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Microsporogenesis, reproductive behavior, and fertility in five *Pennisetum* species

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Summary. Microsporogenesis, reproductive behavior, pollen fertility and seed set were studied in *Pennisetum basedowii* Summerhayes and C. E. Hubbard, 2n = 54; *P. macrostachyum* (Brough.) Trin., 2n = 54; *P. macrourum* Trin., 2n = 36; *P. polystachion* (L.) Schult, 2n = 54; and *P. squamulatum* Fresen 2n = 54. Meiosis was regular in *P. basedowii* with primarily bivalent pairing. As many as 54 univalents were observed at metaphase I in *P. macrostachyum*. A high frequency of univalents at metaphase I in *P. macrourum* resulted in lagging chromosomes and micronuclei at anaphase I and telophase I, respectively. *Pennisetum polystachion* and *P. squamulatum* showed frequent multivalent chromosome associations. Studies of megasporogenesis and embryo sac development in *P. basedowii* showed sexual reproduction. *Pennisetum macrostachyum* was highly male sterile with predominantly aposporous apomictic embryo sac development. *Pennisetum macrourum, P. polystachion,* and *P. squamulatum* had only aposporous embryo sac development. Seed propagated progenies of these latter three species were uniform and matromorphic, confirming the obligate apomixis nature.

Key words: Cytology – Genome – Apomixis – Fertility – Sexual

Introduction

Pennisetum Rich. is a large tropical and subtropical genus of the tribe *Paniceae* consisting of more than 140 species (Jauhar 1981). Most are constituent of natural tropical savannahs of the Old World. Several species could contribute to the enrichment of the gene pool of cultivated pearl millet, *Pennisetum americanum* L. Leeke., and serve as potential donors of heritable traits such as apomixis, perennial growth habit, disease resistance, and drought tolerance. The potential of crossing pearl millet with wild relatives has been discussed (Hanna 1979, 1981; Hanna and Dujardin 1982).

The present investigation was conducted to document the chromosome number and behavior, method of reproduction and fertility of *P. basedowii* Summerhayes and C. E. Hubbard, *P. macrostachyum* (Brough.) Trin., *P. macrourum* Trin., *P. polystachion* (L.) Schult., and *P. squamulatum* Fresen.

Materials and methods

Pennisetum accessions used in this study included *P. basedowii* (PI 257782), *P. macrostachyum* (PI 354276), *P. macrourum* (PI 315736), *P. polystachion* (PI 189347), and *P. squamulatum* (PI 248534).

Vouchers of each species were deposited in the grass breeding herbarium at Tifton, Georgia under the numbers: PS2 – *P. basedowii,* PS8 – *P. macrostachyum,* PS11 – *P. macrourum,* PS19 – *P. polystachion,* and PS24 – *P. squamulatum.*

Thirty plants established from open pollinated seed of each accession except *P. macrostachyum* were planted in the field in 1982. Observations were made on a single plant of *P. macrostachyum.* Inflorescences for study of microsporogenesis were fixed in Carnoy's solution and stored in 70% ethanol. Pollen mother cells (PMCs) were stained with aceto-carmine. Mitosis was studied in root tips of seedlings growing in 5 cm pots in a greenhouse. Roots were pretreated for 2 h in a saturated solution of 1-monobromonaphthalene, hydrolyzed for 8 min in 5 N HCl at room temperature and stained in Feulgens. Inflorescences at different stages of development were collected for the study of megasporogenesis and embryo sac development and fixed in FAA. Ovaries were dissected, dehydrated in a tertiary butyl alcohol series, and embedded in paraffin. Ovaries were sectioned at 12 μ and stained in Safranin-O-fast-green. Pollen fertility was estimated by determining the percent pollen

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stainability with I_2KI. A minimum of 500 pollen grains was counted for each accession. Seed set was established from 5 inflorescences on each accession allowed to open-pollinate in the field.

Results and discussion

Chromosome behavior

Somatic chromosome number for each species was 2n=54, except *P. macrourum* had 2n=36 chromosomes. The meiotic chromosome behavior of the five species is summarized in Table 1.

Chromosomes of *P. basedowii* paired as 27 bivalents at metaphase I (Fig. 1 A) indicating that it is an allohexaploid species. Only one PMC was observed to have one univalent and one trivalent at metaphase I (MI). Regular 27–27 chromosome segregation was recorded in more than 100 microsporocytes at anaphase I (AI).

The 2n=54 chromosomes observed in *P. macrostachyum* differs from a previous report of 2n=68 (Shanthamma 1979). A high percentage of univalents was observed in all microsporocytes (Fig. 1 B). Complete asynapsis occurred at diakinesis in 20% of the PMCs analyzed. A maximum of six bivalents and two trivalents was found in the remaining PMCs. Univalents dispersed at random and tended to clump together at AI. A few univalents divided equationally and gave rise to fragments at telophase I (TI). Micronuclei were present at telophase II (T II) and in tetrads. Pollen grains were variable in size. Limited chromosome pairing in this species (probably of hybrid origin) suggests little homology between the constituent genomes.

Chromosome counts of 2n=36 for *P. macrourum* in our study confirmed previous reports (Jauhar 1981). Chromosomes paired on the average of 6.21 univalents plus 14.60 bivalents at MI. The chromosome associations suggest this species is allotetraploid with incomplete homology between the two genomes. Up to 12 univalents lagged at AI and several divided equationally. Approximately 70% of the tetrads had micronuclei.

The 2n=54 chromosomes observed in *P. polystachion* is the most frequent number reported in this species (Dujardin 1978). Chromosomes paired at MI on

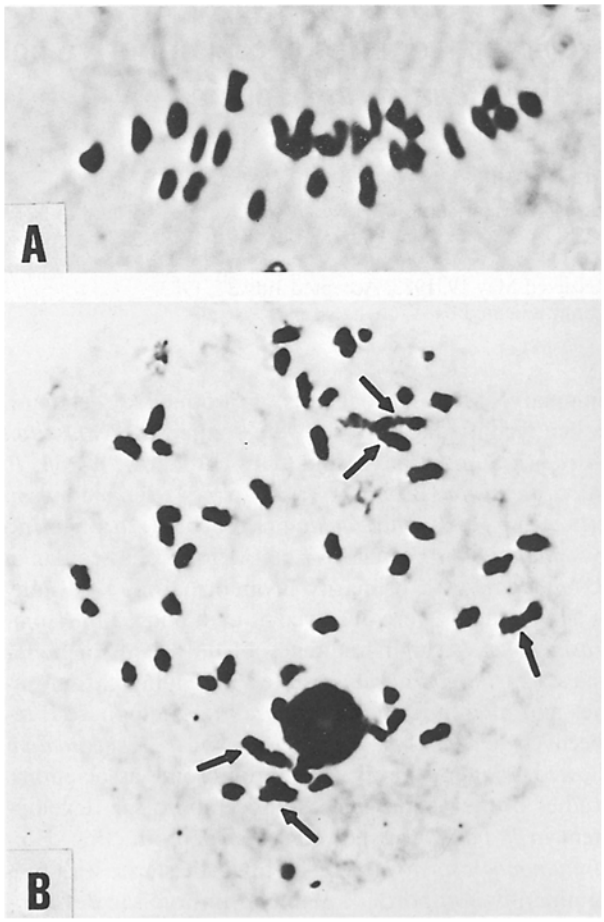

Fig. 1 A, B. Meiotic chromosomes of *Pennisetum basedowii* and *P. macrostachyum*. **A** Metaphase I of *P. basedowii* showing 27 bivalents. **B** Diakinesis of *P. macrostachyum* showing 44I and 5II (*arrows*). ca. ×1800

Table 1. Chromosome associations at diakinesis and metaphase I in five *Pennisetum* species

	No. PMCs[a] scored	Chromosome association						
		I	II	III	IV	V	VI	VIII
P. basedowii	91	0.01	26.98	0.01	–	–	–	–
		0–1	25–27	0–1	–	–	–	–
P. macrostachyum	78	48.49	2.34	0.29	–	–	–	–
		38–54	0–6	0–2	–	–	–	–
P. macrourum	75	6.21	14.60	0.15	–	–	–	–
		2–12	10–17	0–2	–	–	–	–
P. polystachion	72	0.65	15.58	0.30	3.43	0.10	0.90	0.14
		0–5	10–23	0–3	0–6	0–2	0–3	0–2
P. squamulatum	80	0.57	11.56	0.22	5.46	0.11	0.76	0.36
		0–4	6–23	0–2	1–10	0–1	0–3	0–2

[a] PMCs = Pollen mother cells

the average as 15.58 bivalents and 3.43 quadrivalents, however, a few higher multivalents were observed. The maximum number of six quadrivalents was similar to the frequency observed in a previous report (Rangaswamy 1972). In this study, PMCs with only bivalents were not observed and bivalent pairing was lower in our accession than in other previously reported accessions (Hrishi 1952; Sisodia 1970). Our studies indicate this species is autoallohexaploid.

Univalents, bivalents, trivalents, quadrivalents, hexavalents, and octovalents were present at diakinesis and MI in *P. squamulatum,* 2n=54. The most frequent association of bivalents and quadrivalents at MI was 11 and 7, respectively, with a maximum of 23 bivalents and 10 quadrivalents. Despite multivalents, disjunction at anaphase I was quite regular with 27 chromosomes at each pole. An occasional bridge was observed.

Similar chromosome associations have been observed previously for *P. squamulatum* (Krishnaswami and Thulasidas 1962; Patil et al. 1961; Rangaswamy 1972; Sisodia 1970). Jauhar (1981) reported up to 27 II and suggested this species is of allohexaploid origin. Segmental homology between the basic genomes and/or interchange heterozygosity could have caused the multivalent associations.

Method of reproduction

Megasporogenesis and embryo sac development in 117 sectioned ovules of *P. basedowii* showed sexual reproduction. The archesporial cell developed from a single hypodermal cell of the nucellus and underwent meiosis to produce a linear tetrad of megaspores. The three megaspores nearest the micropyle degenerated, leaving the chalazal megaspore as the functional magaspore. The functional megaspore enlarged and divided three times to form a mature eight nucleate female gametophyte. The mature embryo sac was of the *Polygonum* type with two synergids, one egg cell, two polars, and three antipodals. At anthesis, the synergids had degenerated and the antipodals had proliferated (Fig. 2A). Only 3% of the ovules aborted. Progeny of this species showed variation for height and maturity.

Although sexual method or reproduction had been reported for *P. macrostachyum* (Shanthamma 1979), our introduction appeared predominantly aposporous. Of 195 mature ovules observed, 123 had no embryo sac, undoubtedly as a result of the high meiotic irregularities. In some ovules archesporial cells collapsed without dividing. In other ovules, the sexual tissue collapsed during meiosis and the formation of the megaspores. In 4% of the ovules, degeneration of the embryo sac was delayed until the 4 nucleate stage. Mature 8-nucleate embryo sacs were not observed. In 33% of the ovules examined, active nucellar and integumental cells developed into mature aposporous embryo sacs. Apparently cells in all areas of the ovule from the micropyle to the chalazal end and the integuments were able to develop simultaneously into multiple mature embryo sacs (Fig. 2B).

Megasporogenesis and embryo sac development were similar in all 206 ovules of *P. macrourum,* in 199 ovules of *P. polystachion* and in 279 ovules of *P. squamulatum.* In these three species, aposporic initials differentiated very early in the ovules. Remnants of degenerated sporogenous tissue were noted but no dyads or linear tetrads were observed. In *P. macrourum* and *P. squamulatum,* one to 8 nucellar cells became active, whereas only one to four were found in *P. polystachion.* In each, two or three aposporous cells continued development to form mature 4 nucleate embryo sacs (Fig. 2C–E). *Pennisetum macrourum, P. polystachion,* and *P. squamulatum* showed 2, 8, and 8%, respectively, aborted embryo sac at anthesis. In another study, *P. polystachion* has been recently reported to reproduce by apomixis (Birari 1981). Progenies of these three species showed no morphological variability. Early degeneration of sexual tissue, absence of 8 nucleate embryo sacs, and uniformity of progeny demonstrated obligate apomictic method of reproduction in these three species.

Pollen stainability and seed set

The percentage of stainable pollen grains and seed set under open-pollinated conditions are summarized in Table 2. Pollen stainability was similar and relatively high in *P. basedowii, P. polystachion,* and *P. squamulatum.* The lower pollen stainability (60%) observed in *P. macrourum* and especially *P. macrostachyum* (15%) was probably due to the irregular segregation of univalents at meiosis. The lower than expected seed set based on pollen stainability in the apomictic species *P. macrourum* and *P. squamulatum* could be due to some self-incompatibility mechanism that prevented stimulation of pseudogamy. Although seed set was determined on inflorescences allowed to open-pollinate in the field, seed set was essentially a result of selfing in the apomictic species since the genotype of all 30 plants within each species were identical. The low seed set and pollen stainability in *P. macrostachyum* resulted from the high frequency of univalents and irregular chromosome be-

Table 2. Percent stainable pollen and seed set of five *Pennisetum* species

	Stainable pollen (%)	Open-pollinated seed (%)
P. basedowii	80	55
P. macrostachyum	15	<1
P. macrourum	60	<1
P. polystachion	77	80
P. squamulatum	82	34

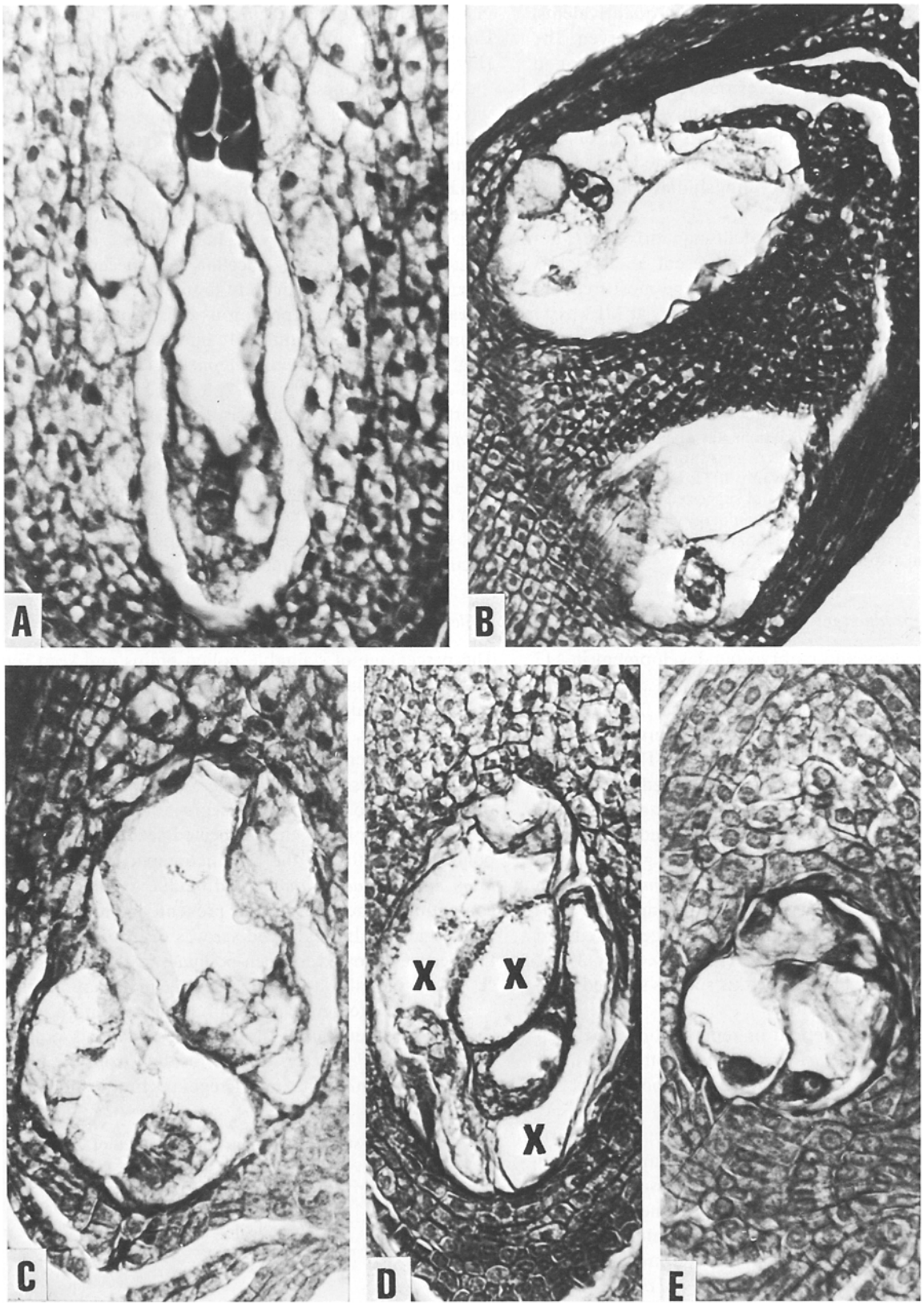

Fig. 2 A–E. Embryo sacs in ovules of five *Pennisetum* species. **A** Sexual embryo sac of *P. basedowii* with conical mass of antipodals and two polar nuclei. **B** Two aposporous embryo sacs in *P. macrostachyum.* **C** Two aposporous embryo sacs in *P. macrourum.* **D** Three aposporous embryo sacs (indicated by x) in *P. polystachion.* **E** Three aposporous embryo sacs in *P. squamulatum.* ca. ×300

havior at meiosis and apparent inability of the pollen to stimulate apomictic development in aposporous embryo sacs.

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References

Birari SP (1981) Cytology of apomixis in *Pennisetum polystachyon.* J Maharashtra Agric Univ 6:205–208

Dujardin M (1978) Chromosome numbers of some tropical African grasses from western Zaire. Can J Bot 46:2138–2152

Hanna WW (1979) Interspecific hybrids between pearl millet and fountain grass. J Hered 70:425–427

Hanna WW (1981) Method of reproduction in Napiergrass and in the 3× and 6× alloploid hybrids with pearl millet. Crop Sci 21:123–126

Hanna WW, Dujardin M (1982) Apomictic interspecific hybrids between pearl millet and *Pennisetum orientale* L. C. Rich. Crop Sci 22:857–859

Hrishi NJ (1952) Studies on the cytogenetics of some species of *Pennisetum* and their comparative morphology and anatomy. Genetics 26:280–356

Jauhar PP (1981) Cytogenetics and breeding of pearl millet and related species. Alan Liss, New York

Krishnaswami S, Thulasidas G (1962) Meiosis in *Pennisetum squamulatum* Fresen. Madras Agric J 49:55–56

Patil BD, Hardas MW, Joshi AB (1961) Auto-alloploid nature of *Pennisetum squamulatum* Fresen. Nature 189:419–420

Rangaswamy SRS (1972) Cytological studies in diploid and polyploid taxa of the genus *Pennisetum* Rich. Genetica 43:257–273

Shanthamma C (1979) Reproductive behavior of *Pennisetum macrostachyum* Benth., and a new basic number in the genus *Pennisetum.* Bull Torrey Bot Club 106:73–78

Sisodia KPS (1970) Cytological studies on some species in genus *Pennisetum.* Theor Appl Genet 40:26–31

Note added in proof

According to Dr. G. Davidse, the accession (PI 354276) from New Guinea and reported in the present study as *Pennisetum macrostachyum* (Brough.) Trin. is definitely not that species, but is probably an undescribed species related to *Pennisetum setaceum* (Forssk.) Chiov.