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# Cytogenetics of double cross hybrids between *Pennisetum americanum* – *P. purpureum* amphiploids and *P. americanum* × *Pennisetum squamulatum* interspecific hybrids

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**Summary.** Pearl millet, *Pennisetum americanum* L. Leeke-napiergrass, *Pennisetum purpureum* Schum. amphiploids ($2n=42$) were crossed with pearl millet × *Pennisetum squamulatum* Fresen. interspecific hybrids ($2n=41$) to study the potential of germplasm transfer from wild *Pennisetum species* to pearl millet. These two interspecific hybrids were highly cross-compatible and more than two thousand trispecific progenies were produced from 17 double crosses. All double-cross hybrids were perennial and showed a wide range of morphological variations intermediate to both parents in vegetative and inflorescence characteristics. Some crosses resulted in sublethal progenies. Chromosomes paired mainly as bivalents ($\bar{x}=15.88$) or remained as univalents. At metaphase I, trivalents, quadrivalents, an occasional hexavalent and a high frequency of bivalents indicated some homeology among the genomes of the three species. Delayed separation of bivalents, unequal segregation of multivalents, lagging chromosomes, and chromatin bridges were observed at anaphase I. Although approximately 93% of the double-cross hybrids were male-sterile, pollen stainability in male-fertile plants ranged up to 94%. Seed set ranged from 0 to 37 seed per inflorescence in 71 plants under open-pollinated conditions. Apomictic embryo sac development was observed in double-cross progenies when crosses involved a pearl millet × *P. squamulatum* apomictic hybrid as pollen parent. These new double-cross hybrids may serve as 'bridging hybrids' to transfer genes controlling apomixis and other plant characteristics from the wild *Pennisetum* species to pearl millet.

**Key words:** Interspecific hybrids – Pearl millet – Apomixis – Trispecific hybrids – *Pennisetum*

## Introduction

Cultivated pearl millet, *Pennisetum americanum* (L.) Leeke is a diploid ($2n=14$, genome AA) annual species. Napiergrass, *Pennisetum purpureum* Schum. ($2n=28$, genome A'A'BB), is a perennial species in which the A' genome is partially homologous to the A genome of pearl millet. *Pennisetum squamulatum* Fresen. is a hexaploid perennial grass ($2n=54$, genome SSSSSS) that has partial chromosome homeology with pearl millet. Both *P. americanum* and *P. purpureum* reproduce sexually (Hanna 1981) while *P. squamulatum* is an obligate apomict (Dujardin and Hanna 1983).

Interspecific hybrids between pearl millet and napiergrass were first reported in 1944 (Burton 1944) and numerous subsequent reports have been summarized in a review (Januar 1981). Pearl millet x napiergrass triploids ($3x=21$, AA'B) are male- and female-sterile but induced hexaploids ($6x=42$, AAA'A'BB) are male- and female-fertile (Hanna 1981) and can be used in a cross-breeding program. Sexual and apomictic partially male-fertile interspecific hybrids between pearl millet and *P. squamulatum* ($2n=41$, genome AASSS), also were reported (Patil et al. 1961; Dujardin and Hanna 1983). Trispecific hybrids ($2n=48$, genome AA'BSSS) between pearl millet-napiergrass hexaploids and *P. squamulatum* have been reported (Rangaswamy et al. 1971; Rangaswamy and Ponnaiya 1963) and also produced in our laboratory (unpublished results).

The present study is part of a research program investigating the feasibility of transferring germplasm from wild *Pennisetum* species to pearl millet with major emphasis on apomixis. New hybrids were produced by crossing the pearl millet-napiergrass amphiploids ($2n=42$, genome AAA'A'BB) with pearl millet × *P. squamulatum* interspecific hybrids ($2n=41$,

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genome AASSS). We will report on crossability, chromosome associations, reproductive behavior, and fertility in the double-cross hybrids between *P. americanum – P. purpureum* amphiploids and *P. americanum* × *P. squamulatum* interspecific hybrids.

## Materials and methods

Eight pearl millet-napiergrass (MN) hexaploids ($2n=6x=42$) and two pearl millet × *P. squamulatum* interspecific hybrids (SC342-12, an apomictic hybrid, and SC342-17, a sexual hybrid) were used.

The double-cross hybrids, or 'trispecific hybrids', were produced in the greenhouse during the winter of 1982. The partially exserted inflorescences of the female parent were covered with a glassine bag before stigma exsertion. After the stigmas were exserted, the inflorescences were dusted with pollen from the male parent. Eighteen different crosses, including reciprocals, were made (Table 1).

Seed were germinated in petri dishes at 30 °C and seedlings were transplanted to 5 cm pots in the greenhouse.

Somatic chromosome counts were made from root tips pretreated for 2 h in a saturated aqueous solution of monobromonaphthalene, hydrolyzed for 8 min in 5 N HCl at room temperature and stained in Feulgen reagent. Inflorescences were fixed in Carnoy's solution for examination of microsporogenesis and in FAA for embryo sac studies. Dissected ovaries to be sectioned were dehydrated in tertiary butyl alcohol, imbedded in paraffin, sectioned at 12 µm and stained in safranin-fast green. Embryo sacs were also observed with a phase-contrast microscope in ovules cleared with methyl salicylate (Crane 1978).

**Table 1.** Percentage of normal and sublethal trispecific plants from pearl millet-napiergrass hexaploids crossed with pearl millet × *P. squamulatum* interspecific hybrids

| Pedigree[a] | No. of trispecific plants | | |
|---|---|---|---|
| | Total | Normal hybrid % | Sublethal hybrid % |
| MN2 × SC 342-17 | 353 | 48 | 52 |
| MN1 × SC 342-17 | 148 | 100 | 0 |
| MN4 × SC 342-17 | 51 | 100 | 0 |
| MN6 × SC 342-17 | 0 | 0 | 0 |
| MN2 × SC 342-12 | 168 | 1 | 99 |
| MN1 × SC 342-12 | 149 | 100 | 0 |
| MN5 × SC 342-12 | 222 | 91 | 9 |
| MN6 × SC 342-12 | 116 | 100 | 0 |
| MN7 × SC 342-12 | 129 | 100 | 0 |
| MN8 × SC 342-12 | 396 | 42 | 58 |
| SC 342-17 × MN2 | 47 | 8 | 92 |
| SC 342-17 × MN17 | 11 | 100 | 0 |
| SC 342-17 × MN1 | 24 | 100 | 0 |
| SC 342-17 × MN4 | 18 | 100 | 0 |
| SC 342-17 × MN5 | 17 | 88 | 12 |
| SC 342-17 × MN7 | 94 | 100 | 0 |
| SC 342-17 × MN8 | 191 | 17 | 83 |
| SC 342-12 × MN17 | 0 | 0 | 0 |

[a] MN = pearl millet-napiergrass hexaploid; SC = pearl millet × *P. squamulatum* interspecific hybrids

A minimum of twenty ovules were examined for each double-cross hybrid. Pollen fertility was estimated by determining the percentage of stainable pollen with $I_2KI$. Seed set was determined on inflorescences allowed to open-pollinate in the field.

Crossability was calculated by dividing the number of double-cross hybrids by the total number of plants established in the field and expressing that value as a percentage.

## Results and discussion

### *Crossability*

Pearl millet-napiergrass hexaploids pollinated with both sexual and apomictic interspecific pearl millet × *P. squamulatum* hybrids produced 1940 plants of which 1730 were identified as double-cross hybrids (crossability 89%).

The pearl millet × *P. squamulatum* sexual interspecific hybrid (SC342-17) was pollinated with the MN hexaploid pollen and produced 418 plants of which 402 were identified as double-cross hybrids (crossability 96%). Other plants in these crosses resulted from self-pollination of SC342-17 or possibly from facultative apomictic reproduction.

When highly apomictic SC342-12 was pollinated with the MN amphiploids, only morphologically uniform progenies identical to the female hybrid parent (SC342-12) were produced.

### *Morphological characteristics*

All double-cross hybrids were perennial like the interspecific hybrid parents and demonstrated a wide range of variation in vegetative and flowering characteristics. The trispecific hybrids were bushy plants, 3–3.5 m tall, and were intermediate between the two parents in such characteristics as tillering, leaf and stem characteristics, panicle length, density, and shape, number of spikelets per involucre, spikelet size, and bristle length.

Sublethal plants segregated in progenies from seven crosses (Table 1). These sublethal plants had brown leaves and stems. Many died at the seedling stage and were not recorded. Most of the brown plants that were transplanted to the field were weak and grew slowly, but some flowered and a few shed well-stained pollen grains and set seed. Similar sublethal phenotypes were observed previously in triploid pearl millet × napiergrass hybrids (Aken'ova and Chheda 1973; Hanna and Monson 1980).

### *Chromosome behavior*

Somatic chromosome numbers of $2n=41$ and 42 were determined for 23 trispecific hybrids. They probably resulted from the union of a 21-chromosome MN gamete ($7A+7A'+7B$) and a 20- (or 21) chromosome

**Table 2.** Mean number of chromosome associations at diakinesis and metaphase I in trispecific hybrids between pearl millet-napiergrass hexaploids and pearl millet × *P. squamulatum* interspecific hybrids

| Trispecific[a] hybrid no. | Pedigree | 2n chromosome no. | Pollen stainability % | No. PMCs observed | Chromosome associations | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | I | II | III | IV |
| | | | | | x̄ | | | |
| H128-2 (S) | SC 342-17 × MN1 | 42 | 83 | 89 | 4.34<br>(0 – 11) | 16.51<br>(12 – 20) | 0.61<br>(0 – 2) | 0.64<br>(0 – 3) |
| H278-8 (Ap) | MN1 × SC 342-12 | 42 | 35 | 79 | 9.90<br>(3 – 18) | 14.41<br>(9 – 18) | 0.33<br>(0 – 2) | 0.44<br>(0 – 2) |
| H282-6 (S) | MN7 × SC 342-12 | 41 | 40 | 102 | 4.90<br>(1 – 11) | 17.67<br>(13 – 20) | 0.29<br>(0 – 2) | 0.23<br>(0 – 2) |
| H282-5 (Ap) | MN7 × SC 342-12 | 42 | 12 | 83 | 6.89<br>(1 – 13) | 14.42<br>(9 – 19) | 0.61<br>(0 – 3) | 0.86<br>(0 – 3) |

[a] S = sexual; Ap = apomictic

*P. americanum* × *P. squamulatum* gamete (7A + 13S or 14S). The metaphase I (MI) chromosome behavior was observed in four hybrids selected for partial pollen fertility (Table 2). Meiotic chromosome behavior was similar in all four plants. Chromosomes paired mainly as bivalents (Fig. 1) with a maximum frequency of 20 bivalents. Trivalents, quadrivalents, and one hexavalent were observed occasionally in pollen mother cells (PMCs). Similar multivalent associations have been observed in trispecific (*P. americanum* × *P. purpureum*) × *P. squamulatum* hybrids (Rangaswamy et al. 1971). At anaphase I (AI), aberrations such as late division of bivalents, unequal separation of multivalents, lagging chromosomes, and chromatic bridges were observed. As many as four micro-nuclei were present in some tetrads. The high frequency of bivalents in the PMCs and the occurrence of multivalents indicated that some homeology exists between *P. squamulatum* chromosomes and chromosomes of the pearl millet genome and/or the *P. purpureum* A′ or B genome.

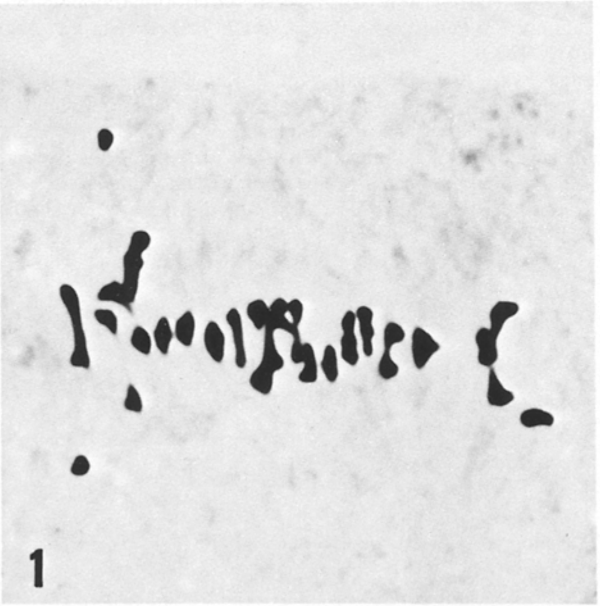

**Fig. 1.** Meiotic chromosomes of a double cross hybrid (2n = 41) between a pearl millet-napiergrass hexaploid and pearl millet × *Pennisetum squamulatum* interspecific hybrids. Metaphase I showing 19 bivalents and 3 univalents (ca × 1,800)

### *Reproductive behavior*

Embryo sac development was studied in plants from crosses involving MN hexaploids crossed with sexual and apomictic hybrids between pearl millet and *P. squamulatum.*

One population of 64 randomly selected double-cross hybrids between MN1 pollinated by apomictic SC342-12 resulted in 30 obligate apomictic plants, 30 sexual plants, one facultative apomictic plant, and three plants with aborted ovules.

No apomictic embryo sac development was observed in 36 plants randomly selected from three crosses between MN1, MN2, and MN4 pollinated with sexual SC342-17 nor in 17 plants from sexual SC342-17 pollinated with MN1.

These data indicate that genes for apomixis can be transferred and are expressed in these double-cross hybrids.

### *Fertility*

Approximately 93% of the double-cross progenies were male-sterile, but several shed pollen with up to 94% stainable pollen, making them valuable for future germplasm transfer research.

**Table 3.** Percentage stainable pollen and seed set in partially malefertile progenies from crosses between pearl millet-napiergrass hexaploids and pearl millet × *P. squamulatum* hybrids

| Trispecific progenies | Reproductive behavior | Stainable pollen % | No. inflorescences | No. seed per OP[a] inflorescence |
|---|---|---|---|---|
| H280-2 | Apomict | 51 | 19 | 9 |
| H280-5 | Sexual | 47 | 8 | 0 |
| H280-6 | Sexual | 48 | 8 | 16 |
| H282-5 | Apomict | 12 | 21 | 7 |
| H282-6 | Sexual | 40 | 14 | 12 |
| H283-3 | Apomict | 73 | 36 | 13 |

[a] OP = open-pollinated

Open-pollinated seed set was determined in 50 male-sterile sexual or apomictic progenies produced from one cross (MN1 × SC342-12). The mean seed number per inflorescence was 12.5 in 30 apomictic progenies versus 0.5 in 20 sexual progenies, with a maximum number of 37 for apomictic and 0.8 for sexual plants. Higher seed set in apomictic progenies than in sexual progenies resulted from pseudogamous aposporous embryo sac development.

Pollen stainability and seed set in a few partially fertile progenies are summarized in Table 3. The chromosome behavior and pollen stainability data indicate that chromosome irregularity is probably not the only cause of male sterility. Genetic incompatibilities among the genomes of the three species may also contribute to the male sterility.

## Conclusion

The pearl millet-napiergrass amphiploids and the pearl millet × *P. squamulatum* interspecific hybrids appeared highly cross-compatible and trispecific hybrids can be produced easily. Male- and female-fertility in some of the sexual and apomictic double-cross (trispecific) hybrids allows these hybrids to be used as 'bridges' in transferring apomixis and other characteristics from the wild species to pearl millet.

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## Note added in proof

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