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An apomictic polyhaploid obtained from a pearl millet × *Pennisetum squamulatum* apomictic interspecific hybrid

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Summary. In a research program to transfer apomixis from *Pennisetum squamulatum* Fresen to pearl millet, *P. americanum* L. Leeke, a polyhaploid plant (2n=21) was discovered in the uniform open-pollinated progeny of an apomictic interspecific hybrid (2n=41) between pearl millet and *P. squamulatum.* The polyhaploid was shorter, less vigorous and was smaller morphologically than its maternal parent. It probably originated by parthenogenetic development of a reduced gametophyte in the apomictic interspecific hybrid. The most common metaphase I chromosome association in the polyhaploid was 4 bivalents plus 13 univalents. Irregular chromosome distribution, tripolar spindles, bridges and fragments were observed at anaphase I and telophase I. The polyhaploid was male-sterile and partially female- fertile having multiple aposporous embryo sacs in 95% of the ovules. Seed set was 3% when open-pollinated and 33% when pollinated with pearl millet pollen. Low seed set was due to competition among multiple embryos developing in the same ovule. Seventeen progeny from seed produced under open-pollination on the polyhaploid each had 2n=21 chromosomes and were morphologically uniform and identical to the female parent. The expression of obligate apomixis in the polyhaploid conditioned by the *P. squamulatum* genome between the simplex and duplex condition indicates that apomictic reproduction is possible in non-polyploid plants.

Key words: Chromosome – Haploid – Interspecific hybrid – *Pennisetum americanum*

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Introduction

Development of unreduced egg cells without fertilization is the normal reproductive process in obligate apomictic plants, whereas parthenogenesis takes place occasionally in reduced eggs in sexual plants. Haploids arise by parthenogenesis but their formation may be promoted by various conditions such as twin seedlings from polyembryonic seeds (Kasha 1974; Kimber and Riley 1963). Results of studies with the *Bothriochloa* – *Dichanthium* complex indicated that functional cytologically reduced and non-reduced gametophytes were produced in facultatively sexual plants, and either gametophyte may develop parthenogenetically or function sexually to produce viable seed (de Wet and Stalker 1974).

Interspecific hybrids (2n=41) between sexual pearl millet, *Pennisetum americanum* L. Leeke and apomictic *P. squamulatum* Fresen. have been produced for the purpose of transferring apomixis to pearl millet (Dujardin and Hanna 1983). The present investigation was conducted to document the chromosome behavior, method of reproduction and fertility of a polyhaploid (2n=21) discovered in the open-pollinated progeny of an apomictic pearl millet × *P. squamulatum* hybrid.

Materials and methods

A previously described (Dujardin and Hanna 1983), highly apomictic interspecific hybrid between pearl millet and *P. squamulatum* (SC 342-12) was progeny tested. The hybrid had 2n=41 chromosomes (14 pearl millet chromosomes + 27 *P. squamulatum* chromosomes). It was partially male- (70% stainable pollen) and female-fertile. Four hundred forty-six morphologically uniform progeny identical to the F_1 interspecific hybrids were established in the field. One offtype plant, less vigorous than the others, was identified, transplanted into the greenhouse, and studied cytogenetically.

Somatic chromosome counts were made from root tips pretreated for 2 h in monobromonaphthalene, hydrolyzed for 8 min in 5 N HCl at room temperature and stained in Feulgen solution. Inflorescences were fixed in Carnoy's solution for microsporogenesis and in FAA for embryo sac studies. Embryo sacs were studied in ovule sections stained in Safranin-Fast Green or observed with a phase contrast microscope in ovules cleared with methylsalicylate.

Panicles of this plant were allowed to open-pollinate in the field or were cross-pollinated with pollen collected in glassine bags from pearl millet (inbred Tift 23BE), *Pennisetum squamulatum* (PI 319196) and the pearl millet × *P. squamulatum* hybrid (82-SC342-12). Seed obtained from open- or cross-pollinations were germinated in petri dishes at 30 °C and the seedlings were transplanted to 5 cm pots in the greenhouse.

Results and discussion

Morphological characteristics

An unusual plant resembling the maternal parent but characterized by reduced vigor, was identified among 446 offspring established from the open-pollinated progeny of an apomictic hybrid between pearl millet and *P. squamulatum*. The offtype plant differed morphologically from its maternal parent and sister offspring by having approximately 50% shorter plant height, narrower leaves and stems, shorter panicles, smaller seeds, and by flowering three weeks later during the summer of 1984.

Chromosome behavior

The somatic chromosome number in the offtype plant was 2n=21 (Fig. 1A), half as many as in its maternal parent and therefore, was classified as a polyhaploid. This aberrant plant probably originated from the parthenogenetic development of a cytologically reduced gametophyte (genome AS ... ASS). Theoretically, this polyhaploid combined 7 pearl millet chromosomes and 14 *P. squamulatum* chromosomes. However, it was not possible to determine how many of the chromosomes of the polyhaploid belonged to either the pearl millet or *P. squamulatum* genomes because several chromosomes of the two species are morphologically similar.

Metaphase I chromosome behavior was highly irregular in the 100 pollen mother cells (PMC's) studied. Chromosomes remained as univalents or paired as bivalents, trivalents, and/or quadrivalents. A mean of 11.80 univalents, 4.06 bivalents, 0.24 trivalent, and 0.09

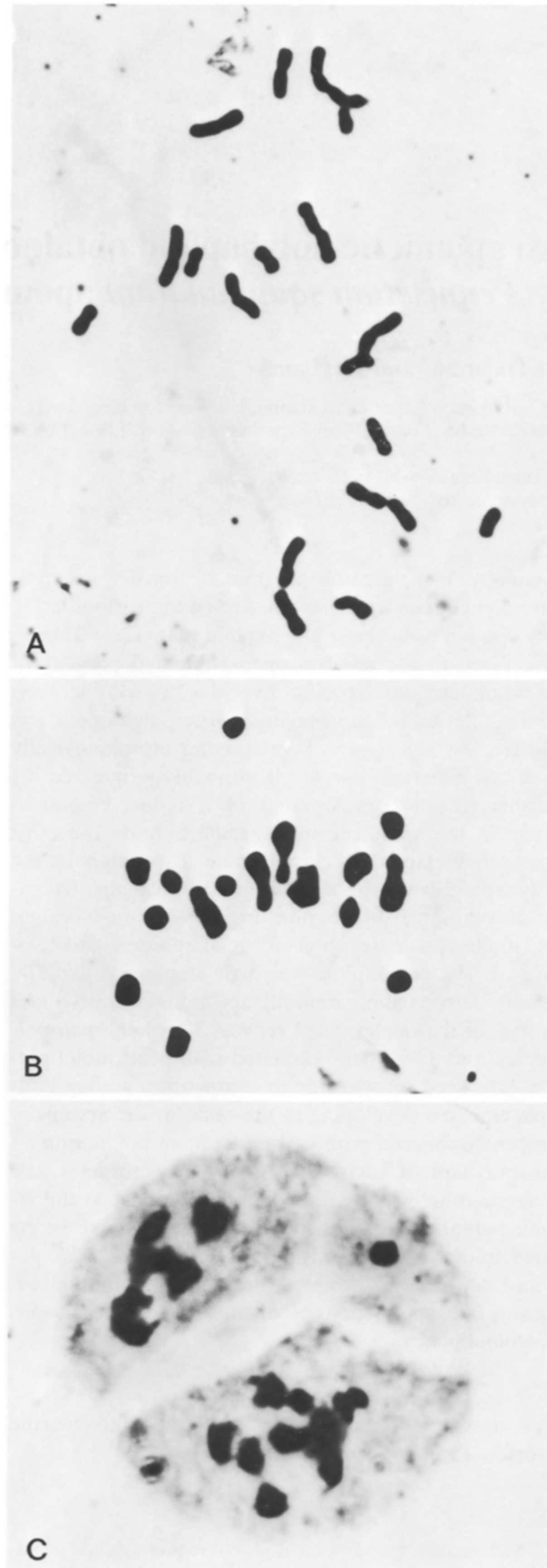

Fig. 1 A–C. Mitotic and meiotic chromosomes of a polyhaploid (2n=21) derived from a pearl millet × *P. squamulatum* interspecific hybrid. **A** Root tip with 21 chromosomes (ca × 2,200); **B** meiotic metaphase I with 6 bivalents and 9 univalents (ca × 2,200); **C** triad resulting from a tripolar spindle (ca × 1,500)

quadrivalent per PMC were recorded. Univalents ranged from 7 to 17 per PMC.

From 2 to 7 bivalents with a mode of 4 bivalents per PMC were observed (Fig. 1B). Bivalent pairing was probably a result of intraploid pairing in the pearl millet genome (Powell et al. 1975) and autosyndetic pairing between the *P. squamulatum* genomes and possibly homeology between the pearl millet and the *P. squamulatum* genomes. One or two trivalents and/or one quadrivalent were observed in 23% and 9% of the PMC's, respectively, indicating some residual homeology between genomes of the two species.

Anaphase I disjunction led to unequal chromosome distribution to the poles. Random chromosome dispersion, tripolar spindles, laggards, chromatin bridges, and fragments were common (Fig. 1C).

Reproductive behavior

Embryo sac development was studied in 218 ovules of the polyhaploid plant. Reproductive behavior of the polyhaploid and its progenitor, the *P. americanum* × *P. squamulatum* F_1 interspecific hybrid (82-SC342-12) is reported in Table 1.

The polyhaploid plant showed mature embryo sacs in 95% of the ovules similar to its parent, 82-SC342-12. Almost all ovules had multiple aposporous four-nucleate embryo sacs. The number of embryo sacs per ovule ranged from 2 to 7. One ovule had a reduced eight-nucleate sexual embryo sac and one ovule had both a mature sexual sac and aposporous embryo sacs. Endosperm development and multiple proembryos were observed only in pollinated ovules, indicating that pollination was necessary for the development of seeds.

Fertility

The polyhaploid plant was male-sterile and partially female-fertile. Anthers dehisced at anthesis, but were shriveled and shed no pollen. Complete abortion of the pollen took place at the microspore stage as a result of the meiotic chromosome irregularities. The polyhaploid plant produced viable seed under open-pollinated conditions or when pollinated with pollen of either pearl millet, *P. squamulatum*, or the pearl millet × *P. squamulatum* hybrid (Table 2).

Although 95% of the ovules possessed mature embryo sacs, the average seed set was low. Seed set failure was possibly due to competition among a number of developing embryos in different embryo sacs within each individual ovule. The competition for nucellar tissue by a number of embryos in each ovule resulted in insufficient nutrition for any one embryo in many cases, resulting in lack of seed set. Seventeen seedlings derived from open-pollinated seed of the polyhaploid were uniform and identical to the female parent, and possessed 2n=21 chromosomes. This indicated that the offspring resulted from apomictic development of unreduced gametophytes in the polyhaploid plant.

Table 1. Embryo sac characteristics of a *Pennisetum americanum* × *P. squamulatum* interspecific hybrid and a polyhaploid derived from the hybrid

Hybrid	Total	No. of ovules[a] (in %)			
		S	Ap	Ap+S	Ab
SC342-12 (2n=41)	121	2	92	1	5
Polyhaploid (2n=21)	218	0.5	94	0.5	5

[a] S=sexual, Ap=aposporous, Ab=aborted

Table 2. Seed set on a *Pennisetum americanum* × *P. squamulatum* polyhaploid (2n=21)

Cross	No. of florets	Seed set (%)
Polyhaploid		
× *P. americanum* Tift 23BE	1,048	32.8
× *P. squamulatum* (PI 319196)	622	6.5
× *P. americanum* × *P. squamulatum* interspecific hybrid (SC342-12)	853	7.5
Open pollinated	1,115	3.3

Conclusion

A male-sterile, partially female-fertile apomictic polyhaploid plant originating from a reduced gametophyte was discovered among the open-pollinated progeny of a partially male- and female-fertile apomictic interspecific hybrid between pearl millet and *P. squamulatum*. Apomictic reproduction was maintained in the plant with the reduced chromosome number even though the *P. squamulatum* genome (with genes controlling apomixis) was in the simplex and, at most, duplex condition. Similar results for the expression of apomixis were previously observed in crosses between pearl millet and *P. orientale* (Dujardin and Hanna 1983). The expression of apomixis at lower ploidy levels indicates that apomictic reproduction should be possible in non-polyploid plants.

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