Cytomorphological studies of a spontaneous triploid in *Pennisetum hohenackeri* Hochst. ex Steud.[1]

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During studies on the genomic relationships of the wild species of the genus *Pennisetum*, one spontaneous triploid ($3x = 27$) plant was identified among the diploid cytotypes ($2n = 18$) of *Pennisetum hohenackeri* Hochst. ex Steud. The triploid plant resembled the diploid in most morphological characters, except for the reduced number of spikelets. Chromosome associations of 9 II + 9 I were observed at diakinesis and metaphase I. The bivalents divided normally, while the univalents lagged and formed a separate nucleus, which was included in one of the daughter cells. On the basis of these studies, this plant was considered to be an allotriploid and might have originated as a spontaneous hybrid between diploid *P. hohenackeri* and an unknown tetraploid (amphidiploid) taxon with one of its genomes homologous to that of diploid *P. hohenackeri*. The possible donor of this genome could be *P. orientale*, which is a tetraploid with a basic chromosome number of $x = 9$.

Key words: allotriploid, meiosis, diakinesis, univalents, micronuclei, laggards.

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Au cours d'études sur les relations génomiques entre les espèces sauvages du genre *Pennisetum*, une plante triploïde spontanée ($3x = 27$) a été identifiée parmi les cytotypes diploïdes ($2n = 18$) de *Pennisetum hohenackeri* Hochst. ex Steud. Cette plante ressemblait aux plantes diploïdes pour la majorité des caractères morphologiques, sauf pour le nombre réduit d'épillets. Des associations chromosomiques de 9 II + 9 I ont été observées à diakinèse et à la métaphase I. Les bivalents se sont divisés normalement, alors que la migration des univalents a été ralentie et a donné lieu à la formation d'un noyau séparé qui fut inclus dans l'une des cellules dérivées. D'après nos études, cette plante a été considérée être une allotriploïde qui pourrait résulter d'une hybridation spontanée entre l'espèce diploïde *P. hohenackeri* et un taxon tétraploïde inconnu (amphidiploïde) dont l'un des génomes aurait été homologue de celui de l'espèce diploïde *P. hohenackeri*. Le donneur possible de ce génome pourrait être le *P. orientale*, qui est tétraploïde et dont le nombre de chromosomes de base est $x = 9$.

Mots clés : allotriploïde, méiose, diakinèse, univalents, micronoyaux traînards.

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Introduction

The genus *Pennisetum* L. Rich., which belongs to the tribe Paniceae of the family Poaceae, comprises about 50 species with basic chromosome numbers of $x = 5$, 7, 8, and 9 (Nath et al. 1970). It includes the economically important species *Pennisetum glaucum* (L.) R. Br., grown for grain in Asia and Africa and as a forage in the United States, *P. purpureum* Schumach, a forage crop, and many other fodder grasses that are widely distributed in the tropics and subtropics of both hemispheres.

Pennisetum hohenackeri Hochst. ex Steud. (synonym *P. alopecuros* Steud.) belongs to the section *Gymnothrix* and has a basic chromosome number of $x = 9$. It is a hardy, perennial wild grass. In addition to its use as a soil binder, its leaves are used as fiber (raw material) in rope making for domestic needs in some parts of Asia.

In nature, *P. hohenackeri* occurs as a diploid ($2n = 2x = 18$), as reported by Darlington and Janaki Ammal (1945), Hrishi (1952), Sree Rangaswamy (1972), and Christopher and Abraham (1976). During studies on the intergenomic relationships of 20 species of *Pennisetum* at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), 1 of 29 plants of *P. hohenackeri* was found to be a triploid ($2n = 3x = 27$), while the rest of the plants were diploids ($2n = 2x = 18$). Naturally occurring triploids are rare (Norrmann and Quarin 1987) and have not been reported earlier in *P. hohenackeri* (Jauhar 1981). The present study, therefore, deals with the morphology and cytogenetical behavior of this natural triploid in *P. hohenackeri*, which is apparently observed and reported for the first time.

Materials and methods

The Genetic Resources Unit, ICRISAT Center, Patancheru, maintains 371 accessions of 20 wild species of *Pennisetum*. Each species is grown in 10-m rows spaced 150 cm apart in field conditions. Although all of them set seed, all the annual species are maintained by seed propagation and the perennials are maintained by vegetative propagation. Seeds of *P. hohenackeri* Hochst. ex Steud. were introduced to the ICRISAT Center from Punjab Agricultural University, Ludhiana, India, in 1975. Since then, the species has been maintained through vegetative propagation. Identification of the material was confirmed by J. N. Brunken, Ohio State University, Columbus, where a voucher specimen of this material is deposited in the herbarium.

Data on 11 morphological characters were collected, according to the standard pearl millet descriptors (International Board for Plant Genetic Resources / International Crops Research Institute for the Semi-Arid Tropics 1981), which included plant height (cm), culm diameter (mm), no. of tillers, leaf blade length (cm), leaf blade width (mm), length of panicle (cm), width of panicle (mm), length of

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TABLE 1. Comparisons for morphological characters in diploid and triploid plants of *P. hohenackeri*

Character	Diploid[a] (mean ± SE)	Triploid[b] (mean ± SE)
Plant height (cm)	117.8±9.7	108
Culm diameter (mm)	3±1	3±1
No. of tillers	64±12	69
Leaf blade length (cm)	61±8	69±7
Leaf blade width (mm)	4±1	4±1
Length of panicle (cm)	11.5±1.2	12±0.8
Width of panicle (mm)	5±0.2	4.5±0.3
Length of bristles (cm)	1.3±0.3	1.4±0.5
Colour of anthers	Red	Red
No. of florets/spike	139±12	98±16
Pollen fertility (%)	92±4	37±16
No. of seeds/spike	28±9	12±3

[a]Data based on 28 plants.
[b]Data based on 1 plant.

bristles (cm), color of anthers, no. of florets/spike, and no. of seeds/spike. To test the significance between the mean values for the morphological data of diploid and triploid plants, Student's *t*-test was used.

Young inflorescences from plants grown in the field were fixed in Carnoy's solution (Johansen 1940) and stored in 70% ethyl alcohol for pollen mother cell (PMC) squashes. For chromosome staining, an anther from a floret of appropriate size was squashed in one drop of 2% acetocarmine solution and photomicrographs were taken using temporary slides. A total of 275 cells at diakinesis, 211 cells at metaphase I, and 87 cells at anaphase I were analysed.

Fertility of pollen grains was determined based on stainability with a 2% iodine and potassium iodide (I_2KI) solution. The slides were left overnight and stainability was recorded from a minimum of 25 or more microscope fields and from at least 200 pollen grains.

Results

Of the 29 *P. hohenackeri* Hochst. ex Steud. plants, 1 plant was identified with reduced seed set accompanied by increased pollen sterility (Table 1). Except for the reduced seed set and pollen fertility, this plant was indistinguishable from other plants in all morphological characters studied. Cytological studies of this plant revealed that it was a triploid, which resembled the diploid in gross morphology (Fig. 1). The differences between the mean values of the triploid and diploid were not significant for plant height, culm diameter, number of tillers, length and width of leaf blades, length and width of panicle, length of bristles and color of the anthers, while significant differences were observed for the number of florets/spike, number of seeds/spike, and pollen fertility (Table 1).

Meiotic chromosome associations at diakinesis in the triploid plant were 9 bivalents + 9 univalents (Figs. 3 and 7). Very rarely a loosely paired trivalent (Figs. 4 and 8) and an extra fragment were observed (Table 2). In diploids chromosomes paired normally to form 9 bivalents (Fig. 2). At late diplotene and early diakinesis in the triploid, the univalents appeared fuzzy and lightly stained in contrast to the bivalents, which were condensed and deeply stained. At early stages, the univalents appeared to lag in their condensation cycle. One bivalent and two univalents were seen attached to the nucleolus in the triploid, while only one bivalent was attached to the nucleolus in the diploids.

The triploid had a mean number of 8.95 bivalents and 9.01 univalents at diakinesis and 8.98 bivalents and 9.01 univalents at metaphase I. All the bivalents aligned on the equatorial plate at metaphase I and exhibited normal disjunction at anaphase I. The univalents lagged (Fig. 5) and formed micronuclei, which were included in one of the daughter cells (Fig. 6), resulting in one uninucleate and one binucleate dyad. A micronucleus was observed in 42% of the cells. A few other meiotic disturbances were observed, such as lack of synchronisation of chromosome movement at anaphase I and, at later stages of meiosis, laggards, chromatin exclusion, and micronuclei. Pollen stainability varied from 22 to 57% in this triploid, while the range was 87–96% in the diploids. The low seed set of the triploid when compared with diploids (Table 1) was not unexpected and was consistent with its low pollen fertility. Seeds produced on the triploid failed to germinate.

TABLE 2. Chromosome associations (%) in a triploid of *P. hohenackeri* at diakinesis and metaphase I

Type of association	Diakinesis (% cells)	Metaphase I (% cells)
9 II + 9 I	95	98
1 III + 8 II + 8 I + 1 fragment	3	1
8 II + 11 I	2	1

Discussion

Regular formation of 9 II + 9 I at diakinesis and metaphase I would immediately suggest that this plant was an allotriploid, as in the case of *P. setaceum* (Simpson and Bashaw 1969) and *Andropogon ternatus* (Norrmann and Quarin 1987).

As the formation of trivalents was rare, there seemed to be no intergenomal pairing, suggesting the presence of a rigid control, which usually permitted pairing between the homologous genomes. The occasional occurrence of a single trivalent may be the result of homoeologous pairing of the univalent with one of the bivalents.

An explanation of the origin of the triploid in *P. hohenackeri* could be spontaneous hybridization between a diploid *P. hohenackeri* plant and an unknown tetraploid (amphidiploid) taxon, with one of its genomes homologous to that of *P. hohenackeri*. The probable schemes are shown below.

HH	×	HHOO		HH	×	OO
(H)		(HO)		(HH)		(O)
	HHO				HHO	
	II + I.				II + I	

HH represents the diploid genome of *P. hohenackeri* and HHOO is the genome of the probable amphidiploid taxon. Another possibility is that the unreduced gamete (HH) was contributed by diploid *P. hohenackeri*.

Of the 20 *Pennisetum* species maintained at ICRISAT, the probable tetraploid parent could be *P. orientale* L. C. Rich. ($2n = 4x = 36$) in the origin of triploid *P. hohenackeri* ($2n = 3x = 27$), for the following reasons. (*i*) *Pennisetum orientale* plants were grown close to the *P. hohenackeri* plants. (*ii*) *Pennisetum orientale* is an amphidiploid with 18 bivalents at diakinesis. (*iii*) *Pennisetum orientale* has three nucleolar-organising bivalents, one of which is probably homologous to the nucleolar-organising chromosome of diploid *P. hohen-*

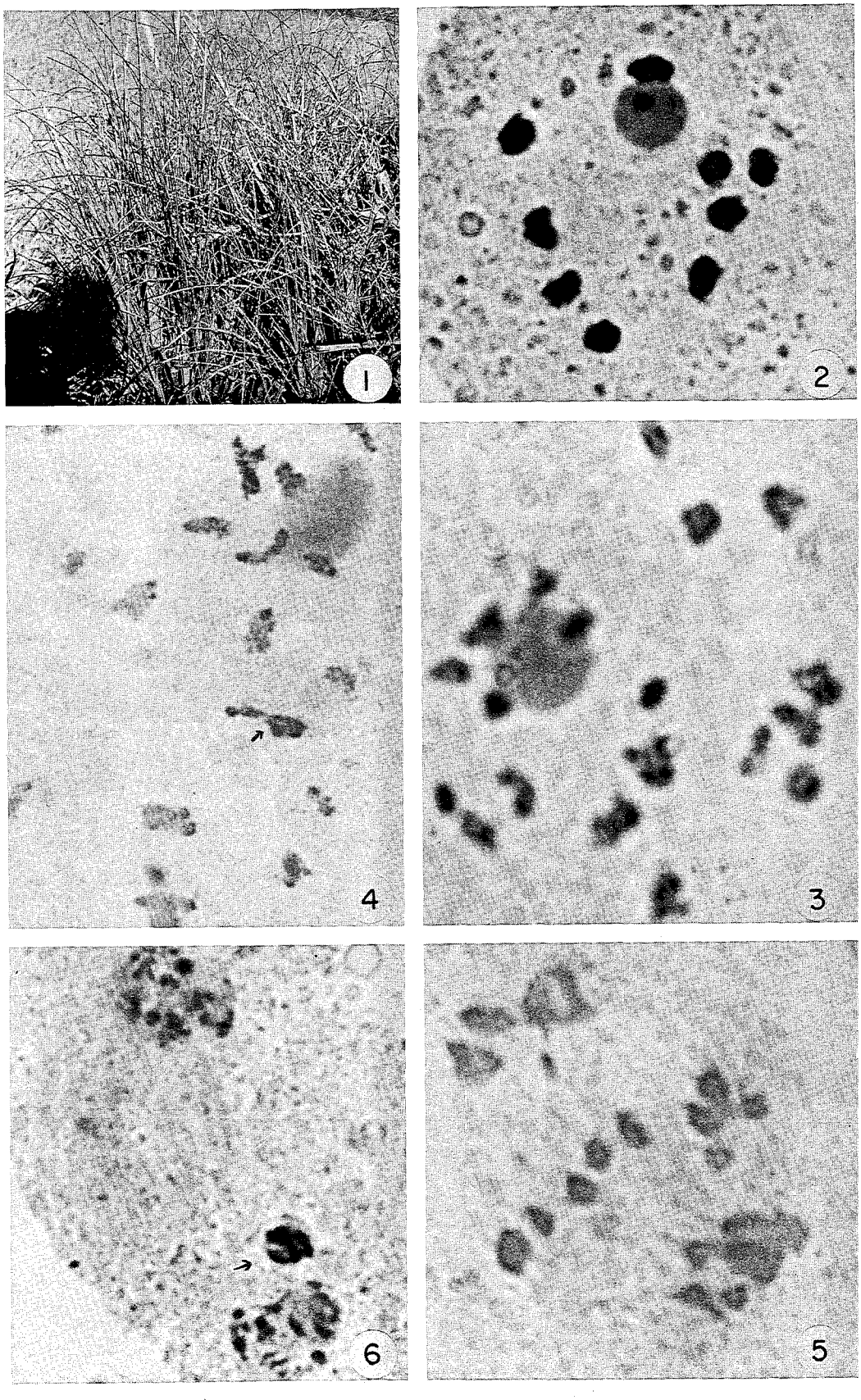

FIG. 1. Morphology of triploid plant of *P. hohenackeri* ($2n = 3x = 27$). FIGS. 2–6. Meiosis in diploid and triploid *P. hohenackeri*. Fig. 2. Diakinesis in diploid showing 9 II. Fig. 3. Diakinesis in triploid showing 9 II + 9 I. Fig. 4. Diakinesis in triploid showing 1 III (arrow) + 8 II + 8 I. Fig. 5. Anaphase I in triploid with 9 laggards. Fig. 6. Telophase I in triploid with extra nucleus (arrow).

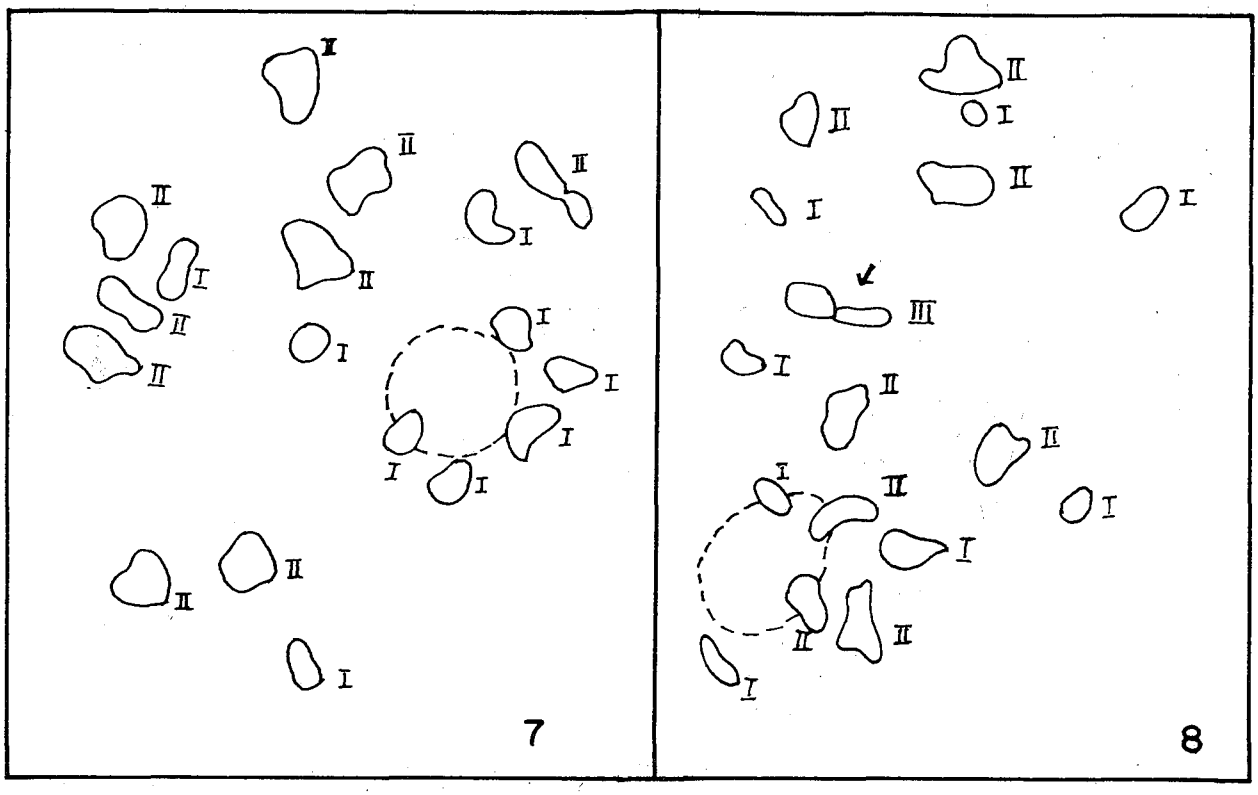

FIG. 7. Explanatory diagram for Fig. 3. FIG. 8. Explanatory diagram for Fig. 4.

ackeri and therefore formed a bivalent in the triploid form. The remaining two univalents associated with the nucleolus in the triploid may represent the other two nucleolar-organising chromosomes of *P. orientale*. (*iv*) The morphology of the unpaired genome of the triploid *P. hohenackeri* closely resembled that of *P. orientale*. Thus, the triploid of *P. hohenackeri* seems to have the genomic constitution HHO so that it generally forms 9 II + 9 I. Further work is needed to confirm this.

Polyploids with uneven genome numbers tend to be infertile whatever their genetic constitution, simply because regular two by two (bivalent) pairing is impossible. Despite the triploid nature and the meiotic irregularities, the perpetuation of the triploid plant was favored by its perennial habit and vegetative mode of propagation. A better understanding of the cytogenetics of the wild species, and their genomic relationships with the cultivated species, would aid the utilization of wild relatives to improve cultivated species.

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