CROSSABILITY OF TRITICALE (X *Triticosecale* Wittmack) WITH MAIZE AND THE EFFECTS OF D-GENOME CHROMOSOMES

M.N. Inagaki[1], M. Mergoum[1], W.H. Pfeiffer[1], A. Mujeeb-Kazi[1] and A.J. Lukaszewski[2]

[1] International Maize and Wheat Improvement Center (CIMMYT), Lisboa 27, Colonia Juarez, Apartado Postal 6 – 641, 06600 Mexico, D. F., Mexico
[2] University of California, Riverside, CA 92521, USA

ABSTRACT. Haploid production in cereal breeding programs can be a valuable tool to fix economic traits in a short time. Hence, an economically feasible technique to produce haploids should be explored. This study was conducted to investigate haploid production in triticale using the maize method. Twenty genotypes from the CIMMYT breeding program and eight D-genome chromosome substitution lines involving the cultivar Rhino were crossed with maize. In crosses involving the 20 triticales, 15 lines produced embryos. The frequency of embryo formation ranged from 0.0 to 5.4%, with an average of 1.1%. From a total of 200 pollinated spikes, 62 plants were regenerated. Most regenerated plants were haploids with 21 chromosomes, and a few aneuhaploids with 22 chromosomes were found. In crosses involving the substitution lines with maize, all the lines produced embryos, while 'Rhino' (control) did not produce embryos. Higher frequencies of embryo formation were obtained in substitution lines with chromosomes 2D and 4D. These results suggest that D-genome chromosomes, particularly chromosome 4D and 2D, in a genetic background can be exploited to increase the frequency of haploid production in triticale x maize crosses.

INTRODUCTION

Most triticale (X *Triticosecale* Wittmack) breeding programs have focused on developing hexaploid triticales (genome composition: AABBRR, $2n = 6x = 42$). These secondary triticales originate from crosses between tetraploid wheat *(Triticum turgidum* L. var. *durum,* genome composition = AABB) and rye *(Secale cereale* L., genome composition = RR) followed by chromosome doubling. In enhancement programs, secondary triticales are frequently crossed with octoploid triticales (genome composition = AABBDDRR) and hexaploid wheat (*T. aestivum* L. em Thell., genome composition = AABBDD) as sources of genetic variability, to improve commercial quality traits by incorporating D-genome chromosomes or segments. The International Maize and Wheat Improvement Center's (CIMMYT's) triticale improvement program has emphasized breeding for resistance to biotic and abiotic stresses, as well as good grain quality (Varughese et al., 1996a,b). This breeding process, particularly of winter growth habit germplasm, could be accelerated by using triticale doubled haploids in which genetic homozygosity ensures accurate selection of recombinant lines with favorable traits. An efficient technique for producing haploids is essential to complement conventional breeding programs. Rapid production of triticale haploids followed by chromosome doubling is required for the application of the maize method in breeding programs. A possible technique for producing haploids is wide hybridization followed by elimination of the pollen donor chromosomes, which has been widely used for producing haploids of wheat (Inagaki, 1996). In this paper, the crossabilities involving triticale genotypes with maize *(Zea mays* L.), and the effects of the presence of D-genome chromosomes in a hexaploid triticale substitution series on crossability with maize were investigated.

MATERIALS AND METHODS

PLANT MATERIAL: Twenty genotypes of triticale selected from CIMMYT's triticale breeding program and eight D-genome chromosome substitution lines of cv. Rhino were used as the female parents. Rhino is a hexaploid spring triticale developed at CIMMYT, that carries a complete set of 14 chromosomes from each of the A, B and R genomes. The hexaploid triticale lines with A or B genome chromosomes substituted by D-genome chromosomes were developed by A. Lukaszewski through selection in the hybrid progenies involving a cross between octoploid and tetraploid triticales, and then transferred through consecutive backcrosses to 'Rhino' (Lukaszewski et al., 1987; Lukaszewski, 1991). For example, a substitution line 1D(1A) carries chromosome 1D instead of chromosome 1A. The maize pollen parent was a single-cross hybrid line, CML-246 x CML-242, developed at CIMMYT. Triticale plants were grown in potted soil in a greenhouse controlled at 29/14°C (max/min). Maize plants were grown in ground soil in a greenhouse without temperature control. Temperature conditions were 35/10°C (max/min) at maize anthesis.

CROSSING: At ear emergence, tillers with spikes were cut off at the base and cultured in a flask containing tap water. Emasculated spikes were pollinated with maize pollen 1 or 2 days before predicted anthesis. After pollination, triticale spikes were cultivated in a solution containing 40 g L^{-1} sucrose, 8 ml L^{-1} sulfurous acid (6% SO_2) and 100 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) until embryo rescue. Procedures of the detached-tiller culture were minor modifications of the method of Inagaki et al. (1997). Five to seven spikes (about 50 florets/spike) randomly selected from 12 plants were used for each crossing treatment, with two replications. Statistical data analysis of embryo formation frequencies were performed after formation.

EMBRYO RESCUE: Fifteen to 17 days after pollination, immature embryos were aseptically excised from seeds, and transferred onto half-strength Murashige and Skoog (1962) culture medium supplemented with 20 g L^{-1} sucrose and 6 g L^{-1} agarose. The embryos were incubated at 25°C, l2-h daylength and *e.* 5000 1ux fluorescent light intensity.

CYTOLOGICAL EXAMINATION: Regenerated plants were cytologically examined in squashed preparations of root-tips stained with acetocarmine, according to the method of Mujeeb-Kazi and Miranda (1985). Some of the preparations were further treated with modified Giemsa-staining procedures (Jahan et al., 1990), and C-banded chromosomes were identified according to the karyotypes of wheat and rye (Lukaszewski and Gustafson, 1983; Lukaszewski et al., 1987).

RESULTS AND DISCUSSION

Seeds that developed after crossing with maize were somewhat smaller than selfed seeds, and filled with an aqueous solution, lacking the solid endosperm found in selfed seeds. Some crossed seeds contained immature embryos. The numbers of seeds set, embryos formed and plants regenerated in crosses of 20 triticale genotypes with maize are shown in the upper part of Table 1. Frequencies of embryo formation ranged from 0.0 to 5.4%. Out of 20 triticale genotypes examined (200 spikes pollinated in total), 15 genotypes produced a total of 107 embryos. From

these embryos, 62 plants were regenerated, resulting in a success rate of 60.8%. Mean frequencies of seed setting, embryo formation and plant regeneration were 4.7%, 1.1% and 0.6%, respectively. A total of 59 plants were used for cytological examination. Among these, 57 plants were triticale haploids carrying a complement of 21 chromosomes. Two plants were aneuhaploids with 22 chromosomes: 14 wheat and 8 rye. The extra rye chromosome in these aneuhaploids was 4R.

Crossability of hexaploid triticale with maize was relatively low and strongly dependent on genotype. Mean frequency of embryo formation across the 20 triticale genotypes examined was 1.1%, that is, one embryo from two triticale spikes pollinated with maize. After plant regeneration, mean frequency of haploid production decreased to 0.6%, that is, only one haploid from four triticale spikes pollinated with maize. Most plants regenerated from hexaploid triticale x maize crosses were haploids with 21 chromosomes. Stable chromosome number of regenerated plants was also obtained in hexaploid wheat x maize crosses (Inagaki and Mujeeb-Kazi, 1995). These facts suggested that triticale haploid production through maize crosses has an advantage for use in breeding and genetic analyses.

All the substitution lines examined produced seeds and embryos at frequencies of 2.9 – 40.1% and 1.3 – 14.4%, respectively, whereas Rhino did not produce seeds or embryos, as shown in the lower part of Table 1. Higher frequencies of embryo formation were obtained in substitution lines 2D(2A), 2D(2B) and 4D(4B). This suggested an effect of individual D-genome chromosomes, in particular, chromosomes 2D and 4D, in increasing the crossability with maize in the genetic background of Rhino. It has been confirmed that many hexaploid triticale genotypes contain chromosomes or segments of the D-genome (in particular, chromosomes 2D and 6D) as a result of crossing with octoploid triticale and hexaploid wheat (Lukaszewski et al., 1987; Lukaszewski, 1988). The frequency of haploid production in hexaploid triticale may increase along with improved grain quality and disease resistance from introduced D-genome chromosomes in triticale. However, the presence of individual D-genome chromosomes in hexaploid triticale does not always indicate high crossability with maize, because breeder's selections were irrespective of crossability, and the effect of individual D-genome chromosomes may not be sufficient to increase crossability in different triticale genetic backgrounds. Thus, technical advances for efficient haploid production cannot be ensured by selecting genotypes which carry D-genome chromosomal material.

Seed setting frequencies in hexaploid triticale crossed with maize were very low (4.7% for the 20 genotypes, 13.7% for the eight substitution lines), suggesting that applying 2,4-D after maize pollination did not sufficiently promote seed development. However, 2,4-D is critical for enhancing seed development in hexaploid wheat, even when fertilization does not occur in wide crosses (Inagaki, 1986; Inagaki and Tahir, 1990). In addition to 2,4-D, we need to identify chemicals such as silver nitrate that enhance seed development in tetraploid wheat x maize crosses (O'Donoughue and Bennett, 1994). As alternative pollen sources, maize-related species such as eastern gamagrass *(Tripsacum dactyloides* L.) and teosinte (Z. *mays* L. spp. *mexicana)* may increase embryo formation frequency, as confirmed in haploid production of tetraploid wheat (Riera-Lizarazu and Mujeeb-Kazi, 1993; Dusautoir et al., 1995). Selection of plant growth regulators and pollen sources is crucial for developing seeds and embryos in hexaploid triticale x maize crosses.

Table 1. Seed set, embryo formation and plant regeneration in 20 hexaploid triticale genotypes and 8 chromosome substitution lines crossed with maize. Numbers followed by the same letter are not significantly different at P=5%.

	Pedigree/Substitution	Number of florets pollinated	Number of seeds set (%)	Number of embryos formed (%)	Number of plants regenerated (%)
1.	Buf 4//Jlo 97/Civet/3/Lamb 1//Reh/ Yogui 1	444	64 (14.4)	24 (5.4)	19
2.	Anoas 3/Tatu 4	500	125 (25.0)	20 (4.0)	12
3.	Musx/Lynx//Yogui 1/3/Fahad 4	506	43 (8.5)	20 (4.0)	7
4	Manati 1	488	70 (14.3)	8 (1.6)	6
5.	Ona 2/Poss 1-2	512	45 (8.8)	7 (1..3)	5
6.	Rondo/Bant 5//Anoas 2	418	9 (2.5)	5 (1.2)	3
7.	Gnu/Asad//Ardi/3/Manati 1	548	19 (3.5)	5 (0.9)	3
8.	Hare 7265/Yogui 1//Bull 2	540	38 (7.0)	4 (0.7)	2
9.	CMH77A.1024/2*Yogui//Lamb 4	546	8 (1.5)	4 (0.7)	3
10.	Bull 10/Manati 1	550	12 (2.2)	2 (0.4)	0
11.	Caracal	432	8 (1.9)	2 (0.4)	1
12.	Zebra 79/Lynx*2//Fahad 1	510	6 (1.8)	2 (0.4)	0
13.	Uron 7//Sika 26/Hare 337	482	3 (0.6)	2 (0.4)	1
14.	Erizo 10/Bull 1-1	498	13 (2.6)	1 (0.2)	0
15.	Fahad 5	442	9 (2.0)	1 (0.2)	0
16.	Lamb 2	442	0 (0.0)	0 (0.0)	0
17.	Rhino 3//Bull 1-1	550	0 (0.0)	0 (0.0)	0
18.	Supi 3//Hare 7265/Yogui 1	576	0 (0.0)	0 (0.0)	0
19.	Ardi 1/Topo 1419//Erizo 9	518	0 (0.0)	0 (0.0)	0
20.	Lasko/2*Erizo 11/3/Yogui 1/Tesmo 5/ Uron 6	516	0 (0.0)	0 (0.0)	0
	Total	10020	472 (4.7)	107 (1.1)	62
	Rhino substitution line				
1.	Rhino 1D(1A)	448	13 (2.9)	11 (2.5)ab	4
2.	Rhino 2D(2A)	424	89 (21.0)	18 (4.2)b	9
3.	Rhino 2D(2B)	472	114 (24. 1)	30 (6.3)bc	11
4.	Rhino 3D(3A)	422	35 (8.3)	11 (2.6)ab	5
5.	Rhino 4D(4B)	604	242 (40.1)	87 (14.4)c	30
6.	Rhino 5D(5A)	664	24 (3.6)	13 (2.0)ab	2
7.	Rhino 6D(6A)	636	38 (6.0)	8 (1.3)ab	4
8.	Rhino 7D(7A)	478	15 (3.1)	12 (2.5)ab	4
9.	Rhino (control)	442	0 (0.0)	0 (0.0)a	0

REFERENCES

Dusautoir, J.C., M. Coumans, F. Kaan, and F. Boutouchent. 1995. Genotype effect and histological events in relation to embryo formation after intergeneric crosses between

durum wheat *(Triticum turgidum* Desf. var *durum)* and maize *(Zea mays* L.) or teosinte *(Zea mays* L. ssp. *mexicana).* J. Genet. Breed. 49:353-358.

Inagaki, M.N. 1986. Crossability of Japanese wheat cultivars with *Hordeum bulbosum* L. Japan. J. Breed. 36:363-370.

Inagaki, M.N. 1996. Technical advances in wheat haploid production using ultra-wide crosses. JIRCAS J. (Japan) 4:51-62.

Inagaki, M.N. and A. Mujeeb-Kazi. 1995. Comparison of polyhaploid production frequencies in crosses of hexaploid wheat with maize, pearl millet and sorghum. Breed. Sci. 45:157-161.

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

Inagaki, M.N., T. Naganaine, and A. Mujeeb-Kazi, 1997. Use of pollen storage and detached-tiller culture in wheat polyhaploid production through wide crosses. Cereal Res. Comm. 25:7-13.

Jahan, Q., N. Ter-Kile, N. Hashmi, M. Aslam, A.A. Vahidy, and A. Mujeeb-Kazi. 1990. The status of the 1B;1R translocation chromosome in some released wheat varieties and the 1989 candidate varieties of Pakistan. Pak.1. Bot. 22:1-10.

Lukaszewski, A.J.. 1988. Chromosome constitution of hexaploid triticale lines in the recent international yield trials. Plant Breeding 100:268-272.

Lukaszewski, A.J.. 1991. Development of aneuploid series in hexaploid triticale. pp. 397-401. In. Proc. 2nd Int. Triticale Symp., CIMMYT, Mexico, D. F.

Lukaszewski, A.J, and J.P. Gustafson. 1983. Translocations and modifications of chromosomes in triticale x wheat hybrids. Theor. Appl. Genet. 64:239-248.

Lukaszewski, A.J., B. Apolinarska, and J.P. Gustafson. 1987. Introduction of the D-genome chromosomes from bread wheat into hexaploid triticale with a complete rye genome. Genome 29:425-430.

Mujeeb-Kazi, A., and J. L. Miranda. 1985. Enhanced resolution of somatic chromosome constrictions as an aid to identifying inter-generic hybrids among some Triticeae. Cytologia 50:701-709.

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

O'Donoughue, L.S. and M.D. Bennett. 1994. Durum wheat haploid production using maize wide-crossing. Theor. Appl. Genet. 89:55-566.

Riera-Lizarazu, O. and A. Mujeeb-Kazi. 1993. Polyhaploid production in the Triticeae: wheat x *Tripsacum* crosses. Crop Sci. 33:973-976.

Varughese, G., W.H. Pfeiffer and R.J. Pena. 1996a. Triticale: A successful alternative crop (part 1). Cereal Foods World 41:474-482.

Varughese, G., W.H. Pfeiffer and R.J. Pena. 1996b. Triticale: A successful alternative crop (part 2). Cereal Foods World 41:635-645.