CSIRO PUBLISHING
www.publish.csiro.au/journals/ajb *Australian Journal of Botany*, 2005, **53**, 579–582

A *Sorghum bicolor* × *S. macrospermum* hybrid recovered by embryo rescue and culture

H. James Price[A,D], *George L. Hodnett*[A], *Byron L. Burson*[B], *Sally L. Dillon*[C] *and William L. Rooney*[A]

[A]Department of Soil & Crop Sciences, Texas A&M University, College Station, TX 77843-2474, USA.
[B]USDA-ARS, Crop Germplasm Research Unit, 430 Heep Center, Texas A&M Univ., College Station, TX 77843-2474, USA.
[C]Australian Tropical Crops and Forages Collection, Queensland Department of Primary Industries and Fisheries, Biloela, Qld 4715, Australia.
[D]Corresponding author. Email: hj-price@tamu.edu

Abstract. Although exotic germplasm is extensively used in sorghum improvement programs, *Sorghum* species classified in sections other than *Eu-sorghum* have not been utilised as germplasm because of strong reproductive barriers involving pollen–pistil incompatibilities. *S. macrospermum* is of particular interest to sorghum breeders because of its close phylogenetic relationship and cytogenetic similarities to *S. bicolor* and its resistance to important sorghum pests and pathogens, such as sorghum midge and sorghum downy mildew. A vegetatively vigorous interspecific hybrid was obtained from a cross between a cytoplasmic male-sterile *S. bicolor* plant and *S. macrospermum* by using embryo rescue and *in vitro* culture techniques. The hybrid was morphologically intermediate to *S. bicolor* and *S. macrospermum* in leaf width, leaf pubescence, plant height, inflorescence morphology, chromosome number and nuclear DNA content. It was male-sterile like its ATx623 parent. The hybrid produced no offspring when used as the female parent in a backcross with *S. bicolor*. This is the first confirmed hybrid between *S. bicolor* and *S. macrospermum*, and to our knowledge, it is the first reported hybrid between *S. bicolor* and any *Sorghum* species outside the *Eu-sorghum* section.

Introduction

Exotic non-cultivated sorghum races have been important sources of genes for sorghum improvement (Duncan *et al*. 1991; Rosenow and Dahlberg 2000). Potential sources of germplasm exist among the twenty-five species of the genus *Sorghum* that are classified into five subgenera or sections, *Eu-sorghum*, *Chaetosorghum*, *Heterosorghum*, *Para-sorghum* and *Stiposorghum* (Garber 1950; Lazarides *et al*. 1991). Species that belong to the *Eu-sorghum* section have a natural distribution that extends from Africa to southern Asia. The *Eu-sorghum* section includes cultivated sorghum, *S. bicolor* (L.) Moench, its subspecies *drummondii* and *arundinaceum*, and the wild species *S. almum* Parodi, *S. propinquum* (Kunth) Hitchc. and *S. halepense* (L.) Pers. (Johnsongrass) (de Wet 1978). *Chaetosorghum* and *Heterosorghum* are monotypic sections with their respective species, *S. laxiflorum* F.M.Bailey and *S. macrospermum* E.D.Garber, restricted to the Australo-Pacific region. The *Para-sorghum* section consists of seven Asian, Australian and central American species (Lazarides *et al*. 1991). Ten species that occur in northern Australia comprise the *Stiposorghum* section (Lazarides *et al*. 1991). No species outside the *Eu-sorghum* section have been utilised as germplasm for improving *S. bicolor* because of strong reproductive barriers (Garber 1950; Doggett 1988), primarily pollen–pistil incompatibilities (Hodnett *et al*. 2005).

Sorghum macrospermum is of particular interest to sorghum breeders (Hacker *et al*. 1992) because of its close phylogenetic relationship (Dillon *et al*. 2004) and cytogenetic similarities to *S. bicolor* (Wu 1990; Price *et al*. 2005). Furthermore, it has been reported to have resistance to sorghum midge, *Stenodiplosis* (*Contarinia*) *sorghicola* (Coquillett) (Sharma and Franzmann 2001), and sorghum downy mildew, *Peronosclerospora sorghi* Weston & Uppal (Shaw) (Kamala *et al*. 2002).

Hodnett *et al*. (2005) observed pollen germination and growth of pollen tubes of several exotic *Sorghum* species in *S. bicolor* pistils. They determined that a limited number of *S. macrospermum* pollen tubes grew into the ovary of *S. bicolor*. Consequently, a number of *S. bicolor* pistils were pollinated with *S. macrospermum* pollen. The objective of this paper is to report the results from these pollinations.

Materials and methods

The *S. bicolor* accession used in this study was the cytoplasmic male-sterile line ATx623. The *S. macrospermum* accession used

10.1071/BT04213 0067-1924/05/060579

was AusTRCF 302367 (Australian Tropical Crops and Forages Collection, Queensland Department of Primary Industries) vouchered as DNA C867 in the Northern Territory Herbarium, Darwin, Northern Territory, Australia. The plants were grown and crossed in a greenhouse at College Station, Texas, without supplemental lighting. ATx623 was used as the female parent in all crosses with *S. macrospermum*. Because the endosperm failed to develop normally in this *S. bicolor* × *S. macrospermum* cross (Hodnett *et al*. 2005), it was necessary to use embryo rescue and culture techniques. An embryo, rescued 15 days after pollination of ATx623 with *S. macrospermum*, was cultured on a Murashige and Skoog (1962) medium with 5.0% sucrose that was solidified with 0.7% agar (plant tissue culture grade, Phytotechnology Laboratories, Shawnee Mission, Kansas), and maintained in an environmental chamber (16 h light and 8 h dark at 24°C). The resulting seedling was transferred into soil in a pot and grown under greenhouse conditions. Chromosome number of the hybrid was determined by examining 20 cells at metaphase in actively growing root tips by using a modified Jewell and Islam-Faridi (1994) technique as described in Price *et al*. (2005). Nuclear DNA content was determined by co-chopping leaf tissue of *S. macrospermum* and the *S. bicolor* ATx623 × *S. macrospermum* hybrid, with leaf tissue of *S. bicolor* ATx623 (2C DNA content = 1.67 pg) as a standard, using procedures described by Price *et al*. (2005). Filtered macerates were stained with propidium iodide (Price *et al*. 2005). The mean fluorescence of nuclei was determined with a Partec Cy-flow flow cytometer (Partec GmbH, Munster, Germany) equipped with a 100-mW green laser.

Results

More than 1200 florets of ATx623 were pollinated with *S. macrospermum* pollen and, at ~15 days post-pollination, the ovules in these florets were examined under a dissecting microscope for embryo development. Only one ovule had a developed embryo. This embryo was rescued and placed on Murashige and Skoog (1962) medium in a culture tube. The differentiated seedling grew vigorously in the greenhouse after it was transferred to soil. The plant was morphologically compared to *S. bicolor* and *S. macrospermum* (Table 1, Fig. 1). It was initially suspected as being a hybrid on the basis of its morphology. The height and leaf width of the hybrid were intermediate to the parents, and there was pubescence, similar to that of the *S. macrospermum* parent, on its leaves. The spikelet morphology also suggested that the plant was a hybrid. The spikelets were ovoid in *S. bicolor* and lanceolate in the hybrid and *S. macrospermum*. The lemmas of *S. bicolor* ATx623 are awnless, whereas those of *S. macrospermum* have long awns. The hybrid had lemmas with awns of intermediate length. The hybrid was male-sterile, like its ATx623 parent. Several hundred florets of the hybrid were pollinated with pollen from the male-fertile *S. bicolor* line, BTx623, but no embryos were produced.

The hybrid was also confirmed cytologically. An intermediate nuclear DNA content supports its hybrid nature (Table 1). The chromosome numbers of *S. bicolor* and of *S. macrospermum* are $2n = 2x = 20$ and $2n = 4x = 40$, respectively; whereas, the hybrid has the expected chromosome number of $2n = 3x = 30$ (Fig. 2). The similarity in size and the lack of distinctive karyological features did not permit the two genomes to be distinguished in the metaphase spreads of the hybrid.

Discussion

The native Australian *Sorghum* species have been of interest to sorghum breeders because they possess important traits, e.g. resistance to biotic and abiotic stresses, that have not been available for sorghum improvement (Hacker *et al*. 1992). Since *S. macrospermum* is resistant to important sorghum pests and pathogens (Sharma and Franzmann 2001; Kamala *et al*. 2002), phylogenetically close (Dillon *et al*. 2004), and chromosomally similar (Wu 1990; Price *et al*. 2005) to *S. bicolor*, it is of interest to sorghum breeders as a source of agronomically important genes. Reported herein is the first confirmed hybrid between *S. bicolor* and *S. macrospermum* and, to our knowledge, the first hybrid between *S. bicolor* and any *Sorghum* species outside the *Eu-sorghum* section. The recovery and analysis of additional *S. bicolor* × *S. macrospermum* hybrids is of importance for long-term sorghum improvement programs, but more research will be required to establish reliable methods to increase crossability, induce fertility in the hybrids and introgress genes from *S. macrospermum* into *S. bicolor*.

Table 1. Some cytological and morphological characteristics of *Sorghum bicolor* ATx623, *S. macrospermum* and their F_1 hybrid
Mean height was measured from ground level to the flag leaf. Mbp = 10^9 base pairs

Plant	Chromosome number ($2n$)	2C DNA content[A] ± s.e. (pg)	1C DNA content (Mbp)	Mean height ± s.e. (m)	Leaf morphology and width ± s.e. (mm)	Spikelet shape, length ± s.e. (mm)	Lemma awn length ± s.e. (mm)
S. bicolor ATx623	20	1.67 ± 0.01	818	0.84 ± 0.02	Glabrous, 54.00 ± 0.64	Ovoid, 5.00 ± 0.01	Absent
S. macrospermum	40	2.27 ± 0.01	1112	2.37 ± 0.15	Pubescent, 29.64 ± 0.71	Lanceolate, 11.00 ± 0.25	36.73 ± 0.59
S. bicolor ATx623 × *S. macrospermum*	30	1.98 ± 0.01	970	1.83	Pubescent, 47.67 ± 2.96	Lanceolate, 8.00 ± 0.01	22.05 ± 0.65

[A]Price *et al*. (2005) determined the 2C DNA content of *S. bicolor* Tx623 to be 1.67 pg, using *Arabidopsis thaliana* Columbia (2C = 0.32 pg DNA; Bennett *et al*. 2003) as a standard.

Fig. 1. Inflorescences of *Sorghum bicolor* ATx623 (left), *S. macrospermum* (right) and the *S. bicolor* × *S. macrospermum* hybrid (centre).

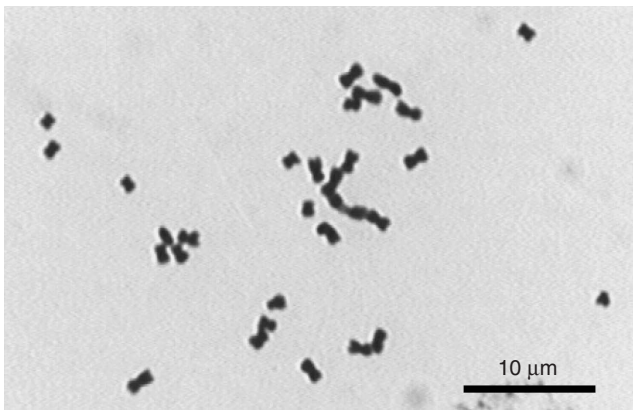

Fig. 2. Chromosomes of the hybrid *S. bicolor* ($2n = 20$) × *S. macrospermerm* ($2n = 4x = 40$), showing the expected chromosome number of $2n = 3x = 30$.

Hodnett *et al*. (2005) reported that the primary barrier to hybridisation between *S. bicolor* and several divergent *Sorghum* species was the failure of alien pollen tubes to grow through the stigma and style of *S. bicolor*. They also determined that in rare cases pollen tubes entered the female gametophyte. However, when fertilisation occurred, the endosperm deteriorated within 15 days that resulted in eventual embryo abortion. This secondary reproductive barrier can be circumvented by embryo rescue and culture, as demonstrated herein.

Since the formation of *S. bicolor* × *S. macrospermum* hybrids is an extremely rare event, the first priority is to find ways to increase the frequency of hybridisation. A logical approach for increasing the frequency of interspecific hybridisation is to screen cultivated and non-cultivated accessions of *S. bicolor* with different genetic backgrounds to discover any that have the ability to overcome or greatly reduce pollen–pistil incompatibilities. There are published reports suggesting that such an approach may be fruitful. For example, in *Sorghum*, genetic variation exists that influences the pollen–pistil incompatibilities in wide-species crosses. The growth of *S. versicolor* Anderss. pollen tubes in sorghum pistils is influenced by the genotype of the sorghum plant (Sun *et al*. 1991). *S. versicolor* pollen tubes grew more in ATx623 pistils, than in pistils of lines KS36A and KS5A. Even though hybrids were not recovered from crosses between these two *Sorghum* species, the limited screening by Sun *et al*. (1991) indicated that there are differences among *S. bicolor* lines controlling the growth of alien pollen tubes.

Genetic control of crossability has been reported in other grass genera. Riley and Chapman (1967) described crossability genes, Kr_1 and Kr_2, that influence interspecific crossability in wheat, *Triticum aestivum* L. The dominant alleles retarded and inhibited pollen-tube growth at the base of the style and in the ovary wall in the crosses, *T. aestivum* × *Secale cereale* L. and *T. aestivum* × *Hordeum bulbosum* L. (Lange and Wojciechowska 1976; Snape *et al*. 1979; Jalani and Moss 1980). Genetic variation also exists in *H. bulbosum* that overrides the action of the Kr_1 allele, thus allowing growth of pollen tubes in wheat pistils (Sitch and Snape 1986).

If genes that reduce pollen–pistil incompatibilities can be found in sorghum, it would have far-reaching utility by allowing hybrids to be made between *S. bicolor* and divergent *Sorghum* species. The production of these hybrids may permit the direct or indirect introgression of agronomically important genes from wild *Sorghum* species into *S. bicolor*.

Acknowledgments

This research was supported by the USDA–CSREES Competitive Grant 2004-35300-14686, the Sorghum Germplasm Committee, and the Texas Agricultural Experiment Station.

References

Bennett MD, Leitch IJ, Price HJ, Johnston JS (2003) Comparisons with *Chaenorhabditis* (~100 Mb) and drosophila (~175 Mb) using flow cytometry show genome size in arabidopsis to be ~157 Mb and thus ~25% larger than the Arabidopsis Genome Initiative estimate of ~125 Mb. *Annals of Botany* **91**, 547–557. doi: 10.1093/aob/mcg057

de Wet JMJ (1978) Systematics and evolution of *Sorghum* sect. *Sorghum* (Gramineae). *American Journal of Botany* **65**, 477–484.

Dillon SL, Lawrence PK, Henry RJ, Ross L, Price HJ, Johnston JS (2004) *Sorghum laxiflorum* and *S. macrospermum*, the Australian native species most closely related to the cultivated *S. bicolor* based on ITS1 and *ndh*F sequence analysis of 25 *Sorghum* species. *Plant Systematics and Evolution* **249**, 233–246. doi: 10.1007/s00606-004-0210-7

Doggett H (1988) '*Sorghum*.' 2nd edn. (John Wiley & Sons: New York)

Duncan RR, Bramel-Cox PJ, Miller FR (1991) Contributions of sorghum germplasm to hybrid development in the USA. In 'Use of plant introduction in cultivar development, part I'. (Eds HL Shands, LE Weisner) pp. 69–102. Special. Publication 117. (Crop Science Society of America: Madison, WI)

Garber ED (1950) Cytotaxonomic studies in the genus *Sorghum*. *University of California Publications in Botany* **23**, 283–361.

Hacker JB, Lazarides M, Andrew MH (1992) Australian native sorghums—A potential breeding resource for the improvement of grain sorghum. In 'Proceedings of the 2nd Australian sorghum conference'. (Eds MA Foale, RG Henzell, PN Vance) pp. 333–337. Occasional Publication 68. (Australian Institute of Agricultural Science: Melbourne)

Hodnett GL, Burson BL, Rooney WL, Dillon SL, Price HJ (2005) Pollen–pistil interactions result in reproductive isolation between *Sorghum bicolor* and divergent *Sorghum* species. *Crop Science* **45**, 1403–1409.

Jalani BS, Moss JP (1980) The site of action of the crossability genes (Kr_1, Kr_2) between *Triticum* and *Secale*. I. Pollen germination, pollen tube growth, and the number of pollen tubes. *Euphytica* **29**, 571–579. doi: 10.1007/BF00023204

Jewell DC, Islam-Faridi MN (1994) A technique for somatic chromosome preparation and C-banding of maize. In 'The maize handbook'. (Eds M Freeling, V Walbot) pp. 484–493. (Springer-Verlag: New York)

Kamala VS, Singh D, Bramel PJ, Rao DM (2002) Sources of resistance to downy mildew in wild and weedy species. *Crop Science* **42**, 1357–1360.

Lange W, Wojciechowska B (1976) The crossing of common wheat (*Triticum aestivum* L.) with cultivated rye (*Secale cereale* L.). I. Crossability, pollen grain germination and pollen tube growth. *Euphytica* **25**, 609–620. doi: 10.1007/BF00041598

Lazarides MJ, Hacker B, Andrew MH (1991) Taxonomy, cytology and ecology of indigenous Australian sorghums (*Sorghum* Moench: Andropogoneae: Poaceae). *Australian Systematic Botany* **4**, 591–635. doi: 10.1071/SB9910591

Murashige T, Skoog F (1962) A revised method for rapid growth and bioassay with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473–497.

Price HJ, Dillon SL, Hodnett G, Rooney WL, Ross L, Johnston JS (2005) Genome evolution in the genus *Sorghum* (Poaceae). *Annals of Botany* **95**, 219–227. doi: 10.1093/aob/mci015

Riley R, Chapman V (1967) The inheritance in wheat of crossability with rye. *Genetical Research* **9**, 259–267.

Rosenow DT, Dahlberg JA (2000) Collection, conversion, and utilization of sorghum. In 'Sorghum: origin, history, technology, and production'. (Eds CW Smith, RA Frederiksen) pp. 309–328. (John Wiley & Sons: New York)

Sharma HC, Franzmann BA (2001) Host–plant preference and oviposition responses of the sorghum midge, *Stenodiplosis sorghicola* (Coquilllett) (Dipt., Cecidomyiidae) towards wild relatives of sorghum. *Journal of Applied Entomology* **125**, 109–114. doi: 10.1046/j.1439-0418.2001.00524.x

Sitch LA, Snape JW (1986) The influences of the *Hordeum bulbosum* and wheat genotype on haploid production in wheat (*Triticum aestivum*). *Zeitschtift fuer Pflanzenzuechtung* **96**, 304–319.

Snape JW, Chapman V, Moss J, Blanchard CE, Miller TE (1979) The crossabilities of wheat varieties with *Hordeum bulbosum*. *Heredity* **42**, 291–298.

Sun Y, Suksayretrup K, Kirkham MB, Liang GH (1991) Pollen tube growth in reciprocal interspecific pollinations of *Sorghum bicolor* and *S. versicolor*. *Plant Breeding* **107**, 197–202.

Wu T (1990) *Sorghum macrospermum* and its relationship to the cultivated species *S. bicolor*. *Cytologia* **55**, 141–151.

Manuscript received 8 December 2004, accepted 26 May 2005