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Transfer of germ plasm from the secondary to the primary gene pool in pennisetum

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Summary. Germ plasm from the A′-genome of *Pennisetum purpureum* Schum. (A′A′BB) of the secondary gene pool was transferred to cultivated pearl millet (AA) [*P. glaucum* (L.) R. Br.] by pollinating cytoplasmic-nuclear male-sterile (cms) pearl millet with fertile allohexaploid pearl millet × *P. purpureum* hybrids (AAA′A′BB). Certain allohexaploids used as pollinators on cms pearl millet resulted in 14-chromosome diploid pearl millet progenies. Three types of diploid pearl millet plants were produced in addition to the expected 28-chromosome AAA′B-genome plants: (1) cms plants with only the A-genome, (2) cms plants with the A- and A′-genomes, and (3) fertile plants with the A- and A′-genomes. The latter group has allowed the utilization of genes for fertility restoration, stiff stalk, maturity, height, and morphological characteristics from the A′-genome of *P. purpureum* in the pearl millet breeding program. Production of monoploid gametes by the allohexaploids appeared to be genetically controlled.

Key words: *Pennisetum glaucum* – *Pennisetum purpureum* – Pearl millet – Genome segregation – Germ plasm

Introduction

Valuable germ plasm is often present in the wild relatives of the cultivated species. Utilization of this germ plasm depends on successful production of interspecific hybrids with enough fertility to allow transfer of gene(s) for and/or chromosomes with the desired characteristic to the cultivated species.

Chromosome elimination has been observed in hybrids between distant genera in the tribe Triticeae (Barclay 1975; von Bothmer et al. 1984; Wang 1987) and between *Zea* and *Triticum* (Laurie and Bennett 1988). Spontaneous chromosome elimination leading to barley haploids has been reported (Kasha and Rao 1970) and utilized in breeding programs. Reversion of polyploids to lower ploidy levels has also been documented (Kimber and Riley 1963; deWet 1971).

The primary, secondary, and tertiary gene pool system (Harlan and deWet 1971) provides a useful classification for grouping wild species based on their relatedness to a cultivated crop species. *Pennisetum purpureum* Schum., napier grass, is the only known species in the secondary gene pool of cultivated pearl millet, *P. glaucum* (L.) R. Br. (Harlan 1975). Pearl millet is a diploid (2n = 14) with the A-genome. *Pennisetum purpureum* is an allotetraploid (2n = 4x = 28) with the A′- and B-genomes. Studies have shown that the A- and A′-genomes are homologous, while the B genome is nonhomologous to the A- or A′-genomes (summarized by both Muldoon and Pearson 1979 and Jauhar 1981). Interspecific triploid (2n = 3x = 21) hybrids between pearl millet and *P. purpureum* are highly male- and female-sterile, but colchicine-induced allohexaploids from these hybrids are both male- and female-fertile (Gonzalez and Hanna 1984; Hanna 1981). Three successive backcrosses of the hexaploid interspecific hybrid to diploid peral millet resulted in some 2n = 14 A-genome plants with morphological characteristics like those of the recurrent pearl millet parent (Gildenhuys and Brix 1969).

The objective of this study was to determine the potential for gene transfer from *P. purpureum* to *P. glaucum*.

Materials and methods

Allohexaploids (2n = 6x = 42) used in this study were produced by treating cytoplasmic-nuclear male-sterile (cms) Tift 23 pearl

millet (2n = 14) × napier grass (2n = 4x = 28) interspecific triploid (2n = 3x = 21) hybrids with colchicine to double the chromosome number, as previously described (Gonzalez and Hanna 1984). All crosses were made with plants growing in the greenhouse except crosses in 1985, when pollen was taken from hexaploid plants flowering in the field in October and used to pollinate cms pearl millet growing in the greenhouse. Crosses were made in February 1980, January 1981, March 1982, and February 1983, respectively. Inflorescences of female parents were bagged with glassine bags before stigma exsertion (*Pennisetum* is protogynous). Inflorescences of pollen parents were covered with glassine bags the day before pollen collection, and the bottom of the bags were folded and secured with paper clips to prevent pollen loss. Inflorescences were hand-treshed by rubbing between two pieces of corrugated rubber. Seeds were planted in steam-sterilized soil in wooden flats in the greenhouse. Seedlings 10 cm tall were established in 5-cm clay pots before transplanting to the field in June. Chromosome counts were made from anthers fixed in absolute alcohol:acetic acid (3:1), and microsporocytes were stained in 2% acetocarmine.

Table 1. Seed set and plants established from crosses between diploid and tetraploid pearl millet (PM) and hexaploid pearl millet × *P. purpureum* crosses (MN)

Year	No.			
	Hexaploids used in crosses	Inflorescences		
		Pollinated	With seed	Established plants
1. cms[a] Diploid PM × hexaploid MN				
1980	14	39	11	404
1981	12	14	9	81
1982	20	111	83	491
1985	20	69	34	329
2. Hexaploid MN × fertile diploid PM				
1981	16	22	19	103
1982	20	105	66	69
3. Tetraploid PM × hexaploid MN				
1983	14	40	40	595
4. Hexaploid MN × tetraploid PM				
1983	20	47	47	1,160

[a] cms = cytoplasmic-nuclear male sterile

Results and discussion

Important characteristics of the progenies of diploid (2n = 14, AA-genome) and tetraploid (2n = 4x = 28, AAAA-genome) pearl millet (PM) plants crossed reciprocally with induced hexaploids (MN) (2n = 6x = 42, AAA'A'BB-genomes) from the pearl millet (AA-genome) × *P. purpureum* (2n = 4x = 28, A'A'BB-genomes) interspecific hybrids are summarized in Tables 1–3. It was easier to cross the hexaploid MN hybrids with tetraploid pearl millet than with diploid pearl millet (Table 1). All inflorescences in crosses between the MN hexaploids and tetraploid pearl millet set seed, while only 59% and 67%

Table 2. Chromosome number, genome composition, and number of plants established in each progeny from crosses between pearl millet (PM) and hexaploid pearl millet × *P. purpureum* crosses (MN)

Cross	Progeny group[b]	Cytotypes recovered	Genome composition	No. of plants established
1. cms[a] Diploid PM × hexaploid MN	I	2n = 28	AAA'B	330
	II	2n = 14	AA	346[c]
	III	2n = 14	AA'	313[c]
	IV	2n = 14	AA'	288
2. Hexaploid MN × fertile diploid PM	I	2n = 35	AAAA'B	1
	I	2n = 28	AAA'B	115
	I	2n = 20,21	AA'B or AAB	2
	II	2n = 14	AA	0
	III	2n = 14	AA'	0
	III	2n = 28	AAAA' or AAA'A'	43
	IV	2n = 14	AA'	2
3. Tetraploid PM × hexaploid MN	I	2n = 28–38 (mode = 35)	AAA'B to AAAA'B	588
	II	2n = 14	AA	0
	III	2n = 14	AA'	0
	IV	2n = 14	AA'	7
4. Hexaploid MN × tetraploid PM	I	2n = 28–38 (mode = 35)	AAA'B to AAAA'B	1158
	II	2n = 14	AA	0
	III	2n = 14	AA'	0
	IV	2n = 14	AA'	2

[a] cms = cytoplasmic-nuclear male sterile
[b] progeny group separation is based on chromosome number, genome composition, and pollen fertility
[c] 28 additional plants belonging to either group II or III but not classified in 1980

Table 3. Progeny from crosses between diploid and tetraploid pearl millet (PM) and hexaploid pearl millet × *P. purpureum* crosses (MN)

Year	No. plant types in different groups			
	With B-genome (I)	Pearl millet (2n = 14)		
		cms without A'- or B-genome (II)	Only AA'-genomes	
			cms (III)	male fertile (IV)
1. cms[a] Diploid PM × hexaploid MN				
1980	151[b]	(28)[c]		225[d]
1981	17	62	0	2
1982	71	162[e]	199[e]	59[e]
1985	91[f]	122[g]	114	2
2. Hexaploid MN × fertile diploid PM				
1981	102	0	0	1
1982	16	0	43[h]	1
3. Tetraploid PM × hexaploid MN				
1983	588	0	0	7
4. Hexaploid MN × tetraploid PM				
1983	1,158	0	0	2

[a] cms = cytoplasmic-nuclear male sterile
[b] 145 plants from one 23DA × MN16 cross
[c] All cms but not classified as to with or without A'-genome
[d] All from a 23DA × MN2 and a 23DA × MN4 cross
[e] 96 plants from 6 23DA × MN2 or MN4 crosses
[f] 87 plants from three 23DA × MN27 crosses
[g] All plants from a 23DA × MN4 cross
[h] Chromosome numbers in 30 plants checked was 2n = 28

of the diploid PM × MN crosses and the MN × diploid PM crosses, respectively, set seed. The average number of seeds produced per inflorescence was 9.5, 1.9, 14.9, and 24.7 for diploid PM × MN, MN × diploid PM, tetraploid PM × MN, and MN × tetraploid PM crosses, respectively. Although the seed set aspect was not studied in detail, the improved seed set obtained with tetraploid pearl millet is probably related to better endosperm development and/or to genome balance. Progeny from diploid PM × MN crosses should theoretically have the AAA'B-genomes, giving an unbalanced ploidy level for both the A- or A'- (21 chromosomes) and B- (7 chromosomes) genomes. Progeny from tetraploid PM × MN crosses have the AAA'A'B-genomes resulting in a balanced ploidy level for both the A- and A'-genomes (28 chromosomes) while only the B genome (7 chromosomes) is unbalanced (Table 2).

Although each of the four different types of crosses listed in Table 3 resulted in transfer of germ plasm from the A'- and/or B-genomes of *P. purpureum* to pearl millet, the cytoplasmic-nuclear male-sterile (cms) diploid PM × hexaploid MN crosses produced the most interesting results. Four groups of plants were recovered from these crosses: (I) perennial and short-day sensitive plants with chromosomes from the B-genome of *P. purpureum*, (II) cms pearl millet plants that were morphologically identical to the diploid pearl millet parent, (III) cms pearl millet plants that differed morphologically from the diploid pearl millet parent, and (IV) male-fertile pearl millet plants that differed morphologically from the diploid pearl millet parent (Tables 2 and 3). This latter group appears to offer the most potential for rapid germ plasm transfer from the A'-genome of *P. purpureum* to cultivated pearl millet.

The perennial B-genome plants in group I (Tables 2 and 3) were expected from these crosses and have been described previously (summarized by Jauhar 1981). Plants in this group are theoretically expected to have 2n = 4x = 28 chromosomes (AAA'B-genomes) from the diploid PM × MN and reciprocal crosses, and 2n = 5x = 35 chromosomes (AAAA'B-genomes) from the tetraploid PM × MN and reciprocal crosses. Thirteen of 16 plants studied cytologically in group I from MN × diploid PM crosses had 2n = 28 chromosomes, while 3 additional plants had 2n = 20, 21, and 35 chromosomes. Chromosome studies on 80 Group I plants from MN × tetraploid PM crosses and reciprocals showed that 2, 1, 3, 3, 56, 8, 6, and 1 plants had 2n = 28, 32, 33, 34, 35, 36, 37, and 38 chromosomes, respectively. These B-genome backcross (BC) plants are vigorous and can be vegetatively propagated. They have forage potential for tropical areas but are not cold tolerant and will not perenniate at Tifton/GA. The BC plants are also highly male- and female-sterile. Thirty-seven 2n = 35 BC_1 plants from the tetraploid PM × MN crosses and reciprocals pollinated with diploid PM pollen formed no seeds, while 5 crosses produced 18 shriveled seeds from which no plants were established. Four 2n = 28 BC_1 plants from the cms diploid PM × MN crosses and reciprocals pollinated with tetraploid PM pollen, and two 2n = 28 BC_1 plants pollinated with diploid PM pollen produced no seeds. In a previous study, seeds were produced on the 28-chromosome BC_1 plants by crossing with diploid pearl millet, and diploid pearl millet plants (without the B-genome) were produced by the BC_3 generation (Gildenhuys and Brix 1969).

Plants in the remaining three groups of progenies were diploid PM and probably resulted from the union of 7-chromosome gametes with the A- and/or A'-genomes from the hexaploid (AAA'A'BB) MN interspecific hybrids with 7-chromosome A-genome gametes from pearl millet. Production of tetraploid (2n = 4x = 28) pearl millet from MN × fertile diploid crosses in 1982 (Table 1) may be by a slightly different mechanism, which will be discussed later in this paper. Sixty-three percent (1980) to 86% (1982) of the cms diploid PM × MN crosses produced diploid (2n = 14) pearl millet platns. Observation on progenies from individual crosses indicated that although a number of the MN plants produced 7-chromo-

some (A- and/or A′-genome) gametes, certain MN plants such as MN2 and MN4 in 1980, 1982, and 1985 produced a high frequency of these gametes (Table 3). Most diploid PM × MN crosses resulted in only a few seeds set on each diploid PM inflorescence. However, crosses using MN2 or MN4 as pollinator usually resulted in a larger number of seed on an inflorescence, suggesting the production of a higher frequency of compatible 7-chromosome A- or A′-genome gametes to unit with 7-chromosome A-genome PM gametes. These results indicate variation for genetic control of genome segregation in the MN plants and show the need to test a number of pollinators (20 hexaploids in this study) as well as numerous crosses.

A previous study showed that monoploid pollen was produced by near-tetraploid interspecific hybrids between tetraploid pearl millet, *P. squamulatum* Fresen, and *P. purpureum* (Dujardin and Hanna 1990). The higher frequency of diploid PM plants produced when the MN parents are used as male pollinator rather than as female parent resulted from the greater number of gametes that can be tested (when pollen is placed on the stigmas) for 7-chromosome A- or A′-genome composition in the pollen (thousands) compared to those tested through the egg (200–300) per inflorescence crossed. Tetraploid PM crossed with the MN hyrids produced only a few 14-chromosome pearl millet plants (Table 3). In crosses 3 and 4, both the MN hexaploids and the tetraploid pearl millet would need to produce some 7-chromosome A- or A′-genome gametes to produce 14-chromosome pearl millet plants. We have observed 14-chromosome plants derived from cms 14-chromosome PM pollinated with 28-chromosome (tetraploid) PM, indicating that tetraploid PM did produce some 7-chromosome gametes (W. W. Hanna, unpublished results). Another indication of genetic control of genome segregation in the MN plants is that 87 B-genome plants were produced from three 23DA × MN27 crosses in 1985, the only year this pollinator was tested (Table 3). The 23DA × MN16 crosses also produced only AAA′B-genomes plants. In 1980, 1981, and 1982, this cross produced 145 (from one cross), 3 (from one cross), and 37 (from four crosses) AAA′B-plants, indicating a possible environmental (different frequencies in different years) as well as genetic (production of only AAA′B-plants in this cross) control of genome segregation.

The group II plants were cms and morphologically similar to Tift 23. This group of plants must have received the A-genome chromosomes of Tift $23DA_1$ that were introduced in the original cms Tift $23DA_1$ × *P. purpureum* interspecific hybrid. The genes on the A-genome from this cross are expected to maintain male sterility of the cms A_1 cytoplasm in the cms × MN backcross. The BC plants are expected to look like Tift 23 because this line is a uniform inbred. Plants set seed when pollinated. The BC_3 pearl millet plants reported by Gildenhuys and Brix (1969) probably belonged to this group. Groups III and IV plants were not previously reported (Gildenhuys and Brix 1969), because only a single MN hexaploid was used in the crosses and small populations were studied.

Group III plants (Tables 2 and 3) were also cms but morphologically different from Tift 23. Plants were usually taller, inflorescences longer, and/or leaves wider than those of Tift 23. This group of BC plants probably received gene(s) or chromosomes from the A′-genome of napier grass. It could not be determined if the introduced variation was due to gene recombination or chromosome segregation, because chromosomes from the A- and A′-genomes are similar in morphology. These plants set seed when pollinated. In order to utilize the variation transferred from *P. purpureum* in these crosses, cms plants needs to be pollinated with a restorer line to restore male fertility and must be followed by selfing to release the genetic variability for selection.

Plants in group IV (Tables 2 and 3) were produced by the same mechanism as in group III, except that a gene(s) or chromosome(s) with fertility restoration gene(s), in addition to the morphological variation, was transferred from the A′-genome of *P. purpureum*. In 1980, inflorescence length was measured on AA′-progeny to obtain an indication of the genetic variability transferred from the A′-genome. Mean inflorescence length (range) was 29 cm (17–46) and 17 cm (16–18) for the Tift 23DA × MN crosses (209 plants) and Tift 23DA (20 plants), respectively. In 1981, 20-plant progenies of 204 selfed plants from the 1980 group IV backcrosses were established in the field. All 1980 selfed BC plants (except 15) segregated for cms. Segregation for cms was expected, since the BC plants should have been heterozygous for the fertility restoration gene(s) transferred from *P. purpureum*. Selfing the BC plants has resulted in variation for fertility restoration, maturity, inflorescence length, plant height, stiff stalk, and vegetative characteristics such as leaf width, leaf angle, and leaf length. The gene(s) controlling fertility restoration and stiff stalk have been especially useful in our new program to develop pearl millet grain hybrids. Inbreds (8–10) developed from the AA′-backcross germ plasm and used to pollinate an early cytoplasmic-nuclear male-sterile line have resulted in high-yielding F_1 grain hybrids (W. W. Hanna, unpublished results).

Napier grass (*P. purpureum*, A′A′BB) appears to be a valuable source of genetic variation for pearl millet (AA). The dominant nature of the B-genome along with the perennial growth habit and vegetative propagation of *P. purpureum* has allowed genetic variation to accumulate over time on the A′-genome with limited selection pressure. Production of monoploid gametes by separating the A′-genome from the B-genome allows the genetic variation on the A′-genome to be phenotypically expressed in the diploid PM progenies. Results of this study

show that napier grass serves as a gene bank for pearl millet through this unique mechanism.

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