Technical Advances in Wheat Haploid Production Using Ultra-wide Crosses

Masanori INAGAKI

Biological Resources Division,
Japan International Research Center for Agricultural Sciences (JIRCAS)
(Tsukuba, Ibaraki, 305 Japan)

Received November 12, 1996

Abstract

A technique for producing wheat haploids through ultra-wide crosses followed by embryo rescue was improved by successfully selecting pollen donors from different subfamilial species and applying a plant growth regulator. Efficient crossing procedures were developed by using stored pollen and detached-tiller culture. Production of wheat haploids from diverse genotypes is efficient enough to obtain two haploids per wheat spike. This technique could be useful as a strategy for reducing the time required to obtain homozygous breeding lines and improving selection efficiency of wheat breeding programs. Procedures for doubled haploid production are described.

Additional key words : *Triticum aestivum*, chromosome elimination, embryo rescue, breeding method

Introduction

Practical breeding programs for self-pollinating crops, such as wheat (*Triticum aestivum* L., $2n$=$6x$=42), must include a process of genetic fixation for uniformity of agronomic traits after genetic recombination to increase variation. Repeated selection of heterozygous materials can increase uniformity, but many generation cycles are required to reach homozygosity in loci associated with agronomic traits. Of great interest to plant breeders, haploid production followed by chromosome doubling offers the quickest method for developing homozygous breeding lines. Doubled haploids derived from heterozygous materials show complete uniformity when used as recombinant inbred lines in selection procedures. An efficient technique for producing doubled haploids thus complements conventional breeding programs.

Two major methods for producing wheat haploids (polyhaploids, $2n$=$3x$=21), one using microspores (pollen) and one using megaspores (egg cells), have been examined over the last two decades. The method for producing haploids from cultured pollen has developed as an anther- or

pollen-culture technique, and is still constrained by the difference in the responses of wheat genotypes[24, 51]. On the other hand, the method using megaspores in ultra-wide crosses with Panicoides subfamilial species followed by embryo rescue has recently been developes[16].

This paper reports on the progress achieved in developing a method for wheat haploid production through ultra-wide crosses since the publication of the previous review[12] on the production of wheat haploids through the bulbosum technique. The achievements of the last decade can be attributed to the collaborative research projects of JIRCAS with the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria, and with the International Maize and Wheat Improvement Center (CIMMYT), Mexico.

Pollen source

Wide crosses of wheat with a bulbous wild barley (*Hordeum bulbosum* L.) result in the production of immature haploid embryos of wheat after preferential elimination of *H. bulbosum* chromosomes from hybrid zygotes[3]. However, the crossability of wheat with *H. bulbosum* is genetically controlled by the genes *Kr1* and *Kr2* located on chromosomes 5B and 5A, respectively[43, 7, 42]. According to the pedigrees of wheat varieties, crossable genotypes can be traced to the variety Chinese Spring or to materials of Asian origin[6]. Both Japanese and Chinese wheat varieties, and in particular, local varieties, are highly crossable with *H. bulbosum*[9, 10, 34]. Wheat genotypes carrying the dominant *Kr* gene(s) are not crossable with *H. bulbosum* and cannot produce haploid embryos. The lack of hybridization of wheat genotypes with *H. bulbosum* is due to the failure of the pollen tube to penetrate the embryo sac[44]. Application of plant growth regulator 2,4-dichlorophenoxyacetic acid (2,4-D) promotes seed setting and embryo formation when crossable genotypes are used for crosses, but cannot break the barrier of cross-incompatibility[10]. Since the efficiency of wheat haploid production is greatly influenced by the crossability of *H. bulbosum* onto wheat, the method using *H. bulbosum* crosses is restricted to crossable wheat genotypes.

Ultra-wide crosses of wheat with members of the Panicoides subfamily have been attempted in alien genetic transfer[50]. Maize (*Zea mays* L.) pollen can successfully hybridize with wheat egg cells and produce hybrid zygotes[25], irrespective of the presence of *Kr* gene(s)[26]. The maize chromosomes are rapidly eliminated from hybrid zygotes, requiring artificial rescue of proembryos at early developmental stage[27, 28, 5]. 2,4-D treatment after pollination is critical to the enhancement of embryo development in wheat x maize crosses[46]. Maize pollination and subsequent 2,4-D treatment onto wheat results in the production of immature wheat embryos capable of regenerating haploid

Table 1. Effect of 2,4-D treatment on embryo formation frequencies (%) in crosses of wheat with *H. bulbosum* and maize

Pollen donor	2,4-D treatment	Wheat variety			
		Chinese Spring	Norin 61	Mexipak 65	Highbury
None	-	0.0	0.0	0.0	0.0
None	+	0.0	0.0	0.0	0.0
H. bulbosum	-	16.9	23.6	0.0	0.0
H. bulbosum	+	25.0	38.5	0.0	0.0
Maize	-	0.0	0.0	0.0	0.0
Maize	+	21.1	17.5	18.9	8.3

Source: Inagaki & Tahir (1990)[13]

plants, even for wheat varieties that are cross-incompatible with *H. bulbosum* (Table 1)[13]. Wheat haploid production through maize crosses has been confirmed using diverse wheat varieties[13, 33, 40]. Some species related to maize, such as teosinte (*Zea mays* L. spp. *mexicana*)[49] and eastern gamagrass (*Tripsacum dactyloides* (L.) L.)[41], are alternative pollen donors for wheat haploid production.

Cytological evidence indicates successful fertilization and elimination of paternal chromosomes from hybrid zygotes in sorghum (*Sorghum bicolor* (L.) Moench) and pearl millet (*Pennisetum glaucum* (L.) R.Br.) crosses, which suggests that sorghum and pearl millet are potential pollen sources for wheat haploid production[29, 31, 1, 5]. Wheat haploids were obtained at high frequencies from sorghum[39] and pearl millet[18] crosses followed by 2,4-D treatment after pollination. However, sorghum crosses expressed a strong genotypic barrier of wheat to embryo formation (Table 2)[19]. Therefore, haploid production through crosses with maize and pearl millet appears more stable than other methods because of its less pronounced genotypic effect on haploid embryo formation.

Pollen storage and detached-tiller culture

Methodologies using ultra-wide crosses require viable pollen at the time of crossing, that is, flowering of wheat and pollen donors must be synchronized. This method can only be applied in seasons and places where both wheat and pollen donors grow. In addition, the environmental conditions where wheat plants will develop after crossing are critical to the efficiency of wheat haploid production. Adequate techniques for long-term pollen storage and for culturing wheat tillers with spikes under controlled conditions are helpful for peforming ultra-wide crosses without having to synchronize flowering times of both parents.

Maize and pearl millet pollen have been successfully preserved at ultra-low temperatures for long periods[4, 8]. Maize pollen dried to optimum water content range and stored at -80°C produced wheat haploid embryos, but the frequencies decreased to half in the case of fresh pollen[17]. Pearl millet is an alternative pollen source not only for haploid production but also for long-term pollen storage. The process of storing pollen involves both drying and freezing. Understanding the effects of drying and freezing on pollen viability is essential for achieving successful long-term pollen storage. Pearl millet pollen is relatively tolerant to drying and freezing, in contrast to maize pollen, which has a narrow water content range for maintaining viability during drying and freezing (Fig. 1)[17, 21]. As a result, pollen storage at ultra-low temperatures does not affect embryo formation

Table 2. Effect of pollen donors on embryo formation frequencies (%) in crosses of wheat with maize, pearl millet and sorghum

Pollen donor	Wheat variety		
	Chinese Spring	Norin 61	Siete Cerros
<Maize>			
Population-93A	26.0	22.4	12.6
Hybrid-33	18.4	26.8	13.1
<Pearl millet>			
NEC-7006	39.4	37.6	13.6
NEC-7268	23.3	15.6	0.8
<Sorghum>			
ICSR-LM-90166	22.1	42.1	0.0
Toluca-1	20.4	41.1	0.0

Source: Inagaki & Mujeeb-Kazi (1995)[19]

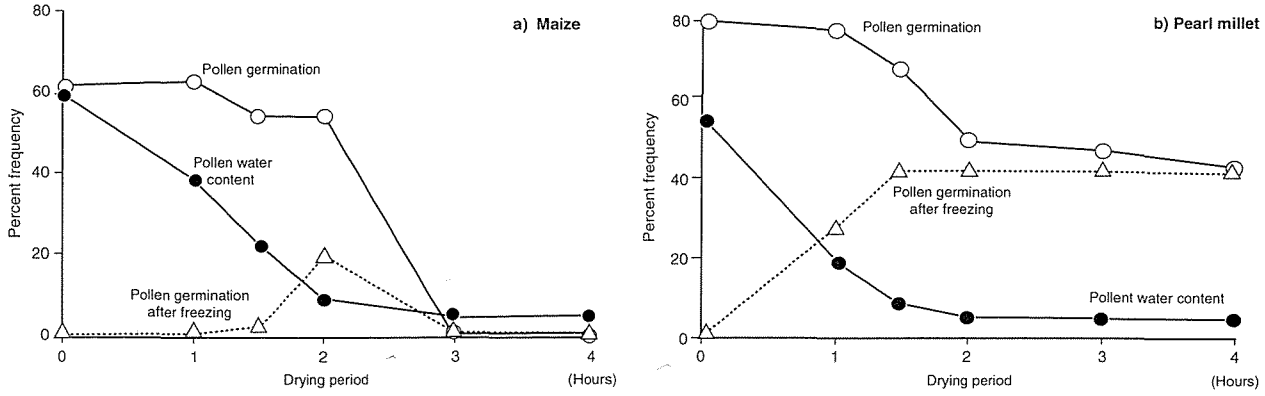

Fig. 1. Water content and germination frequencies of maize (a) and pearl millet (b) pollen after drying and freezing. Maize; F_1 hybrid line (CML-246 x CML-242), pearl millet; inbred line NEC-7006. Source: Inagaki & Mujeeb-Kazi (1994)[17], (1996)[21]

frequency in wheat x pearl millet crosses, but greatly reduces the frequency in wheat x maize crosses[22]. Stored pearl millet pollen can be used as an alternative medium for producing wheat haploids when fresh pollen is not available.

A technique for artificially culturing detached wheat tillers has been developed through physiological research on immature seed vernalization[23]. Major components of the culture solution are sucrose as a nutrient and sulfurous acid for preventing fungal contamination. Supplementing the culture solution with 2,4-D is essential for developing haploid embryos from wheat x maize crosses[49]. Detached-tiller culture has been extensively applied in wheat haploid production using maize crosses at the National Agriculture Research Center (NARC), Japan[37]. The effectiveness of detached-tiller culture in combination with pollen storage in wheat haploid production has also been confirmed (Table 3)[22]. Detached-tiller culture enables to collect spikes from wheat plants growing in distant sites and handle them in a laboratory.

These techniques also enable to avoid having to synchronize flowering times of both parents and result in considerable savings in terms of labor and space required for growing parent plants. They also provide greater flexibility in when and where wheat haploid production through ultra-wide crosses can be performed.

Haploid production

Wheat haploid production through ultra-wide crosses consists of two consecutive steps: formation of immature embryos by crossing and regeneration of haploid plants from rescued embryos. Factors such as the development stage of the wheat florets used for crosses and of the embryos rescued for plant regeneration are critical to haploid production efficiency[30, 35, 18]. In general, crossing at an early development stage of wheat florets results in higher frequencies of embryo formation. Artificial rescue at the appropriate embryo development stage is required to attain higher frequencies of plant regeneration. Cytological examination of regenerated plants indicates that most of them are euhaploids with 21 chromosomes[13, 40, 19]. Retention of pollen donor chromosomes was detected at the proembryo stage in crosses with maize and sorghum[25, 29], and at the tillering stage of plants in crosses with pearl millet and maize[1, 5]. This does not eliminate the possibility of translocating chromosome segments of these pollen donors to the wheat genome background through somatic associations. However, variations in agronomic traits of doubled haploids were negligible in *H. bulbosum*[11, 45] and maize crosses[32, 47]. No significant distortion of segregation ratios of genetic markers was found in doubled haploids produced from hybrid progenies

Table 3. Effects of pollen storage and detached-tiller culture on haploid production frequencies (%) in crosses of wheat with maize and pearl millet

Pollen donor	Tiller culture	Embryo formation (a)	Plant regeneration (b)	Haploid production (axb)
<Maize>				
Fresh	On plant	20.4	67.0	13.7
	Detached	19.4	42.5	8.3
Stored	On plant	2.8	65.0	1.8
	Detached	7.0	46.5	3.3
<Pearl millet>				
Fresh	On plant	19.7	45.8	9.0
	Detached	21.2	56.7	12.0
Stored	On plant	20.4	44.3	9.0
	Detached	27.7	54.5	15.0

Wheat; variety Norin 61, maize; F_1 hybrid line (CML-246 x CML-242), pearl millet; inbred line NEC-7006. Source: Inagaki, Nagamine & Mujeeb-Kazi (1997)[22]

through *H. bulbosum*[14, 15] or maize[48] crosses.

Efficiency of wheat haploid production over a three-year period at CIMMYT is shown in Table 4. The production scheme is summarized in Table 5. Production of wheat haploids is efficient enough to obtain two haploid plants per wheat spike (1.5 doubled haploids per wheat spike after chromosome doubling). The process takes approximately nine months, from sowing of plant materials to harvesting of doubled haploid grains. However, it must be noted that production efficiency varies due to the use of environment-controlled facilities.

In case of haploid production of durum wheat (*T. turgidum* L. var. *durum*) through wide crosses with maize, frequency variation among durum wheat genotypes has been reported[40, 2, 20]. However, significant difference in fertilization frequency was not found[38]. These facts suggest that the absence of the D genome in durum wheat causes differences in development of haploid embryos from hybrid zygotes, resulting in frequency variation of haploid production.

Procedures for doubled haploid production

Steps in doubled haploid production of wheat through ultra-wide crosses are described including the improved techniques.

1) Plant materials

Sowing times of seed materials are determined in order to synchronize flowering times of wheat and pollen donors. A continuous supply of pollen from pollen parents sown at one or two week intervals should be ensured.

2) Crossing

At ear emergence, wheat spikes are emasculated following the removal of the central florets of each spikelet and the apical and basal spikelets (Fig. 2a). Emasculated spikes with 25 - 30 florets are enclosed in glassine bags. Polyethylene bags may be used for maintaining humidity after emasculation. One day before predicted wheat anthesis, emasculated spikes are pollinated with freshly collected donor pollen (Fig. 2b). On two consecutive days after pollination, the uppermost internodes of wheat culms with pollinated spikes are needle-injected with a 100 mg/l 2,4-D solution (Fig. 2c). Most pollinated florets produce plump seeds, but the seeds do not always contain embryos. Four embryos per wheat spike on average can be obtained.

Table 4. Efficiency of wheat haploid production using maize crosses

Year	Wheat materials	No. spikes pollinated	No. embryos obtained	No. plants regenerated
1993	F_1 plants	284	1449	810
1994	F_3 plants	456	1219	898
1995	F_1 plants	501	1894	1267
Total		*1241*	*4562*	*2975*

Table 5. Scheme of doubled haploid production in wheat using ultra-wide crosses

Month	Procedure	Efficiency/day
0	Sow wheat materials	
2.5	Cross wheat with pollen donors	10 spikes
3.0	Embryo rescue	40 embryos
4.0	Transfer haploid plants to soil	25 plants
5.0	Treat plants with colchicine	25 plants
7.5	Cover ears with glassine bags	25 plants
9.0	Harvest doubled haploid grains	15 plants

3) Pollen storage

Stored pollen can be used for crossing on wheat when fresh pollen is not available. Pollen is collected from donors at anthesis and screened through a sieve to remove anthers. Ten grams of pollen are spread on a paper tray and dried with gentle ventilation at 35°C and 35 - 40% relative humidity. Pollen water content is determined from a 0.5 g pollen sample dried at 95°C for five hours. Optimum water content for stored pollen is 10 - 12% for maize and 5 - 7% for pearl millet (Fig. 2d). The dried pollen is distributed among cryopreservation tubes. The sealed tubes are stored in liquid nitrogen (-196°C) until use. After thawing sample tubes containing pollen in a water-bath at 38°C for five minutes, pollen is checked for its germination viability and used for crossing. The drying and freezing process reduces pollen viability and may affect embryo formation frequency in wide crosses.

4) Detached-tiller culture

In the case of wheat plants growing in distant sites, detaching tillers with spikes is feasible for wide crosses. At ear emergence wheat tillers are cut off at the base and cultured in a flask containing tap water. Wheat spikes are emasculated as described above. After pollination, tillers are cultured in a solution containing 40 g/l sucrose, 8 ml/l sulfurous acid (6% SO_2) and 100 mg/l 2,4-D for two days. They are then transferred to a solution containing only sucrose and sulfurous acid, and cultured until embryo rescue. This process takes approximately 12 days (in maize crosses) and 14 days (in pearl millet crosses) after pollination (Fig. 2e). The culture conditions are 22.5°C, 12-hr daylength and 60 - 70% relative humidity in a growth chamber.

5) Embryo culture

Seed set in wheat crossed with pollen donors is somewhat smaller than in selfed seed, and seeds are filled with an aqueous solution instead of solid endosperm as found in selfed seed (Fig. 2f). After 14 (in maize crosses) or 18 days (in pearl millet crosses) of spike growth on wheat plants, wheat seeds are removed from spikes and sterilized in a

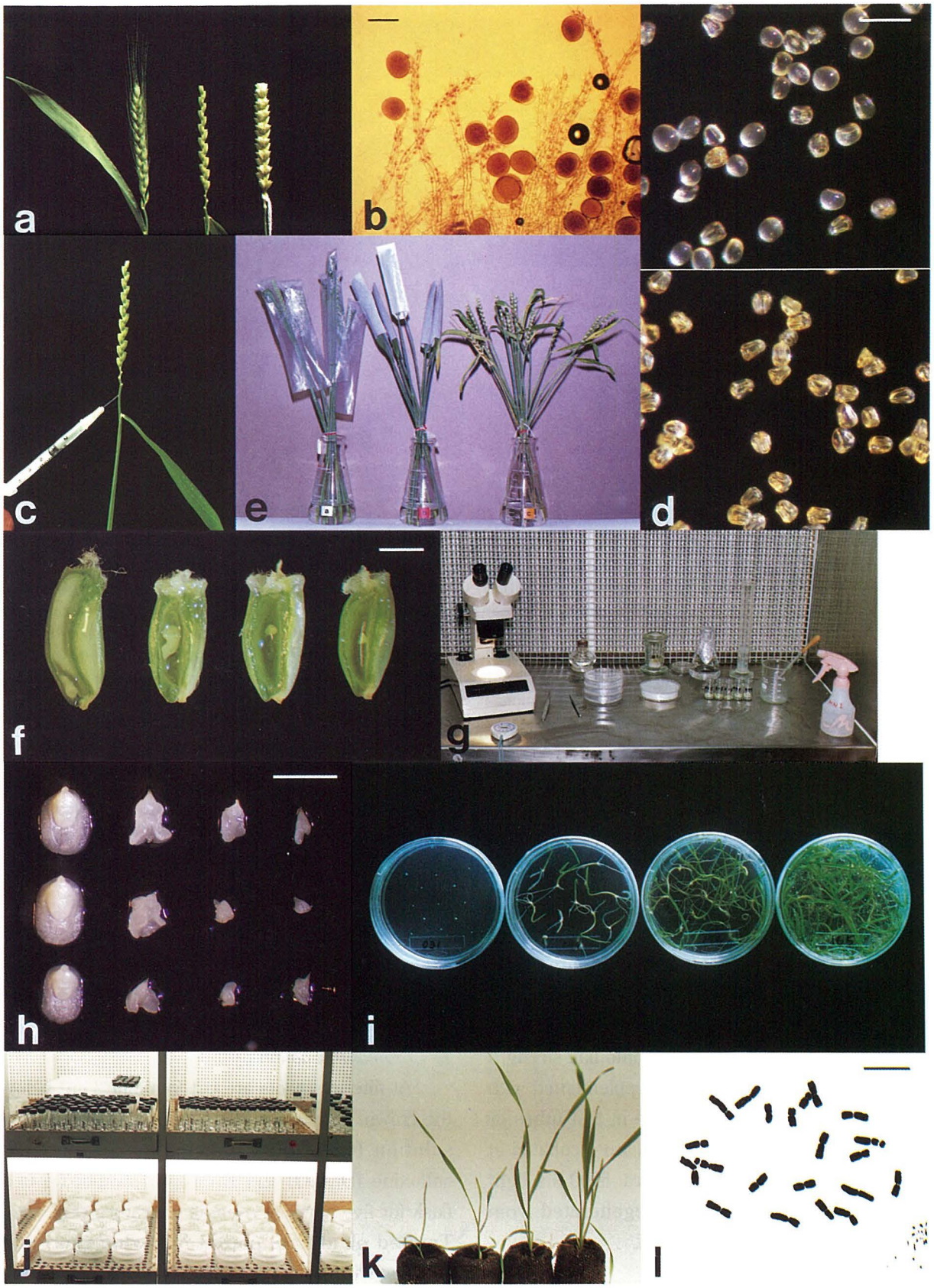

Fig. 2a - l. (See captions p.58)

Fig. 2a - q. Stages in production of wheat doubled haploids through ultra-wide crosses. a) Wheat spikes at ear emergence (*left*), emasculation (*center*) and seed setting (*right*). b) Germination of maize pollen on wheat stigma. Bar 0.1 mm. c) Injection of 2,4-D solution into wheat culm using a syringe. d) Pearl millet pollen at collection (*upper*) and after being dried for two hours (*lower*). Bar 0.1 mm. e) Detached-tiller culture of wheat at emasculation (*left*), pollination (*center*) and seed setting (*right*). f) Wheat seeds obtained from self-pollination, and from crosses with maize, pearl millet and sorghum (*from left to right*). Bar 2 mm. g) Apparatus used for embryo rescue on a laminar flow bench. h) Wheat embryos obtained from self-pollination, and from crosses with maize pearl millet and sorghum (*from left to right*), showing differences in size and shape among crosses. Bar 2 mm. i) Plants developing from cultured embryos (*from left to right*; 0, 1, 2 and 3 weeks after incubation). j) Culture room accommodating test tubes and plastic dishes. k) Wheat haploid plants transferred to potted soil for further development. l) Somatic chromosomes ($2n=3x=21$) of a wheat haploid plant. Bar 10μm. m) Colchicine treatment of wheat haploid plants at tillering. n) Wheat plants transferred to potted soil after colchicine treatment. o) Wheat spikes of colchicine-treated plants, covered with glassine bag (*left*), and producing doubled haploid grains (*right*). p) Doubled haploid plants grown for seed multiplication. q) Doubled haploid lines grown in the field, showing uniformity within lines

solution (1 - 2% sodium hypochloride of CLOROX and few drops/l Tween-20) for 10 minutes (Fig. 2g). After rinsing with sterilized water, embryos (approximately 1.0 mm in diameter) are aseptically excised (Fig. 2h) and transferred onto half strength Murashige & Skoog[36] medium supplemented with 20 g/l sucrose and 6 g/l agarose in test tubes or plastic dishes (Fig. 2i). Embryos are incubated at 25°C, using 16-hr daylength and ca. 5000 lux light intensity (Fig.2j). Plants are regenerated from embryos at frequencies of 50 - 70%.

6) Plant regeneration

Cultured plants with fully developed roots and leaves are transplanted to potted soil and grown further (Fig. 2k). The chromosome number can be examined in squashed preparations of root-tips stained with acetocarmine or aceto-orcein (Fig. 2l).

7) Chromosome doubling

At tillering, roots are cut to about 2 cm below the crown. Plants are then immersed in colchicine solution (0.05 - 0.10% colchicine, 2% dimethyl sulfoxide (DMSO) and 15 drops/l Tween-20) in a flask for five hours at room temperature (Fig. 2m). Treated plants are potted following thorough washing with tap water (Fig. 2n). Potted plants are grown under favorable, tillering-enhancing conditions. Wheat spikes from colchicine-treated plants are enclosed in glassine bags to prevent

outcrossing. Wheat grains are harvested at a 60 - 70% success rate (Fig. 2o).

After multiplying harvested grains for one generation cycle (Fig. 2p), doubled haploid lines are planted in the field for evaluating genetic variation (Fig. 2q).

Conclusion

A technique for producing wheat haploids using ultra-wide crosses followed by embryo rescue has been developed over the last two decades. Significant technical advances have been achieved by using pollen selected from different subfamilial species and applying plant growth regulators. Efficient crossing procedures were developed using stored pollen and detached-tiller culture. At present, doubled haploids derived from hybrid progenies can be used as recombinant inbred lines with favorable uniformity in breeding selection. This technique could thus complement conventional breeding programs and accelerate the release of new varieties in developed countries, as well as in developing countries where rapid varietal development is critical for sustainable wheat production systems.

Acknowledgements

The author would like to thank Dr. M. Tahir, ICARDA, and Dr. A. Mujeeb-Kazi, CIMMYT, for research collaboration on wheat haploids, and Ms. Alma L. McNab, wheat program editor, CIMMYT, for her editorial review of the manuscript.

References

1) Ahmad, F. & Comeau, A. (1990). Wheat × pearl millet hybridization: consequence and potential. *Euphytica* **50**: 181-190.
2) Amrani, N. *et al.* (1993). Genetic variability for haploid production in crosses between tetraploid and hexaploid wheats with maize. *Plant Breed.* **110**: 123-128.
3) Barclay, I.R. (1975). High frequencies of haploid production in wheat (*Triticum aestivum* L.) by chromosome elimination. *Nature (London)* **256**: 410-411.
4) Barnabás, B. & Rajki, E. (1981). Fertility of deep-frozen maize (*Zea mays* L.) pollen. *Ann. Bot.* **48**: 861-864.
5) Comeau, A. *et al.* (1992). Media for the in ovulo culture of proembryos of wheat and wheat-derived interspecific hybrids or haploids. *Plant Sci.* **81**: 117-125.
6) Falk, D.E. & Kasha, K.J. (1981). Comparison of the crossability of rye (*Secale cereale*) and *Hordeum bulbosum* onto wheat (*Triticum aestivum*). *Can. J. Genet. Cytol.* **23**: 81-88.
7) Falk, D.E. & Kasha, K.J. (1983). Genetic studies of the crossability of hexaploid wheat with rye and *Hordeum bulbosum*. *Theor. Appl. Genet.* **64**: 303-307.
8) Hanna, W.W. (1990). Long-term storage of *Pennisetum glaucum* (L.) R. Br. pollen. *Theor. Appl. Genet.* **79**: 605-608.
9) Inagaki, M.N. & Snape, J.W. (1982). Frequencies of haploid production in Japanese wheat varieties crossed with tetraploid *Hordeum bulbosum* L. *Japan. J. Breed.* **32**: 341-347.
10) Inagaki, M.N. (1986). Crossability of Japanese wheat cultivars with *Hordeum bulbosum* L. *Japan. J. Breed.* **36**: 363-370.
11) Inagaki, M.N. (1987). Variation in plant height of doubled haploid lines of wheat derived from intergeneric crosses with *Hordeum bulbosum* L. *Japan. J. Breed.* **37**: 275-282.
12) Inagaki, M.N. (1990). Wheat haploids through the bulbosum technique. *In* 'Biotechnology in Agriculture and Forestry, vol. 13, Wheat', Bajaj, Y.P.S. (ed.), Springer-Verlag, Berlin, 448-459.
13) Inagaki, M.N. & Tahir, M. (1990). Comparison of haploid production frequencies in wheat varieties crossed with *Hordeum bulbosum* L. and maize. *Japan. J. Breed.* **40**: 209-216.
14) Inagaki, M.N. & Tahir, M. (1991). Effects of semi-dwarfing genes *Rht1* and *Rht2* on yield in doubled haploid lines of wheat. *Japan. J. Breed.*

41: 163-167.

15) Inagaki, M.N. & Egawa, Y. (1994). Association between high-molecular weight subunit genotypes of seed storage glutenin and agronomic traits in a doubled haploid population of bread wheat. *Breed. Sci.* **44**: 41-45.

16) Inagaki, M.N. & Mujeeb-Kazi, A. (1994). Progress in polyhaploid production techniques of hexaploid wheat through wide crosses. Proc. 2nd Int. Triticeae Symp., Logan, Utah, USA, 65-69.

17) Inagaki, M.N. & Mujeeb-Kazi, A. (1994). Storage of maize pollen for use in haploid production of hexaploid wheat. *Breed. Sci.* **44**: 387-390.

18) Inagaki, M.N. & Bohorova, N. (1995). Factors affecting the frequencies of embryo formation and haploid plant regeneration in crosses of hexaploid wheat with pearl millet. *Breed. Sci.* **45**: 21-24.

19) Inagaki, M.N. & 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.

20) Inagaki, M.N. & Tahir, M. (1995). Comparison of crossabilties of tetraploid wheat with *Hordeum bulbosum* and maize. *Cereal Res. Commun.* **23** : 339-343.

21) Inagaki, M.N. & Mujeeb-Kazi, A. (1996). Production of polyhaploids of hexaploid wheat using stored pearl millet pollen. Proc. 5th Int. Wheat Conference, Ankara, Turkey. (in press).

22) Inagaki, M.N. *et al.* (1997). Use of pollen storage and detached-tiller culture in wheat polyhaploid production through wide crosses. *Cereal Res. Commun.* **25** (in press).

23) Kato, K. *et al.* (1990). Simplified culture method of detached ears and its application to vernalization in wheat. *Euphytica* **49**: 161-168.

24) Kisana, N.S. *et al.* (1993). Production of doubled haploids by anther culture and wheat × maize method in a wheat breeding programme. *Plant Breed.* **110**: 96-102.

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

26) Laurie, D.A. & Bennett, M.D. (1987). The effect of the crossability loci *Kr1* and *Kr2* on fertilization frequency in hexaploid wheat × maize crosses. *Theor. Appl. Genet.* **73**: 403-409.

27) Laurie, D.A. & Bennett, M.D. (1987). The timing of chromosome elimination in hexaploid wheat × maize crosses. *Genome* **32**: 953-961.

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

29) Laurie, D.A. & Bennett, M.D. (1988). Cytological evidence for fertilization in hexaploid wheat × sorghum crosses. *Plant Breed.* **100**: 73-82.

30) Laurie, D.A. (1989). Factors affecting fertilization frequency in crosses of *Triticum aestivum* cv. 'Highbury' × *Zea mays* cv. 'Seneca 60'. *Plant Breed.* **103**: 133-140.

31) Laurie, D.A. (1989). The frequency of fertilization in wheat × pearl millet crosses. *Genome* **32**: 1063-1067.

32) Laurie, D.A. & Snape, J.W. (1990). The agronomic performance of wheat doubled haploid lines derived from wheat × maize crosses. *Theor. Appl. Genet.* **79**: 813-816.

33) Laurie, D.A. & 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.

34) Li, D.W. & Hu, Q.D. (1986). The crossability of *Triticum aestivum* with *Hordeum bulbosum*. *Acta Bot. Sinica* **28**: 461-471.

35) Matzk, F. & Mahn, A. (1994). Improved techniques for haploid production in wheat using chromosome elimination. *Plant Breed.* **113**: 125-129.

36) Murashige, T. & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15**: 473-

497.

37) Nagamine, T. *et al.* (1995). Improvement in "maize method" for efficient and labor-saving wheat haploid production. Proc. 2nd Asian Crop Science Conference, Fukui, Japan, 772-773.

38) O'Donoughue, L.S. & Bennett, M.D. (1994). Comparative responses of tetraploid wheats pollinated with *Zea mays* L. and *Hordeum bulbosum* L. *Theor. Appl. Genet.* **87**: 673-680.

39) Ohkawa, Y. *et al.* (1992). Production of haploid wheat plants through pollination of sorghum pollen. *Japan. J. Breed.* **42**: 891-894.

40) Riera-Lizarazu, O. *et al.* (1992). Maize (*Zea mays* L.) mediated polyhaploid production in some Triticeae using a detached tiller method. *J. Genet. Breed.* **46**: 335-346.

41) Riera-Lizarazu, O. & Mujeeb-Kazi, A. (1993). Polyhaploid production in the Triticeae: wheat × *Tripsacum* crosses. *Crop Sci.* **33**: 973-976.

42) Sitch, L.A. *et al.* (1985). Intrachromosomal mapping of crossability genes in wheat (*Triticum aestivum*). *Theor. Appl. Genet.* **70**: 309-314.

43) Snape, J.W. *et al.* (1979). The crossability of wheat varieties with *Hordeum bulbosum*. *Heredity* **42**: 291-298.

44) Snape, J.W. *et al.* (1980). Post-pollination events in crosses of hexaploid wheat with tetraploid *Hordeum bulbosum*. *Z. Pflanzenzüchtg.* **85**: 200-204.

45) Snape, J.W. *et al.* (1988). Tests for the presence of gametoclonal variation in barley and wheat doubled haploids produced using the *Hordeum bulbosum* system. *Theor. Appl. Genet.* **75**: 509-513.

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

47) Suenaga, K. & Nakajima, K. (1993). Variation in doubled haploid plants of wheat obtained through wheat (*Triticum aestivum*) × maize (*Zea mays*) crosses. *Plant Breed.* **111**: 120-124.

48) Suenaga, K. & Nakajima, K. (1993). Segregation of genetic markers among wheat doubled haploid lines derived from wheat × maize crosses. *Euphytica* **65**: 145-152.

49) Ushiyama, T. *et al.* (1991). High frequency of haploid production of wheat through intergeneric cross with teosinte. *Japan. J. Breed.* **41**: 353-357.

50) Zenkteler, M. & Nitzsche, W. (1984). Wide hybridization experiments in cereals. *Theor. Appl. Genet.* **68**: 311-315.

51) Zhou, H. (1996). Genetics of green plant regeneration from anther culture in cereals. *In 'In vitro* Haploid Production in Higher Plants, vol. 2, Applications', Jain, S.M. *et al.* (eds.), Kluwer Academic Publishers, Dordrecht, 169-187.

超遠縁交雑を利用するコムギ半数体の作出に関する技術開発

稲垣正典

国際農林水産業研究センター生物資源部
（〒305　茨城県つくば市大わし１－２）

摘　要

コムギの品種改良に要する年限を短縮する方法のひとつは，半数体を利用して遺伝的に均一である純系を即時に作出し，これらを選抜に供試することである。とくに，開発途上国においては，諸障害に抵抗性の品種を早期に育成しその生産性を安定向上させる必要性が高い。これらの観点から，国際農林水産業研究センターは，国際乾燥地域農業研究センター（シリア）および国際トウモロコシ・コムギ改良センター（メキシコ）との間でコムギ半数体の作出と利用に関する共同研究を1986年より実施してきた。本報告は，この共同研究の成果をもとにした。

コムギの半数体作出法としては，すでに葯（花粉）培養法が知られている。しかし，半数体作出頻度はコムギの品種によって大きく異なり，一部の品種から作出ができないという欠点は現在でも解決されていない。これに対して，遠縁交雑を利用する半数体作出法は，異属あるいは異亜科の植物の花粉と交雑させ，その受精卵から花粉親の染色体が消失することにより半数性のコムギ胚を形成させることにある。

花粉親として異属のオオムギ野生種*Hordeum bulbosum* L.を用いる場合，その交雑和合性にコムギ品種間で大きな差がみられ，一部の品種では半数体が作出できない。これを打破する方法として，*H. bulbosum*の系統選抜および植物生長調節物質2,4-Dの利用を試みた。交雑和合性の品種では結実率および胚形成率の向上が得られたが，交雑不和合性を十分に打破することはできなかった。

その後，亜科の異なるトウモロコシを花粉親としてコムギに交雑すると受精はするがトウモロコシの染色体が急速に消失し胚形成に至らないことが報告された。そこで，コムギにトウモロコシ花粉を授粉した後に2,4-Dを処理した結果，*H. bulbosum*と交雑不和合性のコムギ品種からも半数性の胚を高率で獲得することに成功した。さらに，他の花粉親について検討した結果，トウジンビエはトウモロコシと同様な効果を有するが，モロコシは*H. bulbosum*に似てコムギ品種によっては交雑不和合性を示したので，花粉親としてトウモロコシおよびトウジンビエが有効であることを明らかにした。

トウモロコシおよびトウジンビエとの超遠縁交雑を利用するコムギ半数体の作出では，交雑時にこれらの新鮮花粉が常時必要であり，また，コムギの材料が離れた場所にある場合には交雑を実施しにくいという交雑時期および場所についての改良すべき問題点がある。これらを解決する方法として，花粉の凍結保存およびコムギの切り穂培養について検討した。トウモロコシの花粉は，乾燥および凍結処理により発芽率が低下し，コムギに授粉した場合，新鮮花粉使用の場合に比べて胚形成率が半分以下に減少した。これに対して，トウジンビエの花粉は乾燥および凍結処理に耐性があり，凍結保存花粉使用の場合でもコムギの胚形成率の減少は認められなかった。一方，切り穂培養の効果については，切り穂としない通常の交雑方法と比較してコムギの半数体作出頻度に変化がないことをトウモロコシおよびトウジンビエの新鮮および貯蔵花粉を用いて確認した。以上の結果から，凍結保存花粉およびコムギの切り穂を用いてコムギ半数体作出に必要な交雑操作を効率化することができた。

超遠縁交雑を利用するコムギ半数体作出の効率は，現在のところ，コムギ１穂当たり２個の半数体（染色体倍加処理後には1.5個体の倍加半数体）の作出である。今後，コムギの品種改良において育種年限の短縮の一方法として利用されると期待される。最後に，コムギ材料の準備から交雑，胚培養，染色体倍加の過程を経て倍加半数体の作出までの操作について詳述した。

キーワード：コムギ，トウモロコシ，トウジンビエ，半数体，遠縁交雑，胚培養，染色体倍加，花粉貯蔵，切り穂培養，育種法