PRODUCTION OF WHEAT HAPLOIDS USING ANTHER CULTURE AND WHEAT X MAIZE HYBRIDIZATION TECHNIQUES

R. S. Sadasivaiah, B. R. Orshinsky and G. C. Kozub
Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada T1J 4B1

SUMMARY

Ten spring wheat breeding lines derived from diverse crosses differed significantly in their response to anther culture and wheat x maize hybridization techniques for the production of haploids. Depending on the genotype, the frequency of embryo induction from cultured anthers ranged from 0.1 to 55.6 per 100 anthers with a mean of 16.3. Plant regeneration from cultured embryos also varied with the genotype. Although the embryos from one line failed to regenerate, those from the nine other lines produced both green and albino plantlets. The frequency of green plants produced per 100 cultured anthers ranged from 0 to 4.73 with a mean of 1.64. Depending on the genotype, 25 to 75% of the anther-derived green plants showed spontaneous chromosome doubling. The wheat x maize hybridization technique was highly effective in producing haploids in all ten breeding lines, although the frequency varied with genotype. The percentage of seed set ranged from 52.7 to 71.6 with a mean of 62.5, but only 19.3% of the seeds contained embryos. About 75% of the cultured embryos developed into green plants. The number of green plants produced per 100 pollinated florets ranged from 2.40 to 12.66 with a mean of 6.29, all having the haploid chromosome number of 21. The line recalcitrant to anther culture produced the highest number of haploids with the wheat x maize hybridization method. There was no evidence of albinism or spontaneous chromosome doubling. The reduced genotype specificity, absence of albinism and ease of application, makes the wheat x maize hybridization technique more efficient than anther culture for the production of haploids in common wheat.

Key words: *Triticum aestivum,* haploids, anther culture, wheat x maize hybridization.

INTRODUCTION

Interest in haploids as a genetic and breeding tool is increasing concomitant with the development of more efficient methods for their production. The two major techniques for haploid production in cereals are anther/microspore culture and wide hybridization.

In wheat, use of anther culture in breeding programs is limited by strong genotype specificity, low frequency of haploids, poor embryo quality, and a high rate of albinism in regenerants (Kisana et al., 1993; Orshinsky and Sadasivaiah, 1994; Lefebvre and Devaux, 1996). Despite these problems, the anther culture technique has been successfully applied in some wheat breeding programs, resulting in new cultivars (Hu et al., 1986; De Buyser et al., 1987; R. Graf, 1997, personal comm.).

Production of haploids by wide hybridization followed by the preferential (spontaneous) elimination of the chromosomes of the pollen parent during embryogenesis has been the subject of considerable study since it was first reported from studies of reciprocal interspecific crosses between a wild (*Hordeum bulbosum* L.) and cultivated barley (*Hordeum vulgare* L.) (Symko, 1969; Kasha and Kao, 1970; Lange, 1971; Subrahmanyam and Kasha, 1973). Barclay (1975) recovered a high frequency of haploids of wheat (*Triticum aestivum* L. var. Chinese Spring) through a similar chromosome elimination process in crosses with *H. bulbosum*. However, most

wheat varieties have very low crossability to *H. bulbosum* because of the presence of dominant alleles of the Kr1 and Kr2 gene(s) which restrict the application of the *H. bulbosum* technique to haploid production in wheat (Snape et al., 1979; Falk and Kasha, 1981, 1983). The discovery by Laurie and Bennett (1986) that intergeneric crosses between wheat and maize (*Zea mays* L.) resulted in the production of wheat haploids and that maize appeared insensitive to the action of dominant alleles at wheat crossability loci (Kr1 and Kr2) indicated that wheat x maize crosses could be a valuable alternative to wheat x *H. bulbosum* for production of wheat haploids via chromosome elimination (Laurie and Bennett, 1987, 1988; Suenaga and Nakajima, 1989; Inagaki and Tahir, 1990; Laurie and Reymondie, 1991; Suenaga et al., 1991).

This paper will report on success rates of wheat haploid production using both anther culture and wheat x maize hybridization techniques and briefly discuss their potential as tools in wheat cultivar development programs.

MATERIAL AND METHODS

Plant growth conditions

Ten breeding lines derived from various crosses of soft white spring wheat were used (Table 1). Anther and embryo donor plants were grown in a growth cabinet at 19/16°C day/night temperature and 16/8 h photoperiod. Hybrid (CL30/CL34) maize plants used as pollen source were grown in a growth room maintained at 23/20°C day/night temperature and 16/8 h photoperiod.

Table 1. Material used in the study

Line	Pedigree
B-353	AC Reed//Owens/IDO159
B-359	AC Reed//SWS-103/SWS-18
B-700x	89-B33/AC Reed
B-788y	Dirkwin/Treasure//Blanca/Fielder
B-792y	Dirkwin/Treasure//Blanca/Fielder
B-799	Dirkwin/8021-V2//Treasure/Blanca
B-812	HY-355/L(Ind)-19//Wadual/L(Ind)-21
B-854x	89-B33/AC Reed
B-878	SWS-15/SWS-18//Blanca/Treasure
B-91034	AC Reed/Centennial
Maize hybrid	CL30/CL34

x, y, denotes sister lines.

Anther culture

Anthers from primary and secondary florets containing mid-to-late uninucleate microspores were aseptically removed and cultured for 4 to 7 weeks at 30°C in the dark in MN6 liquid medium (Orshinsky and Sadasivaiah, 1994). Embryo induction was recorded as the number of embryos produced per 100 anthers. Small (less than 1 mm in diameter), white or translucent, and poorly developed embryos were not cultured. Firm, well-developed embryos that were 2-3 mm in diameter were transferred onto MS (Murashige and Skoog, 1962) agar-solidified regeneration

medium containing sucrose (20g/l) + 1 mg/l indole-3-acetic acid + 1 mg/l kinetin + 146 mg/l glutamine (Schaeffer et al., 1979), and cultured at 25 °C with a 16/8h photoperiod for 4 to 5 weeks. Regenerated green plantlets were transferred to plastic trays containing Cornell mix (Boodley and Sheldrake, 1977). Once the plantlets were established with good shoot and root systems, they were transferred to root trainers. Plant production was recorded as the number of green plants produced per 100 cultured anthers. The percentages of embryos produced and their subsequent developmental fate (Table 2) were determined for each line and compared statistically (Fleiss, 1981). The CATMOD procedure for categorical modelling in SAS (SAS Institute Inc., 1989) was used to carry out the statistical analyses.

Wheat x maize hybridization procedures

Spikes were hand-emasculated 1-2 days prior to anthesis and covered with glassine bags. One to two days after emasculation the spikes were pollinated with freshly collected maize pollen. On the two consecutive days after pollination the spikes were sprayed with a solution of 75 ppm 2,4-D. On days 3 and 4 after pollination the spikes were sprayed with 300 ppm gibberellic acid (GA_3) and re-covered with glassine bags after each spraying.

Embryo rescue

Spikes were harvested at 16-19 days after pollination and the number of intact, non-selfed florets in each was counted and recorded as the number of florets pollinated. Seeds were removed from the florets, sterilized in 70% ethanol for 30 sec., briefly rinsed in sterile distilled water, then sterilized for 2 min. in 2.6% sodium hypochlorite (or 50% Javex) with a final 2 min. rinse in sterile distilled water. Embryos were aseptically excised under a stereomicroscope. Small, poorly developed embryos were counted to determine the total embryos formed but were not cultured. Only well-developed embryos were transferred, keeping the embryo axis upwards, to culture vials containing a half-strength MS basal medium supplemented with sucrose (20 g/l), vitamins (myo-inositol, 100 mg/l; thiamine HCl, 1 mg/l; nicotinic acid, 0.5 mg/l; pyridoxine HCl, 0.5 mg/l), amino acids (glutamine, 146 mg/l; glycine, 2 mg/l) and agar (8 g/l). The pH of the medium was adjusted to 5.8 prior to autoclaving.

Vials containing embryos were kept in the dark at 4 °C for 2 days prior to incubating in the dark at 22 °C for 1-2 weeks and then transferred to a 16/8h light regime at 25 °C. Germinated and non-germinated embryos were counted, and seedlings with well-developed roots and shoots were transplanted to root trainers filled with Cornell mix for subsequent growth in a growth cabinet maintained at 15/12 °C (day/night) and a 16/8h photoperiod. The percentages of florets with seed, seeds with embryos and embryo developmental fate (Table 3) were determined for each line and compared statistically.

RESULTS

Anther culture

The number of embryos per 100 anthers ranged from 0.1 (B-812) to 55.6 (B-854) with a mean of 16.3. The two embryos derived from B-812 failed to regenerate, but cultured embryos from the nine other breeding lines produced both green (4.0% to 41.5%) and albino (21.6% to 35.0%) plantlets. From 36.9% to 67.7% of the embryos failed to establish plantlets. The frequency of green plantlets produced per 100 cultured anthers from individual lines varied from 0 to 4.73

(Table 2) with an overall mean of 1.64. Depending on the genotype, 25 to 75% of the anther-derived green plants (mean of 44.1) showed spontaneous chromosome doubling (data not presented). The effect of genotype on the outcomes of culture and regeneration was highly significant ($P<0.001$) except for the percentage of cultured embryos with albino shoots ($P<0.05$).

Table 2. Effect of genotype on wheat haploid production with the anther culture technique

Line	Embryos/ 100 anthers	% of embryos cultured	% of embryos with green shoots	% of embryos with albino shoots	% of embryos with no shoots	Green plantlets/ 100 anthers
B-353	10.1±0.6* (2341)**	70.8±3.0 (236)	6.6±1.9 (167)	25.7±3.3 (167)	67.7±3.6 (167)	0.47±0.14 (2341)
B-359	15.1±0.9 (1507)	71.5±3.0 (228)	11.0±2.4 (163)	27.6±3.5 (163)	61.4±3.8 (163)	1.19±0.27 (1507)
B-700x	7.9±0.6 (1854)	71.9±3.7 (146)	19.0±3.8 (105)	34.3±4.6 (105)	46.7±4.9 (105)	1.08±0.24 (1854)
B-788y	26.8±1.2 (1311)	56.1±2.6 (351)	9.6±2.1 (197)	35.0±3.4 (197)	55.4±3.5 (197)	1.45±0.33 (1311)
B-792y	32.5±1.2 (1487)	52.2±2.3 (483)	4.0±1.2 (252)	30.6±2.9 (252)	65.4±3.0 (252)	0.67±0.21 (1487)
B-799	3.5±0.4 (1896)	74.6±5.3 (67)	30.0±6.5 (50)	22.0±5.8 (50)	48.0±7.1 (50)	0.79±0.20 (1896)
B-812 †	0.1 (1615)	100.0 (2)	0.0 (2)	0.0 (2)	100.0 (2)	0.00 (1615)
B-854x	55.6±1.3 (1469)	53.9±1.7 (817)	13.6±1.6 (440)	32.5±2.2 (440)	53.9±2.4 (440)	4.08±0.52 (1469)
B-878	14.5±0.9 (1542)	78.6±2.7 (224)	41.5±3.7 (176)	21.6±3.1 (176)	36.9±3.6 (176)	4.73±0.54 (1542)
B-91034	11.3±0.7 (2247)	66.8±3.0 (253)	25.4±3.4 (169)	32.0±3.6 (169)	42.6±3.8 (169)	1.91±0.29 (2247)
P	<0.001	<0.001	<0.001	<0.05	<0.001	<0.001

x, y denotes sister lines; * Standard error; ** Number in parentheses is the sample size N.
† B-812 is omitted from statistical analysis when N = 2.

Wheat x maize hybridization

The percentage of seed set ranged from 52.7 (B-91034) to 71.6 (B-878) with an overall mean of 62.5. However, only 14.7 (B-799) to 28.9% (B-812) of the seed, with a mean of 19.3%,

contained an embryo. From 35.2 (B-854) to 81.6% (B-812) of the embryos obtained were considered culturable and 64.4 (B-799) to 86.9% (B-792) of these, with a mean of 75.6%, developed into green plantlets. The frequency of green plantlets per 100 florets ranged from 2.40 (B-854) to 12.66 (B-812) with a mean 6.29. The line recalcitrant to anther culture (B-812) produced the highest number of haploids (12.66) per 100 florets (Table 3). All randomly selected seedlings that were examined cytologically had the haploid chromosome number of 21. There was no evidence of albinism or spontaneous chromosome doubling. The effect of genotype on all the outcomes assessed was highly significant (P<0.001).

Table 3. Effect of genotype on wheat haploid production with the wheat x maize hybridization technique

Line	% Florets with seed	% seeds with emb. Total	% seeds with emb. culturable	% of total emb. cultured	% of cultured emb. with green shoots	Green plantlets/ 100 florets
B-353	65.5±0.9* (2547)**	19.5±1.0 (1668)	15.1±0.9 (1668)	77.3±2.3 (326)	80.6±2.5 (252)	7.97±0.54 (2547)
B-359	60.0±1.2 (1791)	18.2±1.2 (1075)	11.3±1.0 (1075)	62.2±3.5 (196)	75.4±3.9 (122)	5.14±0.52 (1791)
B-700x	66.0±1.2 (1500)	15.9±1.2 (990)	10.6±1.0 (990)	66.9±3.8 (157)	65.7±4.6 (105)	4.60±0.54 (1500)
B-788y	64.0±1.4 (1124)	20.0±1.5 (719)	13.9±1.3 (719)	69.4±3.8 (144)	68.0±4.7 (100)	6.05±0.71 (1124)
B-792y	61.8±1.4 (1234)	22.0±1.5 (763)	17.0±1.4 (763)	77.4±3.2 (168)	86.9±3.0 (130)	9.16±0.82 (1234)
B-799	60.6±1.0 (2354)	14.7±9.4 (1426)	7.3±0.7 (1426)	49.8±3.5 (209)	64.4±4.7 (104)	2.85±0.34 (2354)
B-812	66.9±1.0 (2354)	28.9±1.1 (1576)	23.6±1.1 (1576)	81.6±1.8 (456)	80.1±2.1 (372)	12.66±0.69 (2354)
B-854x	59.5±1.3 (1376)	14.9±1.2 (819)	5.3±0.8 (819)	35.2±4.3 (122)	76.7±6.4 (43)	2.40±0.41 (1376)
B-878	71.6±1.2 (1375)	21.5±1.3 (985)	15.7±1.1 (985)	73.1±3.0 (212)	68.4±3.7 (155)	7.71±0.72 (1375)
B-91034	52.7±1.0 (2377)	15.2±1.0 (1253)	11.2±0.9 (1253)	73.3±3.2 (191)	73.6±3.7 (140)	4.33±0.42 (2377)
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

x, y denotes sister lines; *Standard error; **Number in parentheses is the sample size N.

DISCUSSION

Embryo induction frequencies from cultured anthers varied widely among the lines used in the present study. Line B-854 had the highest embryo induction response (55.6 embryos/100 anthers) while B-812 was recalcitrant producing only 0.1 embryo/100 anthers. The frequency of plantlet regeneration from cultured embryos also varied with genotype. An overall average of 42.2% (0 to 63.1%) of the cultured embryos regenerated giving a mean yield of 1.64 green (0.47 to 4.73) and 3.24 (0 to 9.73) albino plantlets per 100 anthers. In a study evaluating the anther culture response of 38 different three-way wheat crosses, Kisana et al (1993) also observed significant differences among crosses for plantlet regeneration (0 to 11.9, mean of 0.6/100 anthers). The strong genotypic effects, low frequency of haploids, and a high rate of albino regenerants limits the use of the anther culture technique for haploid production in wheat breeding programs (De Buyser and Henry, 1986; Henry and De Buyser, 1990; Orshinsky and Sadasivaiah, 1994; Lefebvre and Devaux, 1996).

All ten of the lines evaluated produced haploids in crosses with maize although they differed significantly in percentage of seed set, embryo formation and plantlet regeneration. The percentage of seed set ranged from 52.7 to 71.6, with a mean of 62.5, but only 19.3% (14.7 to 28.9%) of the seeds contained embryos. From 64.4 to 86.9% of the cultured embryos (mean of 75.6%) regenerated to yield an average of 6.29 (2.40 to 12.66) green plantlets per 100 florets. There was no evidence of albinism or spontaneous chromosome doubling in the regenerants. The genotype (B-812) which was recalcitrant to the anther culture method proved to be good parental material for the wheat x maize method. The wheat x maize hybridization technique for wheat haploid production appears to be particularly promising for those genotypes which have proved recalcitrant or respond poorly to anther culture (see also Kisana et al., 1993; Lefebvre and Devaux, 1996; Fedak et al., 1997). However, further studies are needed to determine if genotypes that respond poorly to the wheat x maize hybridization technique would indeed respond better with anther culture.

Genotypic differences for wheat haploid production efficiency with the maize hybridization method have been reported for both the wheat and maize parents (Suenaga et al., 1991). Laurie and Reymondie (1991) reported that 19 commercial wheat varieties crossed with the maize hybrid 'Seneca 60' produced an average of 20 haploid plants per 100 pollinated florets. However, Kisana et al (1993) only obtained an average of 4.7 haploid plants (2.7 to 6.3) per 100 florets from wheat x maize crosses. Using 5 maize parents crossed with 18 wheat hybrids, Lefebvre and Devaux (1996) found genotypic differences for haploid production among combinations of parents and the average number of haploids produced per 100 florets was 9.1 which is similar to the frequency (9.5 per 100 florets) reported by Inagaki and Tahir (1990). These results and those obtained in the present study show that the wheat x maize hybridization technique is an effective means of producing wheat haploids from a wide range of genotypes.

In the present study, the wheat x maize method gave a considerably higher yield of haploid plants (6.29/100 pollinated florets) than the anther culture method (1.64/100 cultured anthers). Kisana et al (1993) and Fedak et al (1997) also arrived at similar conclusions from their studies of doubled haploid production by both anther culture and wheat x maize hybridization methods. Furthermore, there is a potential for further improvement in haploid production frequency with the wheat x maize method by the use of more compatible maize genotypes (Lefebvre and Devaux, 1996) and with the improvement of culture media to rescue poorly developed embryos (Comeau

et al., 1992). Another advantage is that wheat x maize-derived plants appear to be cytologically stable whereas some plants derived from anther culture have chromosomal abnormalities (De Buyser and Henry, 1986; Kisana et al., 1993). The reduction or absence of genotype specificity, absence of albinism, greater chromosomal stability of derived plants, reduction in steps and time in culture, and the ease with which it can be applied, makes the wheat x maize hybridization technique much more efficient than the anther culture for the production of wheat haploids.

Homozygous doubled haploid lines derived from spontaneous and/or induced chromosome doubling offer the unique advantage of attaining instant homozygosity which enhances the precision of genetic studies and efficiency of selection in breeding programs. The use of doubled haploids as a breeding tool has the potential to greatly improve selection efficiency, reduce research costs, and deliver improved cultivars up to 4 years earlier than would be possible using conventional breeding methods (Hu et al., 1986; De Buyser et al., 1987). Reduction of the number of generations required for cultivar development is one of the major benefits to be gained by incorporating doubled haploid methods into conventional breeding programs.

ACKNOWLEDGEMENTS

This work was supported in part by the Farming for the Future program (Project #950648) of the Alberta Agricultural Research Institute. The authors are grateful to Dr. J. R. Byers for constructive comments and helpful suggestions. Lethbridge Research Centre contribution No. 387-9845.

REFERENCES

Barclay, I. R. 1975. High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature 256: 410-411.

Boodley, J. W., and Sheldrake, R. 1977. Cornell peat-lite mixes for commercial plant growing. New York State College of Agriculture and Life Sciences, Ithaca, NY, Information Bulletin, 43.

Comeau, A., Nadeau, P., Plourde, A., Simard, R., Maes, O., Kelly, S., Harper, L., Lettre, J., Landry, B., and St-Pierre, C.A. 1992. Media for the in ovulo culture of proembryos of wheat and wheat-derived interspecific hybrids or haploids. Plant Sci. 81: 117-125.

De Buyser, J., and Henry, Y. 1986. Wheat: Production of haploids, performance of doubled haploids, and yield trials. In: Y.P.S. Bajaj (ed.) Biotechnology in Agriculture and Forestry, Vol.2: Crops I. Springer-Verlag, Berlin, Heidelberg. pp 73-88.

De Buyser, J., Henry, Y., Lonnet, P., Hertzog, R., and Hespel, A. 1987. 'Florin': a doubled haploid wheat variety developed by the anther culture method. Plant Breed. 98: 53-56.

Falk, D. E., and 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.

Falk, D. E., and Kasha, K. J. 1983. Genetic studies of the crossability of hexaploid wheat with rye and *Hordeum bulbosum*. Theor. Appl. Genet. 64 : 303-307.

Fedak, G., Burvill, M., and Voldeng, H. D. 1997. A comparison of anther culture and maize pollination for haploid production in wheat. J. Appl. Genet. 38: 407-414.

Fleiss, J. L. 1981. Statistical methods for rates and proportions. 2nd ed. John Wiley and Sons, Inc., New York.

Henry, Y., and De Buyser, J. 1990. Wheat anther culture: Agronomic performance of doubled haploid lines and release of a new variety 'Florin'. In: Y.P.S. Bajaj (ed.) Biotechnology in Agriculture and Forestry, Vol. 13. Spinger-Verlag, New York. pp. 285-352.

Hu, D., Yuan, Z., Tang, Y., and Liu, J. 1986. Jinghua-1. A winter wheat variety derived from the pollen sporophyte. Scientia Sinica (Series B) 29: 733-745.

Inagaki, M., and Tahir, M. 1990. Comparison of haploid production frequencies in wheat varieties crossed with *Hordeum bulbosum* L. and maize. Japan. J. Breed. 40: 209-216.

Kasha, K. J., and Kao, K. N. 1970. High frequency haploid production in barley (*Hordeum vulgare* L.). Nature 225: 874-876.

Kisana, N. S., Nkongolo, K.K., Quick, J. S., and Johnson, D. L. 1993. Production of doubled haploids by anther culture and wheat x maize method in a wheat breeding programme. Plant Breed. 110: 96-102.

Lange, W. 1971. Crosses between *Hordeum vulgare* L. and *H. bulbosum* L. I. Production, morphology and meiosis of hybrids and dihaploids and haploids. Euphytica 20: 14-29.

Laurie, D. A., and Bennett, M. D. 1986. Wheat x maize hybridization. Can. J. Genet. Cytol. 28: 313-316.

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

Laurie, D. A., and Bennett, M. D. 1988. The production of haploid wheat plants from wheat x maize crosses. Theor. Appl. Genet. 76: 393-397.

Laurie, D. A., and 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.

Lefebvre, D., and Devaux, P. 1996. Doubled haploids of wheat from wheat x maize crosses: genotypic influence, fertility and inheritance of the 1BL-1RS chromosome. Theor. Appl. Genet. 93: 1267-1273.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Plant Physiol. 15: 473-497.

Orshinsky, B. R., and Sadasivaiah, R. S. 1994. Effects of media on embryoid induction and plant regeneration from cultured anthers of soft white spring wheats (*Triticum aestivum* L.). Plant Sci. 102: 99-107.

Schaeffer, G. W., Baenziger, P. S., and Worley, J. 1979. Haploid plant development from anthers and in vitro embryo culture of wheat. Crop Sci. 19: 698-702.

SAS Institute Inc. 1989. SAS/STAT User's guide, version 6. 4th ed. SAS Institue, Inc., Cary NC

Snape, J. W., Chapman, V., Moss, J., Blanchard, C. E., and Miller, T. E. 1979. The crossabilities of wheat varieties with *Hordeum bulbosum*. Heredity 42: 291-298.

Subrahmanyam, N. C., and Kasha, K. J. 1973. Selective chromosomal elimination during haploid formation in barley following interspecific hybridization. Chromosoma (Berl.) 42: 111-125.

Suenaga, K., and 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.

Suenaga, K., Tamaki, M., and Nakajima, K. 1991. Influence of wheat (*Triticum aestivum*) and maize (*Zea mays*) genotypes on haploid wheat production in crosses between wheat and maize. Bull. Natl. Inst. Agrobiol. Resour. 6: 131-142.

Symko, S. 1969. Haploid barley from crosses of *Hordeum bulbosum* (2x) x *H. vulgare* (2x). Can. J. Genet. Cytol. 11: 602-608.

Received 11 September, 1998, accepted 9 October, 1998