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Full Length Research Paper

Breeding of newly licensed wheat variety Huapei 8 and improved breeding strategy by anther culture

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Wheat breeding by anther culture has the advantage of reducing breeding time, as well as high efficiency and accuracy in progeny selection. However, low percentage of callus induction, green plantlet regeneration and chromosome doubling of the haploid regenerants has limited its practical application. We improved the techniques in consideration of the three key steps by adjusting the sowing date of donor wheat, adding rare earth elements (REE) to the differentiation medium, and improving the chromosome doubling method. On the average, the induction rate of callus reached 8% for all the genotypes inoculated in the annual breeding. The regeneration rate and percentage of fertile regenerants were above 25 and 80%, respectively. We also designed a specific breeding strategy for anther culture by focusing on the genotypic constitution of the parents, their combination type and the progeny selection method. H_2 was the best selection generation for traits with high heredity ability, and H_3 was the best selection for grain traits and yield test. Consequently, we bred and licensed six new wheat varieties derived from anther culture and significantly reduced breeding time to three to five years. Huapei 8 was the newest released wheat variety bred by anther culture with superior agronomic characters, high yield potential and wide adaptability.

Key words: *Triticum aestivum*, anther culture, rear earth elements, chromosome doubling, wheat breeding.

INTRODUCTION

The scheme of wheat breeding has long been dominated by conventional cross-breeding programs. The development of modern biotechnology has made it possible to alter breeding approaches through the use of cellular engineering, transgenic method and marker-assisted selection. Wheat breeding by anther culture has the potential to reduce breeding time to three to five years since heterozygous progenies could become homozygous in doubled haploid using the chromosome doubling technique. Another advantage of wheat breeding by anther culture in traditional breeding programs is that it is more efficient in pure line selection rather than the heterozygous selection. Anther culture is also a highly efficient approach in obtaining doubled haploid (DH) population for mapping of genetic linkage and quantitative trait locus (QTL) (Zhang et al., 2009). The haploid callus induced in anther culture could also be a superior transgenic acceptor, especially for polyploid crops, as wheat. In China, it has been nearly 40 years since the creation of the first plantlet derived by anther culture. Due to its high efficiency and accuracy in progeny selection, this breeding system has been widely accepted by wheat breeders (Orlov et al., 1993; Dietzmann and Foroughi-Wehr, 1996). However, the genotype dependence of induction response, low percentage of green plants from anther cultures and difficulties in chromosome doubling have limited its practical use in wheat breeding, although these have drawn the attention of many researchers (Zhuang et al., 1983; Zhou et al., 1989; Dietzmann and Foroughi-Wehr 1996; Tuvesson et al., 2000). In recent decades, we focused on the overall design of the breeding scheme using anther culture in order to develop and integrate improved techniques in the key steps, as

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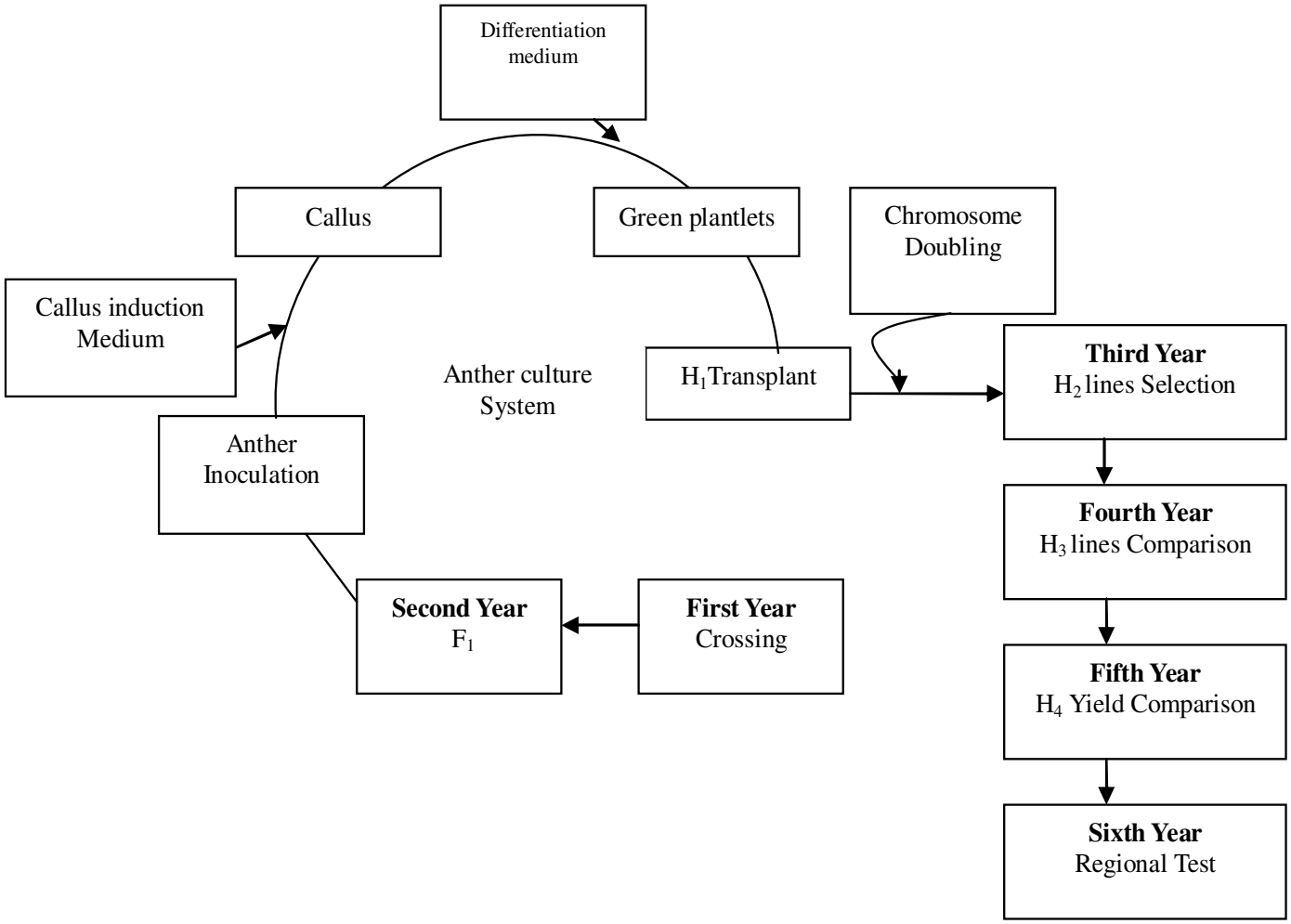

Figure 1. The anther culture breeding system.

shown in Figure 1. The anther culture breeding system is currently being successfully used in our laboratory, and six new varieties derived from wheat anther culture using improved breeding procedures have been licensed since 1999. Most of the breeding period up to the submission process for regional testing averaged four to six years (Table 1).

MATERIALS AND METHODS

Parent combination

The 9824H-1-2 was crossed with Zhengzhou 91138 in 2001, and the F1 was back-crossed with 91138 in 2002. The back-cross was given the combination number of 0247. In late December, 0247 was sown in the experimental field of Henan Academy of Agricultural Sciences in Zhengzhou.

The medium used to induce callus was a Gui medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D, 2.0 mg/L), kinetin (KT, 0.5 mg/L), and sucrose (90 g/L) (Hai et al., 1997). The medium used for plantlet regeneration from the callus was an Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) with the major inorganic salts reduced to half of their original strengths, supplemented with KT 1.0 mg/L, α-naphthalene acetic acid (NAA, 0.5 mg/L), REA (1.0 mg/L) and sucrose (30 g/L). Half of the MS added to KT (1.0 mg/L), MET (3.0 mg/L) and sucrose (80 g/L) was used for the rooting plantlet.

Culture procedures and chromosome doubling

Spikes were sampled before their emergence from the flag leaf sheath. Anthers in the central region of the spikes with pollens at mid to late-uninucleate stages were used for inoculation. Donor spikes were surface-sterilized with 75% ethanol, after which the leaf sheaths was removed. Anthers were taken for inoculation with the hood for the induction medium and cultured in the dark at 28°C until the induced callus became 1 to 1.5 mm. The calluses were then transferred into the differentiation medium. When the regenerated plantlet grew to 1 to 2 cm, they were transferred into the rooting medium. Ten days later, the plantlets in the rooting medium were stored in the fridge at 5°C during the summer season, and were later transferred to the experiment field in October. The following spring, the chromosomes of the haploid plants were doubled with colchicine during the tilling stage using the half root socking method (He et al., 1990).

Table 1. Traits of the newly licensed wheat varieties derived from anther culture (data from the regional test of the corresponding year).

Variety	Combination type	Year of license	Grain yield (kg/hm^2)	Numbers of spikes (×10^4/hm^2)	Grains/ spike	1,000 grain weight (g)	Plant height (cm)
Huapei 1	F_1	2006	8511.4	555-600	33-39	42-45	72
Huapei 3	F_1	2006	8619.0	555-585	32-35	48-50	75
Huapei 5	F_1	2006	7927.5	645-720	30-33	40-45	80
Huapei 6	TC	2008	8238.0	540-570	35-38	50-55	78
Huapei 8	B C1	2009	8071.5	570-600	32-35	50-52	80

Table 2. Three key components of yield of Huapei 8 (data from the regional test of the corresponding year).

Year	Trail	Variety	Numbers of spike (×10^4/hm^2)	Grain /spike	1000 grain weight (g)
2007-2008	Regional trail	Huapei 8	580.0	32.1	49.6
		Yanshi4110	586.5	31.2	47.6
2008-2009	Productivity trail	Huapei 8	615.0	34.5	44.4
		Yanshi4110	663.0	31.1	38.6

Table 3. Yield stability of Huapei 8 (results of the State Regional Spring Water Experimental test).

Year	Total numbers of tested variety	Variety	Suitability degree (%)	HSC (%)	Rank
2007-2008	13	Huapei 8	90.91	95.56	1
		Yanshi4110 (ck)	63.64	90.22	5
2008-2009	10	Huapei 8	90.00	95.86	1
		Yanshi4110 (ck)	20.00	89.90	7

HSC= {(x-s)/1.1 × CK} ×100; x, yield; s, standard deviation.

RESULTS

The first haploid generation (H_1) was transplanted from the test tube. Its growth environment differed from the natural environment. Thus, the single plant selection of F_1 under a traditional breeding procedure was not conducted for the H_1 of the anther culture breeding program. Twelve lines, with no significant poor traits, were harvested. The lines were planted in 2005 for trait selection, and line 0247H-4 was proposed for pre-regional test in 2006. This line passed both regional and productivity tests in 2006 and 2007, respectively, and was approved for release in 2009.

Superior agronomic characters

Huapei 8 is a weak-spring to semi-winter variety with a heavy head. Its height is about 80 cm with strong stem and logging resistance. It was tested for quality parameters in 2008, particularly, for volume weight (795 g/L), protein (dry; 13.27%), wet daugh (28.4%), falling number (367 s), water absorbing rate (54.0 ml/100 g), forming time (2 min), stable time (1.7 min), sediment value (44.0 ml) and hardness (51%).

Coordinated three key components of yield

Regional and productivity tests showed that Huapei 8 had a stable number of spikes, grain numbers per spike and grain weight, indicating its ability for self-adjustment (Table 2).

High yield potential and good stability

In the regional test (2007-2008), Huapei 8 ranked top in the group with a yield of 8071.5 kg/hm^2, and even surpassed the control by 6.37%. In the productivity test (2008 to 2009), its yield also ranked top and surpassed the control by 8.1%. Its high stable coefficient (HSC) reached 95.56 and 95.86% in 2007 and 2008, respectively, indicating yield stability. It also has a high suitability at 90.91 and 90.00% based on data of 2007 and 2008, showing wide adaptability (Table 3).

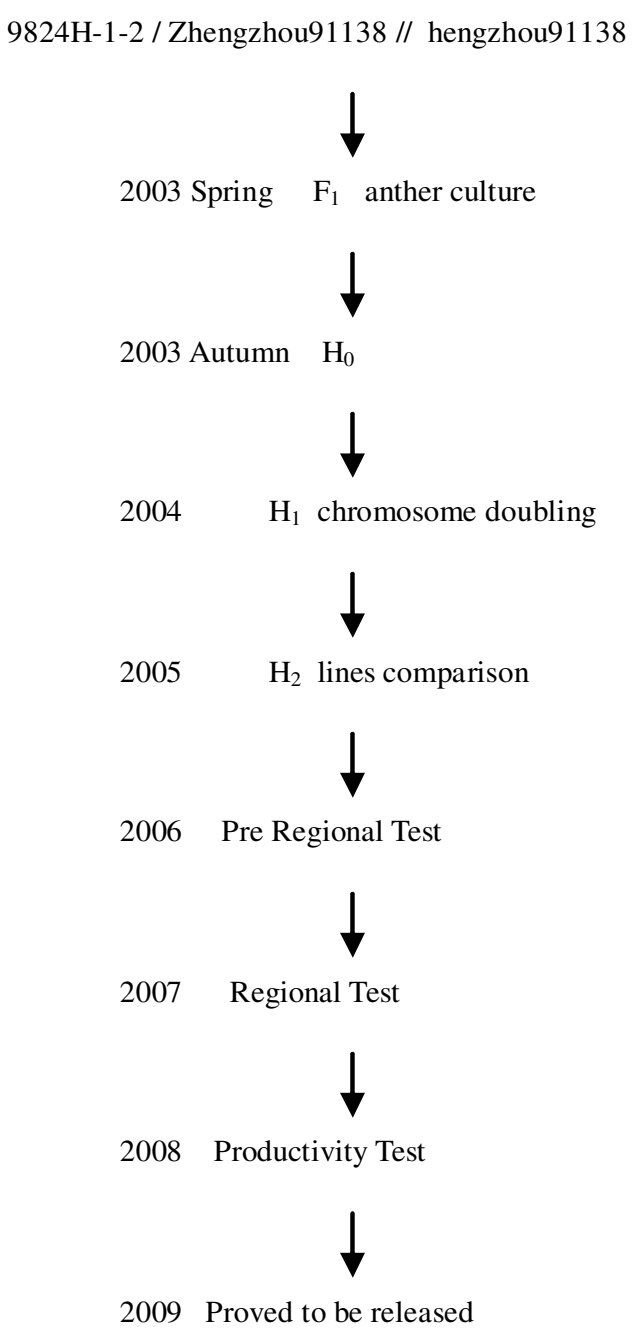

Figure 2. Breeding procedure of Huapei 8.

DISCUSSION

Huapei 8 is derived from the progenies of anther culture for selected F_1 plants using the back-cross combination (9824H-1-2/Zhengzhou91138//Zhengzhou91138) (Figure 2). It has superior agronomic traits and has passed the regional and productivity tests for multi-location trails. The characteristics of Huapei 8 demonstrate productivity, stability and adaptability. Huapei 8 was approved for release in 2009 in Henan Province (License No. 2009005), and has been submitted for Property Protection of Plant New Variety.

Callus induction from anther culture, green plantlet regeneration from callus, and chromosome doubling have been identified as the three limited steps in the breeding application of anther culture. Much attention has been focused on the improvement of the three steps, mostly by adjusting the medium supplements (Zhou et al., 1992a; Zamani et al., 2000; Soriano et al., 2008). However, the effects of some of the supplements were contradictory. While the percentage of regenerable callus increased, the callus production might reduce. When the level of fertility was high, a significant decrease was also observed in plant regeneration ability. Moreover, it has long been known that the anther culture response was genotype dependent (Zhou et al., 1992b; Reynolds and Kitto, 1992; Orlov et al., 1993). From the breeding standpoint, we focused on the practical feasibility to gain large population of regeneration plants for breeding screening. Through years of designed experiments, three improvements on the techniques were developed.

Postponed sowing date meets anther culture requirements for suitable pollen development stage and the environmental temperature in the inoculating period

The development stage of pollen is crucial for callus induction rate during anther culture. It is known that the uninucleate stage has the highest callus induction effect, while culture temperature affects anther culture response (Ouyang et al., 1983; He and Ouyang, 1984). However, we also found that environmental temperature during the inoculating period has affected the development of anther, significantly affecting callus induction (Luo et al., 1999). An environmental temperature of 25°C ensured higher callus induction rates. In Huanghuai, this temperature was apparent during the dates, April 20 to 30. Huanghuai is the largest wheat-producing region in China. Winter wheat is generally sown in the middle of October up to April of the following year. To meet necessary anther culture conditions, we postponed the sowing date of the donor wheat to middle of November since the temperature was about 5 to 10°C. The last days of April (succeeding year) may be suitable for the pollen development stage and environmental temperature. For years of inoculation of anthers at environmental temperatures around 25°C, the callus induction rate is maintained at above 8% on the average.

Rare earth elements (REE) stimulates green plant regeneration

REE has the effect of promoting plant growth, increasing chlorophyll contents and photosynthetic rate (Hong et al., 2002; He and Xue, 2005). Supplementing MS medium

(Murashige and Skoog 1962) with REE for plantlet regeneration can significantly improve the regeneration rates of green plant. In this study, 1.5 mg/L of REE was found to have the highest green plant regeneration rate, with green plant differentiation time reduced to as early as seven to eight days. Green plant regeneration rate increased from 13.72 to 27.51% (Hai et al., 2006a, b).

Chromosome doubling by colchicine using half root soaking treatments

Chromosome doubling is one of the key steps to obtaining seeds from regenerated green plants. The conventional chromosome doubling method involves applying colchicine to the regenerated plants before transplanting them to the soil. Whole roots were soaked in colchicine liquid and the mortality of the treated green plants was high. Others attempted adding colchicine into the induction medium; however, the regeneration rate of some genotypes was affected (Zamani et al., 2000). The half root soaking method involves digging out half of the roots during the tilling stage and treating only half of the roots with colchicine at a concentration of 0.4% for three days (He et al., 1990). The half root soaking method has two advantages when compared with the other methods. First, the seedlings remain in the soil and continue to absorb nutrients, allowing a survival rate up to 98.1%. Second, the percentage of fertile doubled haploid plants can be stabilized up to 80%, which is about twice more potent than other methods.

Apart for the technique improvement of anther culture as a means to increase the efficiency of callus induction, green plantlet regeneration and chromosome doubling, the practical breeding scheme includes choosing the genotypic constitution of the parents, their combination type and the progenies selection strategy. These are the key factors for ensuring a successful breeding program.

The genotypic constitution of the parents

There are two main modules in the choice of parents. First, the parental materials should have comparable superior traits. Second, the parents should differ from each other greatly in ecotype and have less similarity. In the first module, the DH offspring had comprehensive traits and were suitable for breeding use. The second module created many segregate types of offspring and was better for germplasm innovation. Thus, the strategy for anther culture breeding should choose parents with superior agronomic traits. The parents of the newly released varieties of Huapei 1, Huapei 3 and Huapei 5 include Bainong 64, Yumai 21, Yumai 57, Yumai 18 and Yumai 2, which are all popular varieties of the Huanghuai winter wheat. As for the low yield of green haploids for offspring from some elite agronomic parents, compensation could be made by planting of more anthers (Tuvesson et al., 2000).

The combination type

It was shown that anther cultures at different hybrid generations have different breeding efficiencies. The traits of the offspring of F_1 anther culture had large segregations, in which it was easier to find the type superior to the better parent. Breeding time was also significantly reduced. Huapei 1, Huapei 3 and Huapei 5 are all derived from the F_1 anther culture. For a superior combination, the anther culture of the DH offspring from single cross F_3 and double cross F_2 may have more opportunities in achieving the combined desired traits. Huapei 6 was bred by the anther culture of double cross F_2. BC_1 can also be used as an anther donor and combine the comprehensive properties of the recurrent parent and the elite traits from non-recurrent parent. Huapei 8 was derived from the BC_1 anther culture. In Germany, Dietzmann and Foroughi-Wehr (1996) had successfully incorporated resistance to BaYMV and *Rhynchosporium secalis* into eight superior DH lines together with the maintenance of their high agronomic characters by recurrent selection with repeated haploid steps. Ma and Lu (2010) also gain several lines resistant to Fusarium Head Blight through anther culture of offspring from complex combination.

The progeny selection strategy

For the anther culture, H_2 lines are double haploid lines with traits that are stable and easy to select in terms of phenotype. Thus, H_2 is best for generation selection. To ensure the applicability of the H_2 line selection, the planting of H_2 should be the same as in the production field. H_2 selection should focus on traits with high heredity ability (winterness or springness), disease resistance, and plant height. Meanwhile, H_3 could be employed for yield test; it should focus on grain trait selection.

With improved techniques for anther culture, as well as specific strategy of parent selection and the combination module, the selection method for haploid breeding and wheat anther culture breeding schemes can then be applied more practically. Advantages of breeding time have been demonstrated in our breeding practice of wheat varieties derived from anther culture. The superior agronomic performance of these varieties was verified by multi-location trails and during practical production.

REFERENCES

Dietzmann E, Foroughi-Wehr B (1996). Combination of resistances to barley yellow mosaic virus and *Rhynchosporium secalis* by recurrent selection with repeated haploid steps. Plant Breed. 115: 179-182.

Hai Y, He X, Huang B, Wang J (1997). Preparation of Gui culture medium and its application to wheat anther culture. Acta Botanica

Sinica 39: 742-747.
Hai Y, Kang M, Guo J, He N, Zhang D (2006a). Effects of REA and genotypes on the green wheat plantlet induction frequency. Acta. Agriculturae Boreali-Sinica, 21: 34-36.
Hai Y, Kang M, Guo J, He N, Huang B (2006b). Effects of environment, Rare Earth and genotypes on the green plantlet differentiation rate of wheat anther culture callus. J. Henan Agric. Sci. 7: 27-29.
He D, Ouyang J (1984).Callus and plantlet formation from cultured wheat anthers at different developmental stages. Plant Sci. Lett. 33: 71-79.
He X, Wang J, Liu W (1990). Study on Chromosome doubling of pollen derived plant in wheat by half root soaking method. In Hu H and Wang H. Plant cell engineering and breeding. Beijing Industry University Press, Beijing. pp. 407-409.
He Y, Xue L (2005). Biological effects of rare earth elements and their action mechanisms. Chinese J. Appl. Ecol. 16: 1983-1989.
Hong F, Wei Z, Zhao G (2002). Mechanism of lanthanum effect on chlorophyll of spinach. Sci. China Series C, 45: 166-176.
Luo P, Hai Y, Liu W, Huang B, Liu X, He X (1999). Effects of sowing date on callus induction frequency of wheat anther culture. J. Henan Agric. Sci., 9: 3-4.
Ma H, Lu W (2010). Progress on genetic improvement for resistance to Fusarium Head Blight in wheat. Jiangsu. J. Agric. Sci. 26: 197-203.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassaya with tobacco tissue culture. Physiologia Plantarum, 15: 473-479.
Orlov PA, Maurishcheva EB, Palilova AN (1993). Estimation of the response to anther culturing in 60 genotypes of different wheat species. Plant Breed. 111: 339-342.
Ouyang J, Zhou S, Jia S (1983).The response of anther culture to culture temperature in *Triticum aestivum*. Theor. Appl. Genet. 66: 101-109.
Reynolds T, Kitto SL (1992). Identification of embryoid-abundant genes that are temporally expreaaed during pollen embryogenesis in wheat anther cultures. Plant Physiol. 100: 1744-1750.
Soriano M, Cistue L, Castillo AM (2008). Enhanced induction of microspore embryogenesis after n-butanol treatment in wheay (*Triticum aestivum* L.) anther culture. Plant Cell Rep. 27: 805-811.
Tuvesson S, Ljungberg A, Johansson N, Karlsson KE, Suijs LW, Josset JP (2000). Large-scale production of wheat and triticale double haploids through the use of a single-anther culture method. Plant Breed. 119: 455-459.
Zamani I, Kovacs G, Gouli-Vavdinoudi E, Roupakias DG, Barnabas B (2000). Regeneration of fertile doubled haploid plants from colchicine-supplemented media in wheat anther culture. Plant Breed. 119: 461-465.
Zhang K, Tian J, Zhao L, Liu B, Chen G (2009). Detection of quantitative trait loci for heading date based on the doubled haploid progeny of two elite Chinese wheat cultivars. Genetica, 135: 257-265.
Zhou H, and Konzak C F (1989). Improvement of anther culture methods for haploid production in wheat. Crop Sci. 29: 817-821.
Zhou H, Ball ST, Konzak CF (1992a). Functional properties of ficoll and their influence on anther culture responses of wheat. Plant Cell, Tissue and Organ Culture, 30: 77-83.
Zhou H, Konzak CF (1992b). Genetic control of green plant regeneration from anther culture of wheat. Genome, 35: 957-961.
Zhuang J, Jia X (1983). Increasing differentiation frequencies in wheat pollen callus. In: Hu H, and Vega MR (eds.), Cell and Tissue Culture Techniques for Cereal Crop Improvement. Science China Press, Beijing. pp. 413-432.