Plant Breeding 117, 485—487 (1998)

ISSN 0179-9541

Short Communication

Production of haploids in bread wheat, durum wheat and hexaploid triticale crossed with pearl millet

M. N. INAGAKI[1,3] and C. T. HASH[2]

[1]International Maize and Wheat Improvement Center (CIMMYT), Lisboa 27, Colonia Juárez, Apartado Postal 6-641, 06600, Mexico, DF, Mexico; [2]International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, 502324, Andhra Pradesh, India; [3]Present address: Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Ibaraki, 305-8686 Japan

With 1 figure and 1 table

Received February 1, 1998/Accepted March 4, 1998
Communicated by P. Ruckenbauer

Abstract

Pearl millet is an efficient alternative to maize as a pollen source for haploid production in bread wheat. To compare haploid production frequencies in other Triticeae species, the crossabilities of two genotypes each of bread wheat, durum wheat and hexaploid triticale with four pearl millet genotypes and a maize control were examined. Embryos were obtained from crosses of all three species with both pearl millet and maize. However, significant differences in crossability were found among the three species (10.5–79.8% seed development and 1.4–15.8% embryo formation), as well as among genotypes of durum wheat (7.2–23.7% and 2.1–6.4%) and hexaploid triticale (0.3–20.6% and 0.1–2.7%). Crossability of bread wheat with pearl millet was relatively high. Haploid plants were regenerated from crosses of all three species with pearl millet. As in the case of maize crosses, low crossabilities of durum wheat and hexaploid triticale with pearl millet can be attributed to the absence of D-genome chromosomes.

Key words: *Triticum aestivum* — *T. turgidum* var. *durum* — *Pennisetum glaucum* — × *Triticosecale* — haploid — wide crosses

In recent years, the development of haploid production techniques for bread wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD), using chromosome elimination following wide crosses, has advanced rapidly (see Inagaki 1997 for a review). One of the most successful techniques at present uses crosses with maize (*Zea mays* L.) (Laurie and Bennett 1986, 1988) followed by application of 2,4-dichlorophenoxyacetic acid (2,4-D) (Suenaga and Nakajima 1989). This technique can be applied to diverse genotypes of bread wheat for stable haploid production (Inagaki and Tahir 1990) and used for instant production of recombinant inbred lines for both breeding purposes and genetic analyses. Wheat haploid production efficiency has also been improved significantly by pollen storage, detached-tiller culture and hot-water emasculation, considerably reducing the labour and space required for crossing (Inagaki et al. 1997a).

However, recent reports indicate distinct genotypic variation in the haploid production frequencies of durum wheat (*Triticum turgidum* L. var. *durum*, $2n = 4x = 28$, AABB) and hexaploid triticale (× *Triticosecale* Wittmack, $2n = 6x = 42$, AABBRR) crossed with maize (Amrani et al. 1993, O'Donoughue and Bennett 1994, Dusautoir et al. 1995, Inagaki and Tahir 1995, Inagaki et al. 1997b). Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an alternative pollen source for both efficient haploid production in bread wheat (Inagaki and Bohorova 1995, Inagaki and Mujeeb-Kazi 1995) and stable long-term pollen storage (Hanna and Towill 1995, Inagaki and Mujeeb-Kazi 1997). To compare haploid production efficiencies across a wider range of Triticeae, the crossabilities of bread wheat, durum wheat and hexaploid triticale with pearl millet and maize were examined.

Plant materials: Two genotypes each from bread wheat ('Norin 61' and 'Attila'), durum wheat ('Altar-84' and 'Sora/2*Plata-12') and hexaploid triticale ('Anoas-3/Tatu-4' and 'Jilotecpec-96') were used as female parents. Fresh pollen from three very early-flowering pearl millet inbred parents ('LGD-1-10-B', 'H-77/833-2-P5' and '843B') of pearl millet mapping populations (Hash and Witcombe 1994) was used. In addition, pearl millet pollen of 'NEC-7006' stored for 14 months in liquid nitrogen and fresh pollen of the single-cross maize hybrid 'CML-246 × CML-242' were chosen as pollen sources (Inagaki et al. 1997a). Female parents were grown in the field. Temperatures at ear emergence were 28/8°C (maximum/minimum). Pollen parents were grown in a greenhouse at 33/15°C (maximum/minimum temperatures).

Crossing with pearl millet and maize: Spikes on detached tillers of bread wheat, durum wheat and hexaploid triticale were crossed with pearl millet and maize, and then cultured in a nutrient solution containing 40 g/l sucrose, 100 mg/l 2,4-D and 8 ml/l sulphurous acid. Five spikes were used for each crossing treatment, with two replications. Statistical data analyses on seed development and embryo formation percentages were performed after angular transformation.

At 14 days after pollination with pearl millet '843B' and maize 'CML-246 × CML-242', immature embryos were aseptically excised from developing seeds on crossed spikes of cultured detached tillers, and transferred on to Murashige and Skoog (1962) culture medium supplemented with 20 g/l sucrose and 6 g/l agarose. Procedures of detached-tiller culture and embryo rescue were as described by Inagaki et al. (1997a, b). Regenerated plants were cytologically examined in squashed root-tip preparations followed by modified Giemsa-staining procedures (Jahan et al. 1990). C-banded chromosomes were identified based on the karyotypes of wheat and triticale (Friebe and Gill 1994, Lukaszewski and Gustafson 1983).

Seeds developed after pollination of pearl millet and maize

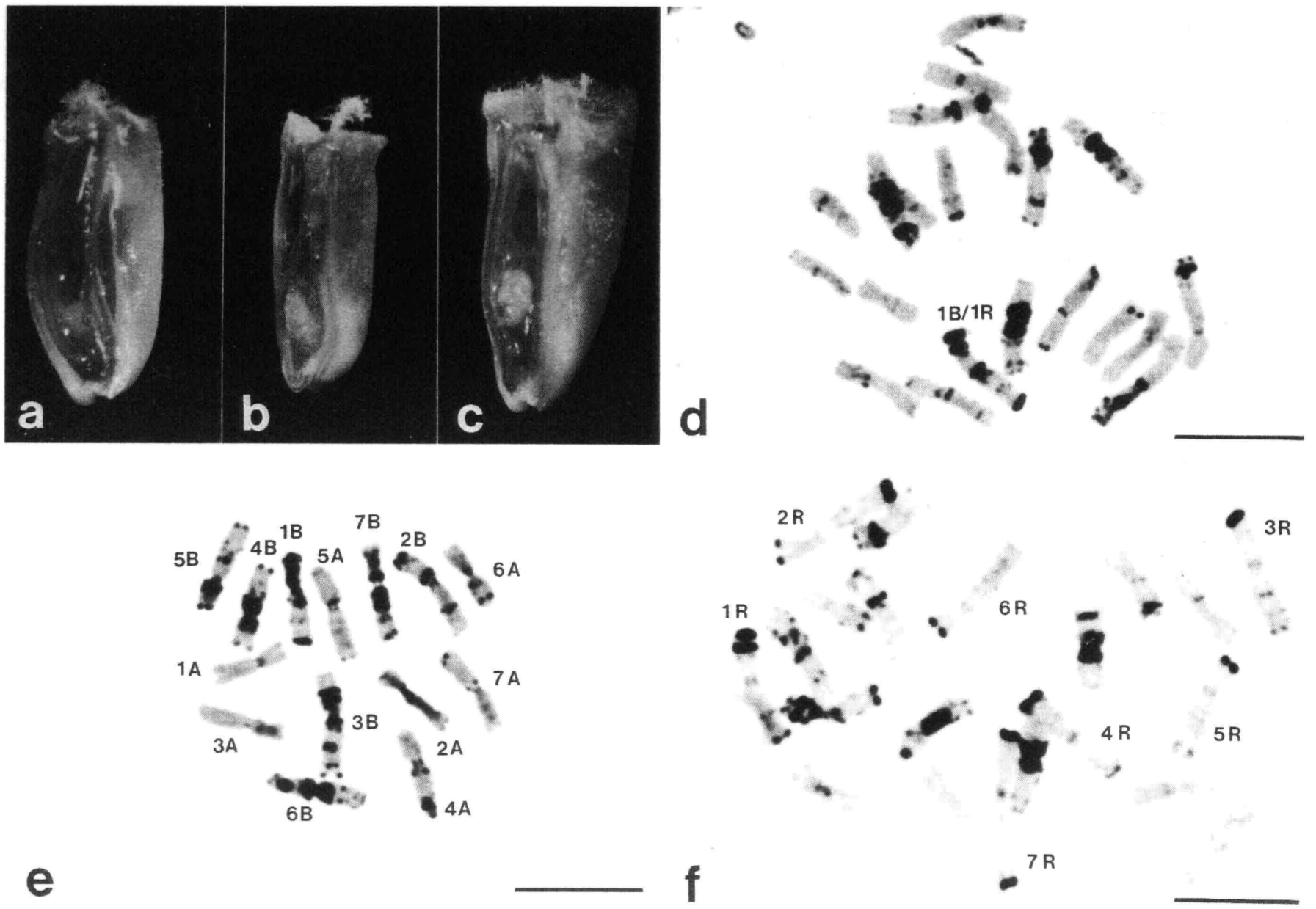

Fig. 1: Seeds containing embryos (a, b and c) and somatic chromosomes of regenerated plants (d, e and f), which were obtained from crosses of bread wheat 'Attila', durum wheat 'Sora/2*Plata-12' and hexaploid triticale 'Anoas-3/Tatu-4' with pearl millet '843B', respectively. Bars indicate 10 μm

were filled with an aqueous solution and lacked solid endosperm (Fig. 1). Some immature embryos were found in developed seeds. Mean seed development percentages (number of seeds developed/number of florets pollinated × 100) were 79.8, 15.5 and 10.5% in bread wheat, durum wheat and hexaploid triticale, respectively. Mean embryo formation percentages (number of embryos formed/number of florets pollinated × 100) were 15.8, 4.3 and 1.4% in bread wheat, durum wheat and hexaploid triticale, respectively (Table 1). There was no significant difference in seed development and embryo formation percentages among pollen sources, except for a pearl millet genotype ('LGD-1-10-B') which produced little pollen. Embryos obtained in pearl millet crosses were smaller than those of maize crosses and were often polyembryos developing multishoots (data not collected). Significant differences in embryo formation percentage were found between the three Triticeae species and among genotypes of each species. Mean percentages of plant regeneration from embryos of these three species were 50.2% and 47.9% in crosses with pearl millet '843B' and maize 'CML-246 × CML-242', respectively.

Table 1: Seed development percentages (embryo formation in parenthesis) in bread wheat, durum wheat and hexaploid triticale crossed with pearl millet and maize

	Pearl millet				Maize	
	'LGD-1-10-B'	'H-77/833-2-P5'	'843B'	'NEC-7006'	'CML-246 × CML-242'	Mean
Bread wheat						
'Norin 61'	73.8 (29.4)	82.4 (9.0)	77.6 (18.0)	73.2 (29.6)	72.5 (17.0)	75.9a (20.6a)
'Attila'	72.8 (0.3)	83.9 (0.6)	94.5 (28.2)	85.6 (10.9)	81.0 (14.9)	83.6a (11.0b)
Durum wheat						
'Sora/2*Plata-12'	3.0 (0.7)	48.9 (14.1)	16.7 (8.5)	20.0 (4.5)	29.7 (4.3)	23.7b (6.4bc)
'Altar-84'	0.0 (0.0)	25.6 (8.1)	2.7 (0.4)	7.6 (2.0)	0.3 (0.0)	7.2c (2.1de)
Hexaploid triticale						
'Anoas-3/Tatu-4'	18.4 (0.0)	41.0 (1.4)	10.3 (5.5)	18.0 (5.2)	15.5 (1.2)	20.6b (2.7cd)
'Jilotecpec-96'	0.0 (0.0)	1.4 (0.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.3c (0.1e)
Mean	28.0b (5.1b)	47.2a (5.6ab)	33.6ab (10.1a)	34.1a (8.7a)	33.2ab (6.2ab)	

Means followed by the same letter within each trait in the column or row are not significantly different by the least significant difference (P = 0.05).

Cytological examination of regenerated plants (at least 10 plants each from bread wheat and durum wheat, two plants from hexaploid triticale) chosen at random from crosses with pearl millet '843B' showed that these plants were haploids (polyhaploids), carrying 21 chromosomes of bread wheat and hexaploid triticale and 14 chromosomes of durum wheat. Haploid plants of bread wheat 'Attila' carried a 1B/1R translocated chromosome. A complement of chromosomes of both the A and B genomes were identified in haploid plants from durum wheat 'Sora/2*Plata-12'. Seven rye chromosomes were found in those of hexaploid triticale 'Anoas-3/Tatu-4' (Fig. 1).

In this study, stored pearl millet pollen was as effective as fresh pollen for haploid production in bread wheat, durum wheat and hexaploid triticale. The crossability of each of these three Triticeae species with pearl millet was similar to that with maize. Haploid plants were obtained in all three species crossed with pearl millet. However, crossability with pearl millet differed significantly among the three species and among genotypes of each species. Two bread wheat genotypes with completely different pedigrees showed relatively high percentages of seed development and embryo formation, as previously reported (Inagaki and Mujeeb-Kazi 1995). Crosses of durum wheat and hexaploid triticale with either pearl millet or maize resulted in low seed development percentages and genotypic differences in embryo formation percentage, as with crosses of durum wheat with maize (Inagaki et al. 1998). These crossabilities may be increased by the addition of silver nitrate to detached-tiller culture solution (O'Donoughue and Bennett 1994, Inagaki et al. 1998). As with maize, the lower crossability of durum wheat and hexaploid triticale with pearl millet may be genetically attributed to their lack of the D-genome chromosomes. Some D-genome chromosomes substituted in genetic backgrounds of durum wheat and hexaploid triticale enhanced crossability with maize (Inagaki et al. 1997b, 1998). In this study, pearl millet offered no advantage over maize as a fresh pollen source for haploid production in durum wheat and hexaploid triticale. However, the shorter life cycle, smaller high-tillering plants and greater ease of pollen storage (data not shown) of pearl millet compared with maize are advantages worth exploiting for bread wheat haploid production. In conclusion, a technique is required to improve the low crossability of durum wheat and hexaploid triticale with pearl millet and/or maize before wider application of haploid techniques in breeding programmes for these species can be recommended.

Acknowledgements

This report is a contribution from collaborative research involving CIMMYT, ICRISAT and JIRCAS. The authors thank V. Rosas, CIMMYT, for his assistance on chromosome banding techniques. The manuscript was approved as ICRISAT JA no. 2236.

References

Amrani, N., A. Sarrafi, and G. Albert, 1993: Genetic variability for haploid production in crosses between tetraploid and hexaploid wheats and maize. Plant Breeding **110,** 123—128.

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.

Friebe, B., and B. S. Gill, 1994: C-band polymorphism and structural rearrangements detected in common wheat (*Triticum aestivum*). Euphytica **78,** 1—5.

Hanna, W. W., and L. E. Towill, 1995: Long-term pollen storage. Plant Breed. Rev. **13,** 179—207.

Hash, C. T., and J. R. Witcombe, 1994: Pearl millet mapping populations at ICRISAT. In: Witcombe, J. R., and R. R. Duncan (eds.), Use of Molecular Markers in Sorghum and Pearl Millet Breeding for Developing Countries, 69—75. Overseas Development Administration, London.

Inagaki, M. N., 1997: Technical advances in wheat haploid production using ultra-wide crosses. JIRCAS J. (Japan) **4,** 51—62.

——, and N. Bohorova, 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.

——, 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.

——, and ——, 1997: Production of polyhaploids of hexaploid wheat using stored pearl millet pollen. In: H.-J. Braun, F. Altay, W. E. Kronstad, S. P. S. Beniwal, and A. McNab (eds), Wheat: Prospects for Global Improvement, 327—333. Kluwer Academic Publishers, Dordrecht.

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

——, and ——, 1995: Comparison of crossabilities of tetraploid wheat with *Hordeum bulbosum* and maize. Cereal Res. Commun. **23,** 339—343.

——, T. Nagamine, and A. Mujeeb-Kazi, 1997a: Use of pollen storage and detached-tiller culture in wheat polyhaploid production through wide crosses. Cereal Res. Commun. **25,** 7—13.

——, W. H. Pfeiffer, M. Mergoum, and A. Mujeeb-Kazi, 1998: Variation of the crossability of durum wheat with maize. Euphytica, in press.

——, ——, ——, ——, and A. J. Lukaszewski, 1997b: Effects of D-genome chromosomes on crossability of hexaploid triticale (× *Triticosecale* Wittmack) with maize. Plant Breeding **116,** 387—389.

Jahan, Q., N. Ter-Kuile, 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. J. Bot. **22,** 1—10.

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

——, and ——, 1988: The production of haploid wheat plants from wheat × maize crosses. Theor. Appl. Genet. **76,** 393—397.

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

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,** 559—566.

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