyang et al. (1994) cultured pollen calli on colchicine containing medium during regeneration and reported an average increase in the frequency of fertile plants from 17% in the control to 54% with the best *in vitro* treatment. Recently, however, it has been reported that high concentrations of colchicine have a toxic effect that reduces the number of embryos derived from the microspore culture (Barnabas et al., 1991; Navarro-Alvarez et al., 1994). Furthermore, the early chromosome doubling event may lead to a possible increased frequency of aneuploids among *in vitro* chromosome doubled wheat haploids.

Several microtubule depolymerising herbicides were also proved to be efficient for *in vitro* chromosome doubling of microspores. Zhao and Simmonds (1995) tested trifluralin and Hansen and Andersen (1996) trifluralin, oryzalin and amiprophos methyl in rapeseed. They achieved the mean rate of diploidization of 60 to 65%, which is comparable with the application of colchicine. Most recently, Pintos et al. (2007, 2010) have reported other effective antimitotic agents such as charcoal and amino acid treatments in cork oak.

STABILITY AND AGRONOMIC PERFORMANCE OF DOUBLED HAPLOIDS

To be used in a breeding programme, DH plants have to be genetically stable with no aberrant genetic variation arising during the process. Therefore, it is important to determine if any genetic variation is introduced during the production of DH lines. Very limited studies have been conducted on this line. Suenaga and Nakajima (1993) evaluated 110 wheat DH lines derived from wheat x maize crosses and found that 15 DH lines were variable for 2 traits like extreme dwarfism, low seed fertility, alteration of spike type and strips. Similarly, Kammholz et al. (1998) also found that expected normal segregation pattern for 6 glutenin loci across the 7 crosses indicated that wheat x maize system is stable across the generations. Laurie and Snape (1990) assessed the agronomic performance of wheat doubled haploid lines derived from wheat x maize crosses. They compared the performance of various DH lines of Chinese Spring, Hope, and lines of a single chromosome substitution of Chinese Spring and their respective parents under field conditions. No significant variation was detected in either population of Chinese Spring DH lines and neither population differed significantly from its parent.

Kisana et al. (1993), compared wheat DHs produced through anther-culture and wheat x maize crosses, and found that anther-derived plants were cytologically unstable, whereas all the plants regenerated from wheat x maize crosses were stable. In contrast, in other experiments, where the extent of variation from intergeneric cross methods and anther culture methods were compared, there were no significant differences in agronomic characters between the methods of DH production (Henry et al., 1988; Bjomstad et al., 1993). In studies of comparing the agronomic performance of best wheat genotypes selected through DH, single seed descent (SSD) and pedigree methods, no significant differences in grain yield were found among any of the

populations when the parental varieties were closely related in their pedigrees (Abd et al., 1993; Inagaki et al., 1998). In two crosses with low coefficients of parentage and large progeny variation, grain yield of selected DH lines was significantly lower than grain yield of SSD and pedigree selected lines (Inagaki et al., 1998). Recently, El-Hennawy et al. (2011) have evaluated the agronomic performance of anther-culture derived doubled haploid wheat genotypes and identified genotypes which are highly stable and superior to the best checks in grain yield performance.

FACTORS TO BE CONSIDERED IN DOUBLED HAPLOID WHEAT BREEDING

For successful implementation of doubled haploid wheat breeding program, different factors such as the filial generation from which doubled haploids are made, the population size and the comparative advantage of DH with other conventional breeding methods need to be determined. Conventionally, in an effort to shorten the breeding cycle, most breeders prefer to produce DH from the F_1 generation. Inducing homozygosity at such an earlier stage may limit the opportunity for recombination events which create potentially useful genetic variation for breeders. Snape and Simpson (1981) examined the theoretical and practical effects of linkage on traits of DH lines derived from F_1, F_2, F_3 and intermated F_2 (S3) generations of barley, and found a significant gain in the genetic variation for spike emergence time, height, grain number/spike and spikes/plant from delaying the production of DHs to the F_2 generation due to the breakup of repulsion linkages and creation of new allelic configurations at unlinked loci (Snape and Simpson, 1981; Choo et al., 1985; Patel et al., 1985; Yonezawa et al., 1987). In contrast, Iyamabo and Hayes (1995) quantified the effects of an additional round of recombination when comparing F_1 and F_2 derived barley DH lines and showed that the additional round of recombination did not lead to large performance differences between the two populations.

Population size plays an important role in the success of any breeding program. Theoretically, if a hybrid has *n* pairs of independently segregating genes, the chance to select a particular homozygous genotype from the F_2 population in a conventional breeding program is $(1/2)^{2n}$, whereas in haploid breeding, it is $(1/2)^n$ (Chu, 1982). Thus, the selection efficiency in haploid breeding is *2n* times better than the conventional methods. Single seed descent (SSD) is similar to the doubled haploid method in that both methods provide rapid generation advancement for producing homozygous lines (Grafius, 1965). In doubled haploid breeding, there is only one opportunity for recombination if F_1 plants are used as donors, while in SSD, recombination can occur in every generation of inbreeding (Grafius, 1965). Minimum population sizes depends on how many unlinked loci were to be fixed, for example, to be sure of fixing two unlinked loci, 16 DHs would be needed. To obtain five unlinked loci, a DH population of 203 would be needed. In the presence of linkage, minimum population sizes would have to increase (Yonezawa et al., 1987; Jansen, 1992) or alternatively, the production of DHs could be delayed in a generation. In general, to improve quantitatively inherited traits such as yield, it is highly important to increase the population size by nominating limited number of elite x elite crosses for DH production.

APPLICATION OF DOUBLED HAPLOIDS

The induction and regeneration of haploids followed by spontaneous or induced doubling of chromosomes are widely used techniques in advanced breeding programs of several agricultural species. In traditional plant breeding, it generally takes at least five generations after crossing before a sufficiently homozygous population is obtained, while DH produces a homozygous plant in one generation. Therefore, DH technology dramatically increases the speed of the inbred developmental processes by reducing several time-intensive generations of inbreeding and by making phenotyping and genotyping more predictive. Doubled haploid methods have been employed in wheat breeding programmes, and new wheat cultivars of DH origin have already been released in many countries such as China, France, Hungary and Canada (Hu et al., 1983; DeBuyser et al., 1987; DePauw et al., 2011). Wheat DH populations have been used also in the creation of molecular marker maps and quantative trait locus (QTL) identifications (Chauhan and Khurana, 2011; Wu et al., 2012).

Due to complete homozygosity, the efficiency of selection for both qualitative and quantitative characters is increased since recessive alleles are fixed in one generation and directly expressed. Additionally, doubled haploids can be used in a recurrent selection scheme in which superior doubled haploids of one cycle represent parents for hybridization for the next cycle. Several cycles of crossing, doubled haploid production and selection are performed and gradual improvement of lines is expected due to the alternation of recombination and selection. Barkley and Chumley (2011) have demonstrated the advantages of a doubled haploid laboratory for Kansas wheat breeding program using economic model analysis (Figure 3). The graph is drawn assuming that the rate of change in yield potential is 150% greater with the use of DH, relative to the baseline scenario of the conventional breeding program. If a DH laboratory were be to built in 2011, a new variety could be released seven years later in 2018 with increased yield potential and/or the same level of genetic potential as varieties released by the conventional breeding program four years later in 2022. As indicated in Figure 3, the large discrete change in

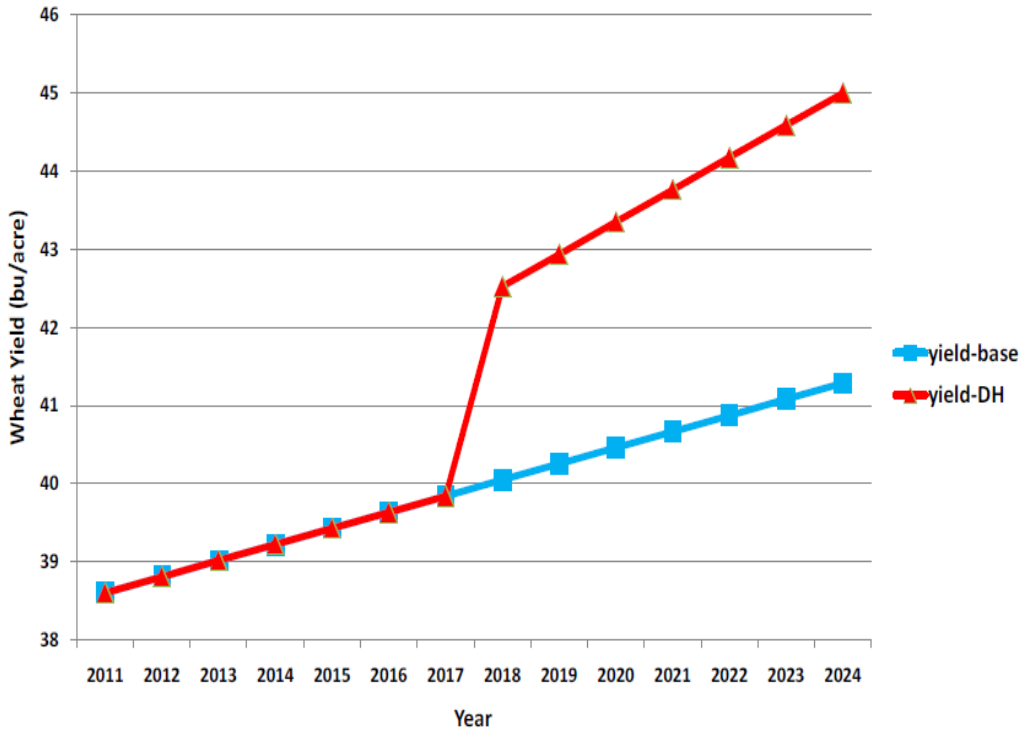

Figure 3. Comparison of conventional and DH wheat variety development (Barkley and Chumley, 2011).

2017 reflects the advantage due to the gain in time and the steeper slope trend indicates the enhanced rate of genetic gain.

DH systems are also the method of choice for mutant selection, due to the ease of selection and fixation of mutations and the desired recombinants, especially when quantitative traits are concerned (DePauw et al., 2011; Wu et al., 2012). Haploid technology has tremendous use for accelerating breeding technologies when combined with marker assisted selection (MAS). MAS, when combined with double haploidy, is a time saving method of performing backcross conversion to select an elite line with a particular trait. By combining molecular markers and DHs, it is possible to stack resistance genes. DH populations can be used as permanent mapping populations because they are stable and constant.

CONCLUSIONS

Doubled haploid breeding is developing more and more into a core technology in crop improvement. The DH technology platform offers a rapid mode of truly homozygous line production that helps to expedite crop breeding programs where homogeneity is an absolutely essential parameter for rapid crop development. Integration of the haploidy technology with other available biotechnological tools such as MAS, induced mutagenesis, and transgene technologies could also effectively expedite the wheat improvement programs. Direct incorporation of cloned genes at the haploid level following subsequent chromosome doubling may help accelerate stable integration of target gene(s) into wheat. Considering the cost and the fact that over-usage of doubled haploidy may reduce genetic variation in breeding germplasm, it is advisable to use DH for very limited and highly desirable crosses in wheat breeding program.

REFERENCES

Abd El-Maksoud MM, Bedö Z (1992). Half-diallel analysis of different characters in wheat anther culture. Acta Agr Hung 41:235-242.

Abd El-Maksoud MM, Karsai I, Bedö 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 42:377-382.

Ahmad J, Chowdhry MA (2005). The efficiency of chromosome doubling in haploids derived from wheat x maize crossing. Pak. J. Biol. Sci. 8:1707-1711.

Almouslem AB, Jauhar PP, Peterson TS, Bommineni VR, Rao MB (1998). Haploid durum wheat production via hybridization with maize. Crop Sci. Soc. Am. 38:1080-1087

Amin AY, Safwat G, El-Emary G (2010). Development of doubled haploid wheat genotypes using chromosome eliminating technique and assessment under salt stress. J. Am. Sci. 6:139-148.

Amrani N, Sarrafi A, Allibert G (1993). Genetic variability for haploid production in crosses between tetraploid and hexaploid wheats with maize. Plant Breed. 110:123-128.

Ayed OS, De Buyser J, Picard E, Trifa1Y, Amara HS (2010). Effect of pre-treatment on isolated microspores culture ability in durum wheat

(*Triticum turgidum* subsp. Durum Desf.). J. Plant Breed. Crop Sci. 2:30-38.
Ayed S, Slama-Ayed O, Teixeira da SJA, Slim-Amara H (2011). Effect of different factors on haploid production through embryo rescue in durum wheat × maize crosses. Int. J. Plant Breed. 5:118-121.
Baenziger PS, DePauw RM (2009). Wheat breeding: Procedures and strategies. In: B.F. Carver (ed.) Wheat: Science and Trade. Wiley-Blackwell Publishing, Ames, IA.
Baenziger PS, Peterson CJ (1992). Genetic variation: its origin and use for breeding self pollinated species. pp: 69-92. *In:* Stalkar TM, Murphy JP, (eds.), Plant breeding in the 1990s. March, 1991, Raleigh, North Carolina.
Baenziger PS, Wesenberg DM, Smail VM, Alexander,WL, Schaeffer GW (1989). Agronomic performance of wheat doubled haploid lines derived from cultivars by anther culture. Plant Breed 103:101-109.
Barclay IR (1975). High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature 256:410-411.
Barkley A, Chumley FG (2011). A doubled haploid laboratory for Kansas wheat breeding: an economic analysis of biotechnology adoption. pp. 1-40. Manhattan, Kansas.
Barnabas B, Phaler PL, Kovacs G (1991). Direct effect of colchicine on the microspore embryogenesis to produce dihaploid plants in wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 81:675-678.
Baum M, Lagudah ES, Appels R (1992). Wide crosses in cereals. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43:117-143.
Blakeslee AF, Belling J, Farhnam ME, Bergner AD (1922). A haploid mutant in the Jimson weed, *Datura stramonium*. Science 55:646-647.
Bueno MA, Gomez A, Boscaiu M, Manzanera JA, Vicente O (1997). Stress-induced formation of haploid plants through anther culture in cork oak (*Quercus suber*). Physiologia Plantarum 99:335-341.
Campbell AW, Griffin WB, Burritt DJ, Conner AJ (2000). The effects of temperature and \ light intensity on embryo numbers in wheat doubled haploid production through wheat x maize crosses. New Zealand J. Crop Hortic. Sci. 28:185-194.
Chauhan H, Khurana P (2011). Use of doubled haploid technology for development of stable drought tolerant bread wheat (*Triticum aestivum* L.) transgenics. Plant Biotechnol. J. 9:408-417.
Chen ZZ, Snyder S, Fan ZG, Loh WH (1994). Efficient production of doubled haploid plants through chromosome doubling of isolated microspores in *Brassica napus*. Plant Breed 113:217-221.
Choo TM, Reinbergs E, Kasha KJ (1985). Use of haploids in breeding barley. Plant Breed. Rev. 3:219-261.
Chu C (1982). Haploids in plant improvement. In: plant improvement and somatic cell genetics. I.K. Vasil, W.R. Scowcroft and K.J. Frey (eds.). Academic Press Inc. pp.129-157.
Cistué L, Romagosa I, Batlle F, Echávarri B (2009). Improvements in the production of doubled haploids in durum wheat (*Triticum turgidum* L.) through isolated microspore culture. Plant Cell Reports 28(5):727-735.
Cistué L, Soriano M, Castillo AM, Vallés MP, Sanz JM, Echàvarri B (2006). Production of doubled haploids in durum wheat (*Triticum turgidum* L.) through isolated microspore. Plant Cell. Rep. 25:257-264.
David JL, Dusautoir JC, Raynauld C, Roumet P (1999). Heritable variation in the ability to produce haploid embryos via pollination with maize and embryo rescue in durum wheat. Genome 42:338-342.
DeBuyser J, Lonnet P, Hertzoc R, Hespel A (1987). "Florin": doubled haploid wheat variety developed by the anther culture method. Plant Breed. 98:53-56.
DePauw RM, Knox RE, Humphreys DG, Thomas JB, Fox SL, P.D. Brown PD, Singh AK, Pozniak C, Randhawa HS, Fowler DB, Graf RJ, Hucl P (2011). New breeding tools impact Canadian commercial farmer fields. Czech J. Genet. Plant Breed. 47:28-34.
El-Hennawy MA, Abdalla AF, Shafey SA, Al-Ashkar IM (2011). Production of doubled haploid wheat lines (*Triticum aestivum* L.) using anther culture technique. Ann. Agric. Sci. 56:63-72.
FAO (2012). FAOSTAT agriculture data. Agricultural production 2009. Available at http://faostat.fao.org (accessed 22 April 2012). FAO, Rome, Italy.
Fehr WR (1993). Principles of Cultivar Development: Theory and Technique. Vol. 1. Macmillan Publishing Company, USA.
Folling L, Olesen A (2002). Transformation of wheat (*Triticum aestivum* L.) microspore-derived callus and microspores by particle bombardment. Plant Cell Rep. 20:629-636.
Gamborg OL, Miller RA, Ojima K (1968). Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:151-158.
Garcia-Llamas C, Ramirez MC, Ballesteros J (2004). Effect of pollinator on haploid production in durum wheat crossed with maize and pearl millet. Plant Breed. 123:201-203.
Grafius JE (1965). Short cuts in plant breeding. Crop Sci. 5:337.
Grauda D, Lepse N, Strazdiņa V, Kokina I, Lapiņa L, Miķelsone1 A, Ļubinskis L, Rashal I (2010). Obtaining of doubled haploid lines by anther culture method for the Latvian wheat breeding. Agron. Res. 8:545-552.
Guha S, Maheshwari SC (1964). *In vitro* production of embryos from anther of *Datura*. Nature 204:497.
Hansen FL, Andersen SB, Due IK, Olesen A (1988). Nitrous oxide as a possible alternative agent for chromosome doubling of wheat haploids. Plant Sci. 54:219-222.
Hansen NJP, Andersen SB (1996). *In vitro* chromosome doubling potential of colchicine, oryzalin, trifluralin, and APM in *Brassica napus* microspore culture. Euphytica 88:156-164.
Hu D, Tang Y, Yuan Z, Wang J (1983). The induction of pollen sporophytes of winter wheat and the development of the new variety Jinghua No. 1. Sci. Agric. Sin. 1:29-35.
Inagaki MN (1997). Technical advances in wheat haploid production using ultra-wide crosses. JIRCAS J. 4:51-62.
Inagaki MN (2003). Doubled haploid production in wheat through wide hybridization. In "Doubled haploid production in crop plants. A manual", Maluszynski, M., Kasha, K.J., Forster, B.P., Szarejko, I. (eds.), Kluwer Academic Publishers, Dordrecht, pp. 53-58.
Inagaki MN, 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.
Inagaki MN, Varughese G, Rajaram S, van Ginkel M, Mujeeb-Kazi A (1998). Comparison of bread wheat lines selected by doubled haploid, single-seed descent and pedigree selection methods. Theor. Appl. Genet. 97:550-556.
Islam SMS (2010). The effect of colchicine pretreatment on isolated microspore culture of wheat (*Triticum aestivum* L.). Aust. J. Crop Sci. 4:660-665.
Iyamabo OE, Hayes PM (1995). Effects of selection and opportunities for recombination in doubled-haploid populations of barley *(Hordeum vulgare* L.). Plant Breed. 114:131-136.
Jacquard C, Nolin F, Hécart C, Grauda D, Rashal I, Dhondt-Cordelier S, Sangwan RS, Devaux P, Mazeyrat-Gourbeyre F, Clément C (2009). Microspore embryogenesis and programmed cell death in barley: effects of copper on albinism in recalcitrant cultivars. Plant Cell Rep. 28:1329-1339.
Jansen RC (1992). On the selection for specific genes in doubled haploids. Heredity 69:92-95.
Kammholz SJ, Grams RA, Banks PM, Sutherland MW (1998). Segregation of glutenins in wheat x maize-derived doubled haploid wheat populations. Aust. J. Agric. Res. 49:1253-1259.
Karimzadeh G, G. Kovács G, Barnabás B (1995). Effects of cold treatment and different culture media on the androgenic capacity of two winter wheat genotypes. Cereal Res. Commun. 23:223-227.
Karsai I, Bedö Z, Hayes PM (1994). Effect of induction medium pH and maltose concentration on *in vitro* androgenesis of hexaploid winter triticale and wheat. Plant Cell Tissue Organ. Cult. 39:49-53.
Khiabani BN, Vedadi C, Rahmani E, Shalmani M (2008). Response of some Iranian wheat genotypes to anther culture system. Indian J. Biotechnol. 7:531-535.
Kihara H (1924). Cytologische und genetische Studien bei wichtigen Getreidearten mit besonderer Rücksicht auf das Verhalten der Chromosomen und die Sterilität in den Bastarden. Mem. Cell Sci. Kyoto Imp. Univ. Ser. B1:1-200.
Kisana NS, Nkongolo KK, Quick JS, Johnson DL (1993). Production of doubled haploids by anther culture and wheat x maize method in a wheat breeding programme. Plant Breed. 110:96-102.
Labbani Z, De Buyser J, Picard E (2007). Effect of mannitol pretreatment to improve green plant regeneration on isolated microspore culture in *Triticum turgidum* ssp. *durum* cv. Plant Breed. 126:565-568.

Laurie DA, Bennett MD (1986). Wheat x maize hybridization. Can. J. Genet. Cytol. 28:313-316.
Laurie DA, Bennett MD (1988). Chromosome behaviour in wheat x maize, wheat x sorghum crosses. Proceeding of Kew chromosome Conference. 3:167-177.
Laurie DA, Reymondie S (1991). High frequencies of fertilization and haploid seedling production in crosses between commercial hexaploid wheat varieties and maize. Plant Breed. 106:182-189.
Laurie DA, Snape JW (1990). The agronomic performances of wheat doubled haploid lines derived from wheat × maize crosses. Theor. Appl. Genet. 79:813-816.
Liang GH, Xu A, Tang H (1987). Direct generation of wheat haploids via anther culture. Crop Sci. 27:336-339.
Liu W, Ming YZ, Polle AE, Konzak CF (2002). Highly efficient doubled-haploid production in wheat (*Triticum aestivum* L.) via induced microspore embryogenesis. Crop Sci. 42:686-692.
Navarro Alvarez W, Baenziger PS, Eskridge KM, Hugo M, Gustafson VD (1994). Addition of colchicine to wheat anther culture media to increase doubled haploid plant production. Plant Breed. 112:192-198.
Niroula RJ, Bimb P (2009). Overview of wheat x maize system of crosses for dihaploid induction in wheat. World Appl. Sci. J. 7:1037-1045.
Niroula, RK, Thapa DB, Bimb HP, Sah BP, Nayak S, (2007). Production of haploid wheat plant from wheat (*Triticum aestivum* L.) x maize (*Zea mays* L.) cross system. Himalayan J. Sci. 5:47-52.
Nistch JP (1969). Experimental androgenesis in *Nicotiana*. Phytomorp. 19:389-404.
O'Donoughue LS, Bennett MD (1994). Durum wheat haploid production using maize-wide crossing. Theor. Appl. Genet. 89:559-566.
Ouyang JW, Liang H, Jia SE, Zhang C, Zhao TH, He LZ, Jia X (1994). Studies on the chromosome doubling of wheat pollen plants. Plant Sci. 98:209-214.
Ouyang YW, Hu CC,. Chuang CC, Tseng CC (1973). Induction of pollen plants from anthers of *Triticum aestivum* L. cultured *in vitro*. Sci. Sin 16:79-95.
Patel JD, Reinbergs E, Fejer SO (1985). Recurrent selection in doubled-haploid populations of barley (*Hordeum vulgare* L.). Can. J. Genet. Cytol. 27:172-177.
Picard E, de Buyser J (1973). Obtention de plantes haploides de *Triticum aestivum* L. a partir de cultures d'anthères *in vitro*. CR Academie des Sciences, Paris, 277:1463-1466.
Pintos B, Manzanera JA, Bueno MA (2007). Antimitotic agents increase the production of doubled-haploid embryos from cork oak anther culture. J. Plant Physiol. 164:1595-1604.
Pintos B, Manzanera JA, Bueno MA, (2010). Oak somatic and gametic embryos maturation is affected by charcoal and specific aminoacids mixture. Ann. For. Sci. 67:205.
Pratap A, Gurdeep SS, Harinder K, Chaudhary (2006). Relative efficiency of anther culture and chromosome elimination techniques for haploid induction in triticale × wheat and triticale × triticale hybrids. Euphytica 150:339-345.
Quisenberry KS, Reitz LP (1967). Wheat and wheat improvement. American Society of Agronomy, Inc. Madison, US.
Raina SK (1997). Doubled haploid breeding in cereals. Plant Breed. Rev. 15:141-186.
Riley R (1974). The status of haploid research, p: 3. *In*: K.J. Kasha (ed.), Haploids in Higher Plants: Advances and Potential. Proceeding of the first international symposium, June 10-14, 1974, Univ. Guelph, Canada.
Sadasivaiah RS, Orshinsky BR, Korzub GC (1999). Production of wheat haploids using anther culture and wheat x maize hybridization technique. Cereal Res. Commun. 27:33-40.
Sakamura T (1918). Kurze Mitteilung über die Chromosomenzahlen und die Verwandtschaftsverhältnisse der *Triticum*-Arten. Bot. Mag. Tokyo 32:151-154.
Schultz A (1913). Die Geschichte der kultivierten Getreide. Nebert, Halle. pp. 1-2
Shirdelmoghanloo H, Moieni A, Mousavi (2009). Effects of embryo induction media and pretreatments in isolated microspore culture of hexaploid wheat (*Triticum aestivum* L. cv. Falat). Afr. J. Biotechnol. 8:6134-6140.
Singh S, Sethi GS, Chaudhary HK (2004). Different responsiveness of winter and spring wheat genotypes to maize-mediated production of haploids. Cereal Res. Commun. 32:201-207
Sitch LA, Snape JW (1987). Factors affecting haploid production in wheat using the *Hordeum bulbosum* system. 1. Genotype and environmental effect on pollen grain germination, pollen tube growth and frequency of fertilization. Euphytica 36:483-496.
Snape JW (1989). Dihaploid breeding: Theoretical basis and practical applications. pp: 20-29. *In*: Mujeeb-Kazi A, Sitch LA, (eds.). Review of advances in plant biotechnolo*gy*, 1985-88. Second International Symposium on Genetic Manipulation in Crops, CIMMYT and IRRI.
Snape JW, Simpson E (1981). The genetical expectations of doubled haploid lines derived from different filial generations. Theor. Appl. Genet. 60:123-128.
Snape JW, Simpson Parker BBE (1986). Criteria for the selection and use of dihaploid systems in cereal breeding programmes, pp: 217-229. *In*: Horn W, Jensen CJ, Odenbach W, Schieder (eds.) O. Genetic manipulation in plant breeding. Walter de Gruyter, Berlin.
Sood S, Dhawan R, Singh K, Bains NS (2003). Development of novel chromosome doubling strategies for wheat x maize system of wheat haploid production. Plant Breed. 122:493-496.
Soriano M Cistue, L, Valle´s MP, Castillo AM (2007). Effects of colchicine on anther and microspore culture of bread wheat (*Triticum aestivum* L.) Plant Cell Tiss. Organ Cult. 91:225-234.
Sourour A, Zoubeir C, Ons T, Youssef T, Hajer S (2012). Performance of durum wheat (*Triticum durum* L.) doubled haploids derived from durum wheat x maize crosses. J. Plant Breed. Crop Sci. 4:32-38.
Suenaga K, Morshedi AR, Darvey NL (1997). Haploid production of Australian wheat (*Triticum aestivum* L.) cultivars through wheat x maize (*Zea mays* L.) crosses. Aust. J. Agric. Res. 48:1207-1211.
Suenaga K, Nakajima K (1993). Variation on in doubled haploid plants of wheat obtained through wheat (*Triticum aestivum*) x maize (*Zea mays*) crosses. Plant Breed. 11:120-124.
Tawkaz S (2011). Response of Some Wheat Genotypes to Anther Culture Technique for Doubled Haploid Production. M.Sc Thesis. Sudan Academy of Science, Kartum.
Touraev A, Vicente O, Heberle-Bors E (1997). Initiation of microspore embryogenesis by stress (a review). Trends Plant Sci. 2:285-323.
Ushiyama T, Kuwabara T, Yoshida T (2007). Effects of various phytohormones on haploid wheat production in wheat × maize crosses. Plant Prod. Sci. 10:36-41.
Wehr BF, Zeller FJ (1990). In vitro microspore reaction of different German wheat cultivars. Theor. Appl. Genet. 79:77-80.
Wei ZM (1982). Pollen callus culture in *Triticum aestivum*. Theor. Appl. Genet. 67:71-73.
Wojnarowiez G, Jacquard C, Devaux P, Sangwan RS, Clément C (2002). Influence of copper sulphate on anther culture in barley (*Hordeum vulgare* L.). Plant Sci. 162:843-847.
Wu X, Chang X, Jing R (2012). Genetic insight into yield-associated traits of wheat grown in multiple rain-fed environments. PLoS One doi:10.1371/journal.pone.0031249.
Xynias IN, Zamani IA, Gouli-Vavdinoudi E, Roupakias DG (2001). Effect of cold pretreatment and incubation temperature on bread wheat (*Triticum aestivum* L.) anther culture. Cereal Res. Comm. 29:331-338.
Yonezawa, Nomura T, Sasaki Y (1987). Conditions favouring doubled haploid breeding over conventional breeding of self-fertilising crops. Euphytica 36:441-453.
Zamani I, Gouli-Vavdinoudi E, Kovacs G, Xynias I, Roupakias D, Barnabás B (2003). Effect of parental genotypes and colchicine treatment on the androgenic response of wheat F1 hybrids. Plant Breed. 122:314-317.
Zhang J, Friebe B, Raupp WJ, Harrison SA, Gill BS (1996). Wheat embryogenesis and haploid production in wheat x maize hybrids. Euphytica 90:315-324.
Zhao J, Simmonds DH (1995). Application of trifluralin to embryogenic microspore cultures to generate doubled haploid plants in *Brassica napus*. Physiologia Plantarum 95:304-309.