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Cytogenetics of a Factor for Syncyte Formation and Male Sterility in *Pennisetum americanum*

M.K. Rao and P.R.K. Koduru
Department of Botany, Andhra University, Waltair (India)

Summary. Male sterility in *Pennisetum americanum* (L.) Leeke, inbred line IP 482, was found to be inherited as a monofactorial recessive phenotype. Homozygosity for the gene designated *ms* 2, produced in addition to pollen abortion, plasmodial tapetum, plasmodial pollen mother cells, delayed and asynchronous meiotic development, desynapsis and blockage of meiosis. Plasmodial PMCs resulted from the fusion of PMCs at pachytene.

Key words: *Pennisetum americanum* – Syncyte formation – Male sterility

Introduction

The proper development and functioning of the sexual organs of an organism is dependent upon several partially synchronized processes controlled by different genes, all of which should be present in a dominant condition for normal functioning. Mutation in any of the genes causes abnormal behaviour at a particular developmental stage and several recessive genes are known to affect various steps of meiosis, leading to either male sterility or both male and female sterility (for reviews see Sjodin 1970; Gottschalk and Kaul 1974; Gottschalk and Klein 1976). The male sterile genes, designated as *ms* genes, are usually recessive and different *ms* genes differ in their mode of action: some of them have no effect on the meiotic development and produce male sterility by aborting the microspores, while others are known to have such pleiotropic effects as induction of plasmodial sporocytes, breakdown of division cycles and degeneration of pollen mother cells (Gottschalk 1976; Gottschalk and Kaul 1974; Smith and Bennett 1973).

In *Pennisetum americanum* (L.) Leeke (2n = 14), an important cereal and fodder crop species, genetic male sterility was reported previously by Gill et al. (1973) and Krishna Rao and Koduru (1978) in which the *ms* genes had no other effect except to cause the abortion of the male germ cells. The genetic male sterility now described is different from the earlier reports for *Pennisetum* in that it is associated with syncyte formation, abnormal meiosis and abnormal tapetal behaviour.

Materials and Methods

In the inbred line IP 482 of *P. americanum* maintained by continued selfing for homozygosity of the hairy lamina, a seedling marker phenotype, two out of the ten plants grown to maturity for seed production were found to be male sterile. Spikes at different stages of development were fixed in methanol-acetic acid (3:1) for 24 hours and stored in 70% alcohol. Cytological events were studied in acetocarmine squashes of pollen mother cells.

Inheritance of male sterility was studied by crossing the sterile plants to male fertiles from the nonsegregating sib families of the isogenic inbred line IP 482 and also to normal plants of nonisogenic inbred lines Vg 212 and IP 1475, in which male sterility has not been recorded so far.

Observations

Morphology

Male sterile plants were more densely hairy and produced more productive tillers than the male fertile sibs. Anthers emerged normally in the male steriles but anthesis was very poor. Selfed ears rarely produced one or two seeds, but there was normal seed set on both hand-pollinated ears and on ears left for open pollination.

Developmental Gradation and Syncyte Formation

In the male fertile plants of IP 482, as in several other inbred lines, meiosis in the pollen mother cells was com-

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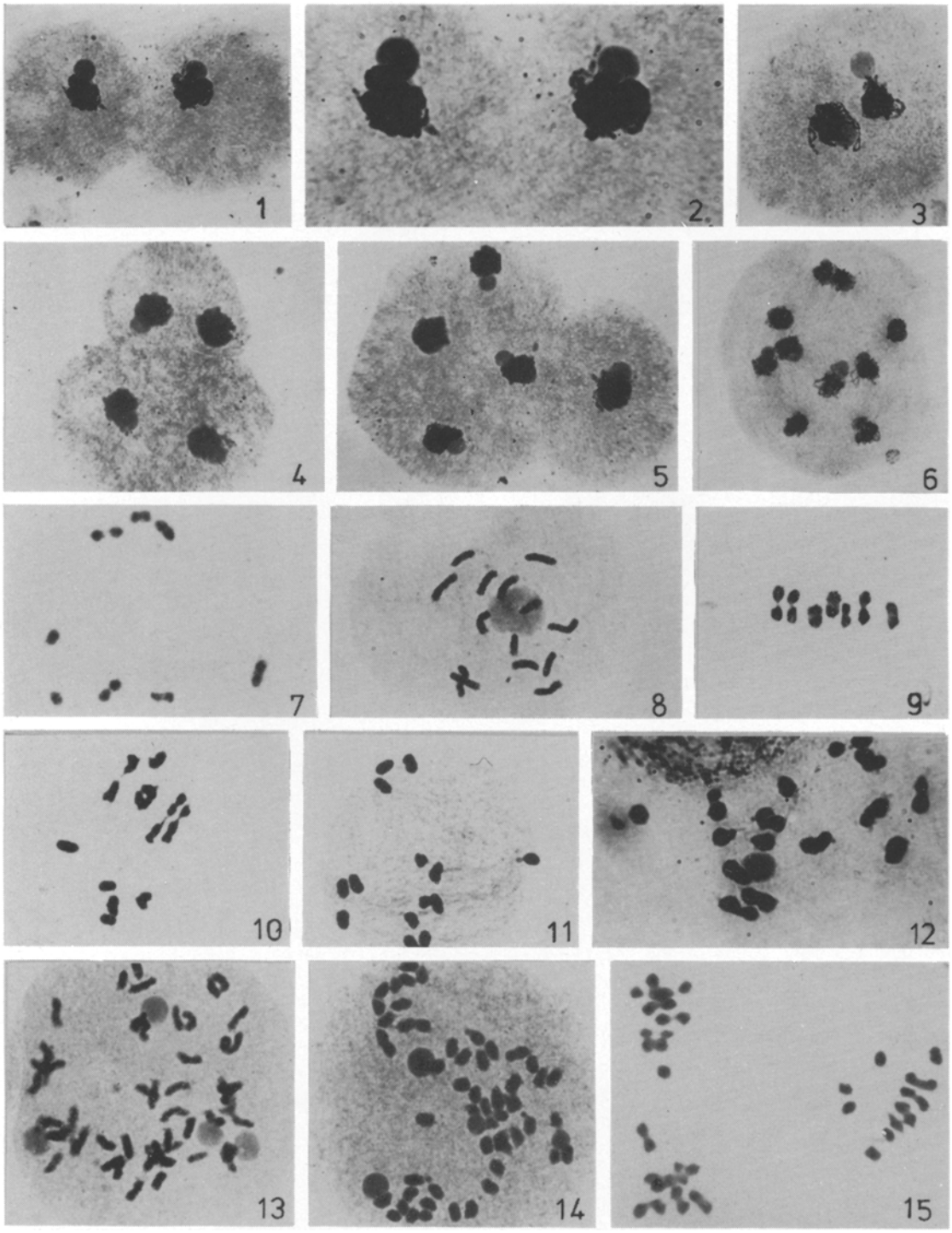

Figs. 1 to 15. Cytology of syncyte formation and meiosis in the PMCs of male sterile pearl millet
Fig. 1. Initiation of syncyte formation. Note the unusually clumped chromosomes
Fig. 2. The nuclei of Fig. 1 magnified to show pairing in chromosomes attached to the nucleolus
Fig. 3. Completion of cell fusion leading to bi-nucleate cells
Figs. 4 to 6. Stages in the formation of multinucleate plasmodia
Fig. 7. Uninucleate PMC showing six rod bivalents and two univalents at diakinesis
Fig. 8. Uninucleate PMC with 14 univalents at diakinesis
Fig. 9. M I orientation of seven bivalents in a uninucleate cell
Fig. 10. M I behaviour of bivalents and univalents in a PMC showing partial desynapsis
Fig. 11. M I in a PMC with all univalents: seven univalents oriented on the equatorial plate; the rest irregularly distributed
Fig. 12. Two nucleate PMC with 11 bivalents and six univalents
Fig. 13. A four nucleate PMC with six bivalents at diakinesis
Fig. 14. A four nucleate PMC with four bivalents and 48 univalents
Fig. 15. M I orientation of bivalents in a three nucleate PMC organizing three separate spindles; note low degree of desynapsis

pleted in all florets by the time the ear emerged completely out of the boot. In male sterile plants of IP 482, however, PMCs at various stages of meiosis were seen in fully emerged ears with stigmas emerging out. Further, in contrast to the male fertile plants, there was no synchronous development among the three anthers of a floret. There was also greater asynchrony between the successive spikelets in the male sterile than in male fertile ears. Thus meiosis in the male steriles was both delayed and asynchronous.

In young anthers, archesporial cells and PMCs at preleptotene were uninucleate and appeared normal. Older anthers, however, contained a mixture of uninucleate cells and plasmodia; there were 2-12 nuclei per plasmodium. Detailed analysis through developmental stages revealed fusion of uninucleate cells, leading to the formation of plasmodia (Figs. 1 to 6). Fusion between PMCs was initiated at pachytene: in these cells nuclei contained closely clumped chromosomes with an eccentric nucleolus; the occasional stretched-out chromosome segments appeared paired (Fig. 2). The fusion initially led to the formation of binucleate cells. Cell fusion increased with maturity of the anthers as can be judged by the progress of meiosis in normal cells. As meiosis proceeded to telophase II in the uninucleate cells, the frequency of plasmodial sporocytes per anther, as well as the number of nuclei in the plasmodial masses, increased because cell fusions continued throughout. (Table 1). The shape and size of the plasmodium was controlled by the number and the extent of fusions between cells.

Meiosis in Uninucleate Cells

There was no synchrony in the meiotic development of uninucleate PMCs within the anther in contrast to the almost synchronous development in normal plants. Frequently PMCs carried seven bivalents of a rod type; occasionally cells with two to four univalents and only rarely cells with fourteen univalents were also present (Figs. 7 and 8). Orientation of bivalents at M I (Fig. 9) and disjunction at A I was normal. However, chromosome orientation (Figs. 10 and 11) and segregation were disturbed with an increase in the number of univalents per PMC. Equational division of the chromosomes occurred at anaphase II and the meiosis was complete leading to the formation of microspores.

Meiosis in Syncytes

Syncytes were rarely seen in the advanced stages of meiosis and even at the time of formation of pollen grains from the uninucleate PMCs they were seldom observed.

Table 1. Percentage frequency of plasmodia with 2-12 nuclei at different stages of meiotic development in the anther

Spikelet number studied	No. of PMCs studied	Frequency of uninucleate PMCs						Number of nuclei in a plasmodium										
		Meta-bolic	Early meiotic phase stages	Diaki-nesis	MI	AI	PII-TII	2	3	4	5	6	7	8	9	10	11-12
1	124	100	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	105	93.3	4.76	–	–	–	–	1.90	–	–	–	–	–	–	–	–	–
3	100	–	75.00	–	–	–	–	19.00	5.0	1.0	–	–	–	–	–	–	–
4	117	–	51.28	–	–	–	–	24.79	11.11	7.69	2.56	1.71	0.85	–	–	–	–
5	151	–	51.67	–	–	–	–	27.15	7.95	5.30	3.97	2.65	1.31	–	–	–	–
6	188	–	48.94	–	–	–	–	25.00	9.57	8.51	3.19	2.66	3.13	–	–	–	–
7	227	–	51.10	–	–	–	–	27.30	8.81	3.52	3.52	2.64	3.08	–	–	–	–
8	233	–	34.33	9.87	0.43	–	–	30.04	9.01	7.30	4.29	3.43	1.29	–	–	–	–
9	287	–	35.50	8.71	5.58	–	–	26.13	6.97	6.97	3.83	3.48	1.39	–	1.05	0.34	–
10	248	–	18.95	10.89	8.06	4.84	2.42	28.23	10.08	5.24	3.22	3.23	3.23	0.40	0.81	0.40	–
11	203	–	17.24	7.39	8.37	4.93	4.93	24.63	9.85	7.88	4.93	0.99	2.46	–	4.43	0.99	0.99
12	135	–	18.52	9.62	11.85	8.15	6.67	14.81	11.85	7.41	5.19	–	–	0.74	3.70	0.74	0.74
13	138	–	18.84	7.25	10.87	8.70	7.25	15.22	10.14	8.70	4.35	1.45	2.17	1.45	2.17	1.45	–

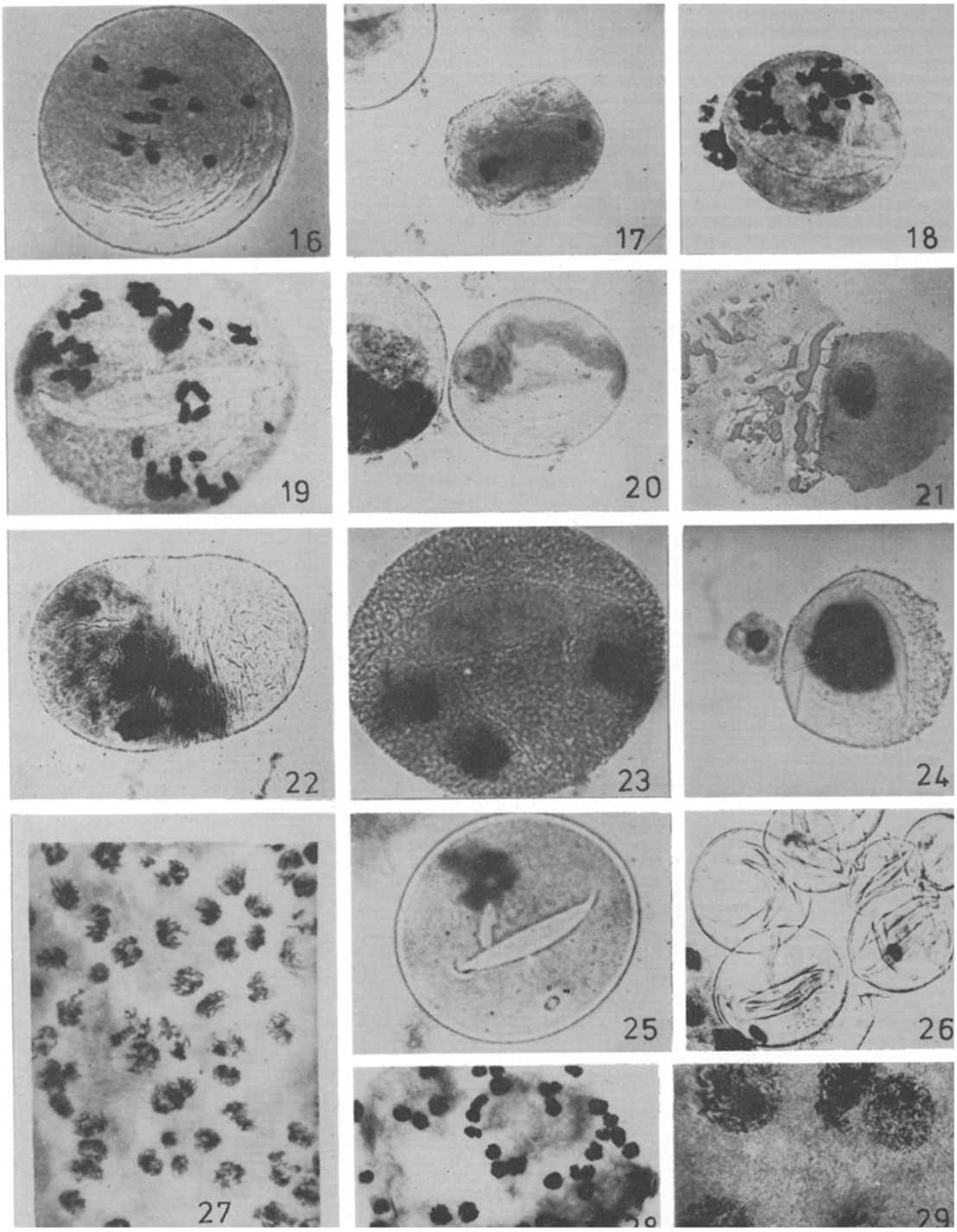

Fig. 16 to 23. Suppression of meiosis in PMCs of male sterile pearl millet
Fig. 16. Uninucleate PMC at M I surrounded by wall
Fig. 17. Uninucleate PMC at A I with degenerating chromatin masses; note the darkly stained cytoplasm
Fig. 18. Two nucleate PMC at diakinesis surrounded by pollen grain wall
Fig. 19. Three nucleate PMC at diakinesis with several univalents, surrounded by wall
Fig. 20. Uninucleate 'pollen grain' with degenerating contents
Fig. 21. A uninucleate 'pollen grain' with cell contents extruded; note the degenerating nucleus and the irregularly thickened wall archetecture
Fig. 22 to 24. 'Pollen grains' formed from plasmodia with different degrees of degeneration of cell contents
Fig. 25. A giant 'pollen grain' formed from a single PMC, with well defined germ pore
Fig. 26. The 'pollen grains' (bigger grains) and microspores (smaller grains) produced by the male sterile plant
Figs. 27 to 29. Stages in the formation of amoeboidal tapetum
Magnification: Figs. 6, 13 to 16, 24, 26 to 28 (× 750μ) Figs. 1, 3 to 5, 7 to 12, 17 to 23 and 29 (× 1000μ) Figs. 2 and 25 (× 1500μ)

Meiotic development between the nuclei of a plasmodium was synchronous but there was asynchrony between the plasmodial masses. Meiotic development beyond diakinesis was observed only in a small proportion of binucleate cells; in all others meiosis was blocked at diakinesis or earlier. Chromosome associations higher than bivalents were not observed (Figs. 12 to 14). Moreover, in syncytes with three or more nuclei, most of the chromosomes were present as univalents (Fig. 14). Only in one exceptional trinucleate did syncyte meiosis proceed up to M I with the bivalents orienting on three separate spindles (Fig. 15). In anthers containing microspores produced from uninucleate cells, thick walls resembling pollen grain walls developed, surrounding the syncytes.

Suppression of Meiosis

Meiosis in both uninucleate and plasmodial sporocytes which were at various stages of meiotic development was suppressed by the deposition of pollen grain walls surrounding them at the time of the formation of pollen grains in the normal cells (Figs. 16 to 19). In most of these PMCs the nuclear contents degenerated (Figs. 20 to 24) while the other cells developed into giant pollen grains with the differentiation of a germ pore (Fig. 25), though in most of them the nuclear contents also showed signs of degeneration (Fig. 26).

Tapetal Behaviour

Tapetal development appeared normal at the early stages of anther development. To start with, the tapetum was uninucleate. Dissolution of intermittent walls in the tapetum leading to plasmodial masses was first noticed when there was formation of syncitial masses of PMCs. By the time wall deposition occurred, surrounding the PMCs and the microspores, the entire tapetum was amoeboidal (Figs. 27 to 29).

Pollen Stainability and Seed Set

Pollen stainability at the time of anthesis was nil. In general the mutant plants did not set seed, however, in the first season six seeds were obtained from the bagged ears. Three seeds germinated and the plants showed the same type of meiotic abnormality and male sterility at maturity as the parent plants. Seed setting in male sterile plants was as good as in male fertile plants, on crossing as well as on open pollination, indicating normal female fertility.

Inheritance

Inheritance of the mutant phenotype was studied both in the selfed families of heterozygous male fertile sib plants

Table 2. Segregation for male sterility versus male fertility in selfed families of heterozygous plants, F_2, testcross and the backcross generation families

Parental genotypes	No. of families	Total No. of plants	Observed Segregation		χ^2 (3:1/1:1)	Homogeneity χ^2
			Male fertility	Male sterility		
Ms ms	Segregating populations					
S_1 generation	1	225	174	51	0.65^d	—
S_2 generation	1	167	120	47	0.88^d	—
S_3 generation	1	150	109	41	0.44^c	—
S_4 generation	1	208	160	48	0.41^c	—
ms2 ms2 × *Ms2 Ms2*	F_2 generation					
IP482 × IP482	3	432	321	111	0.11^b	1.27^c
IP482 × Vg212	4	409	313	96	0.51^d	0.90^b
IP482 × IP1475	3	444	322	122	1.35^e	0.13^a
ms2 ms2 × F_1 (*Ms2 ms2*)	Testcross generation					
IP482 × F_1 (IP482 × IP482)	3	369	179	190	0.33^c	0.90^c
IP482 × F_1 (IP482 × Vg212)	3	368	179	189	0.27^c	0.94^c
IP482 × F_1 (IP482 × IP1475)	3	425	208	217	0.19^c	0.95^c
Ms2 Ms2 × F_1 (*Ms2 ms2*)	Backcross generation					
	7	656	656	000	—	—

P- values: a = 0.95 – 0.9; b = 0.9 – 0.7; c = 0.7 – 0.5; d = 0.5 – 0.3 and e = 0.3 – 0.1

Table 3. Segregation of male sterility 2 in the families of F_3 generation

Parents	F_2 phenotype	Total no. of F_3 families	No. of families		χ^2 (1:2)	p-values
			Non segregating	Segregating		
ms2 ms2 × *Ms2 Ms2*						
IP482 × IP482	Male fertile	29	11(263)*	18(406)	0.27	0.7-0.5
IP482 × Vg212	Male fertile	20	6(154)	14(300)	0.10	0.9-0.7
IP482 × IP1475	Male fertile	32	13(295)	19(454)	0.76	0.5-0.3

* Figures in the brackets represent the total number of plants

and in the crosses of male sterile plants with the nonsegregating male fertile plants from the inbred lines IP 482 (isogenic line), Vg 212 and IP 1475 (non-isogenic lines). All the 920 F_1 plants maintained to maturity were male fertile. Meiosis in 20 plants chosen at random was normal, indicating that the mutant condition was recessive to normal condition. The segregation pattern in the F_2, testcross, backcross and the F_3 generation families suggested monofactorial recessive type of inheritance of the mutant phenotype (Tables 2 and 3). The F_2 and the testcross plants were classified as male fertile and male sterile on the morphological basis of anthesis and seed set on bagged ears. At random, 30 plants per family from the fertile class and ten to fifteen plants from the sterile class were studied at meiosis. All the fertile plants showed normal meiosis. The plants from the sterile class showed the syncyte condition and other meiotic abnormalities as described above. Thus, all the abnormalities of the mutant were inherited as a syndrome controlled by a pair of recessive genes. Since the male sterile gene described now appears to be different from the one described by us earlier (Krishna Rao and Koduru 1978), the gene symbol *ms* 2 is proposed.

Discussion

In a fairly large group of plants the syncyte condition of PMCs has been reported (Gates 1911; Beadle 1932; Snod 1954; Morgan 1956; Price 1956; Sarvella 1958; Kamra 1960; Jain 1962; Sadasivaiah and Magoon 1965, 1966; Bhandari et al 1969; Pradhan and Sen 1971; Mehra and Kalia 1973 and Habib and Chennaveeraiah 1976). Besides gene action (Beadle 1932; Smith 1942; and Pantulu and Manga 1971), external agents such as X-rays (Morgan 1956) and temperature and culture conditions (Jain 1962; Stern 1946; and Lebedeff 1940) were reported to cause syncyte formation in higher plants. Syncyte formation can result either from the failure of cytokinesis during archesporial mitoses (Smith 1942; Pantulu and Manga 1971), from migration of the nucleus from one PMC to the other, as in *Triticum* (Kihara and Lilienfeld 1934) and *Capsicum* (Habib and Chennaveeraiah 1976) or from fusion of uninucleate PMCs, facilitated by the absence or dissolution of the cell walls surrounding the PMCs. Most of the published cases of syncyte formation belong to the third group (Price 1956). In all the reported cases of fusion syncyte formation, cell fusion was observed when the nuclear structure scarcely suggests the onset of meiosis (Mehra and Kalia 1973). However, in the present case syncyte formation was initiated at pachytene.

In *P. americanum*, Burton and Powell (1968) reported on the occurrence of a multiploid condition in some plants of the mutagen treated population, but the genetic basis was not known. Pantulu and Manga (1971) reported the formation of plasmodial sporocytes as a result of the homozygous condition of the gene '*mu*', which suppresses cytokinesis during archesporial mitoses. However, the mutant plants were female sterile as well, producing only a few seeds even on crossing to male fertile lines. Further, the plasmodial condition of PMCs reported now also differs in showing retardation or blockage of meiosis in some PMCs of the anther, desynapsis and abnormal tapetal development. Therefore, the two genes may be presumed to be different. The male sterile gene studied now also differs in the mode of action from the *ms* gene reported earlier (Krishna Rao and Koduru 1978) in that the latter had no effect on the meiotic events but resulted in degeneration of microspores before the onset of first mitotic division. The action of *ms* 2 gene described now resembled the male sterile mutants 71A, 78 and 98A of *Pisum* (Gottschalk and Kaul 1974).

The few seeds produced by the male sterile plants on selfing may suggest the rare functioning of a few pollen grains but the possibility of apomictic seed production cannot be eliminated, particularly in view of the report of apomixis in some lines of *Pennisetum* (Hanna and Powell 1973; Hanna, Powell and Burton 1976).

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Dr. M. Krishna Rao
P.R.K. Koduru
Department of Botany, Andhra University Waltair-530 003 (India)