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VARIABLE STERILITY AND APOSPORY IN PEARL MILLET

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ABSTRACT

In inbred lines of pearl millet, one plant and its selfed progenies showed low and variable seed set in different spikes. Genetic analysis revealed the transmission of variable sterility through male gametes also. Meiosis in PMCs and pollen fertility were normal. However, plants with full seed set were not obtained in any of the segregating generations. Maternal (apomictic) plants were identified at different frequencies in progenies of crosses using genetic markers. In variable steriles, female gametophyte development showed variation within spikes, like (a) normal embryo sacs, (b) diminutive embryo sacs, and (c) multinucleate aposporous initials, one of which formed precocious embryo and endosperm. Studies after pollination with a fertile line also showed variations like (a) normal development of embryo and endosperm, (b) formation of undersized embryos, and (c) degeneration of embryo sacs.

Key words: Sterility, apospory, pearl millet.

The occurrence of apospory, adventive embryony, polyembryony and maternal (asexual) seedlings in crosses is known in quite a few species of *Pennisetum* [1–4]. In pearl millet, induction of female sterility associated with apospory [5] and recovery of maternal seedlings in interstrain crosses [6] have been reported.

A variable sterile plant was isolated in pearl millet (*Pennisetum glaucum* (L.) R. Br., 2n = 14) and the causes underlying this sterility, its transmission to progenies, and occurrence of apospory, precocious embryo and endosperm development and maternal seedlings in crosses were investigated.

MATERIALS AND METHODS

The florets in pearl millet spikes open from tip downwards and are protogynous in which the styles emerge 2 or 3 days prior to anther exsertion. In crosses, the top 1/3 portion

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of the spike was cut off while still in boot leaf and bagged (to prevent selfing) and subsequently hand-pollinated after complete emergence of styles throughout the spike with desirable pollen so that fertilization is effected before the emergence of the anthers in that spike. For studies of prepollination development of female gametophyte, entire spikes were bagged when they were partly exposed from the boot leaf, and from the youngest spike to the ones with full emergence of styles (but before the occurrence of anthesis) were fixed in FAA. For studies of post-pollination development, crossed spikes were fixed at specific time intervals after pollination. The ovules dissected out from the florets were processed using customary methods and serial sections were cut at 4–18 μm thickness and stained in haematoxylin.

Meiosis and pollen stainability were studied from acetocarmine preparations, and pollen germination on styles was assessed following the method of Murthy [7].

For the study of transmission of sterility, the variant plants were selfed and also crossed reciprocally with fully fertile inbred lines. Inbreds with dominant phenotypic markers were used as male in crosses with steriles to identify the maternal (apomictic) seedlings in the progenies.

RESULTS AND DISCUSSION

In the seventh selfed generation of a composite variety Nagarjuna (obtained from Millet Research Station, Vizianagaram, Andhra Pradesh), one plant exhibited sparse seed set on the main culm. The spikes on the remaining culms, showed variation from total failure to moderate seed set. This plant was selfed and the seeds obtained were used to raise the next generation. Of 220 seeds, 50 germinated of which only 30 plants grew to maturity. They also showed the same sterility varying between culms of the same plant. Further, the seed size (normal and small) and the seed germination (none to full) in the sterile plants was also highly variable. The small seed invariably failed to germinate. Meiosis and tetrad formation in PMCs, and pollen stainability and germination on styles were completely normal in all the progeny tested.

TRANSMISSION OF STERILITY

In order to study the transmission of this character to the progenies, two of the plants were selfed and the rest were crossed reciprocally with six fertile inbreds (IP 8008, IP 8210, IP 4807, IP 8214, IP 8166 and IP 7924) obtained from ICRISAT, Patancheru. Seed set was variable. From the incomplete seed set even in inbreds on crossing with the variable sterile as male it was evident that a proportion of the apparently normal pollen in the latter was nonfunctional. Two selfed progenies, and 24 progenies of F_1s using the variable sterile plants as female and 24 other F_1 progenies using the inbreds as female were raised. The progeny size varied from 26 to 280 plants in the former and 54 to 340 in the latter. About 2000 F_1

plants were transplanted from all the crosses. Again, all the 240 plants in the selfed progenies, and F_1s of all the crosses showed inconsistent seed set which contrasted sharply to the uniformly full seed set in all the culms in the parental inbreds. This indicates that the disorder is transmitted to progeny through both male and female gametes. Pollen stainability of all the F_1s was good. Several selfed spikes with good as well as poor seed set were used to raise a large F_2 generation and the F_1 was also back-crossed to two of the corresponding inbreds (IP 4807, IP 8214) to score the segregation of fertile and sterile plants. Fully fertile plants were not found in F_2 or backcross generations. Selfing and selection of ears with comparatively good seed set was done in F_2 and was continued for eight continuous generations, but it had no effect on improvement of seed set. Not a single plant with full fertility in all the culms was obtained in any of the segregating generations. Therefore, the genetic basis of the trait could not be resolved. Since there was no change in the expression of the character in different seasons, in which the successive generations were raised, the possibility of environmental factors causing the sterility was discounted.

OCCURRENCE OF MATERNAL SEEDLINGS

Two of the inbred lines involved in crosses were homozygous dominant, one (IP 8166) for purple plant colour and the other (IP 7924) for hairy plant phenotype, in contrast to green plant colour and glabrous condition in the variable sterile. The expression of the marker genes could be scored in five-week-old seedlings in the seed beds. The F_1 plants of the reciprocal crosses were all expected to be purple in the former and hairy in the latter. However, where the marker stocks were involved as male, 23 out of 256 progeny from purple inbred showed green plant colour and 12 out of 178 progeny from the hairy inbred showed glabrous condition, arousing suspicion that the seedlings with maternal character originated asexually. In order to verify this, in the subsequent generation, using marker stocks as male, 50 spikes were crossed (25 with each of the same two inbreds). Seed set was again variable. Twenty two progenies of F_1 from crosses with purple inbred and 25 with hairy inbred were raised. Seed germination was incomplete. The size of each of the progenies varied from 4 to 293 plants. The frequency of maternal plants varied from 6 in 86 (6.9%) to 102 in 263 (38.7%) in the crosses with purple inbred, and from 24 in 293 (8.1%) to 98 in 256 (38.2%) in crosses with hairy inbred. The overall frequency of maternal seedlings was 28.0%. This confirmed the occurrence of maternal seedlings suspected earlier. Such seedlings must have occurred also in progenies of other crosses, but could not be identified.

PREPOLLINATION DEVELOPMENT

Embryo sac development was studied from 130 randomly chosen unpollinated ovules of various stages coming from five different variable sterile plants. The development was found to be normal in some (Table 1, items 1, 4 to 7) which conformed to the earlier report [8]. Diminutive embryo sac (Table 1, item 8, Fig. 1F), where present, was about one third (115 x 50 μm) the size of normal embryo sac (380 x 80 μm). In ovules with enlarged

multinucleate nucellar cells (Table 1, item 2, Fig. 1A) there was no indication of the presence of archesporium or its derivatives. Of the 1 to 6 aposporous initials (Table 1, item 3, Fig. 1B) with 2 to 5 nuclei each, only one presumably developed into embryo and endosperm precociously (Table 1, item 9 and 10, Fig. 1C–E). The occurrence of monopolar aposporous embryo sacs as distinct from 8-nucleate sexual embryo sacs, frequently reported in Poaceae

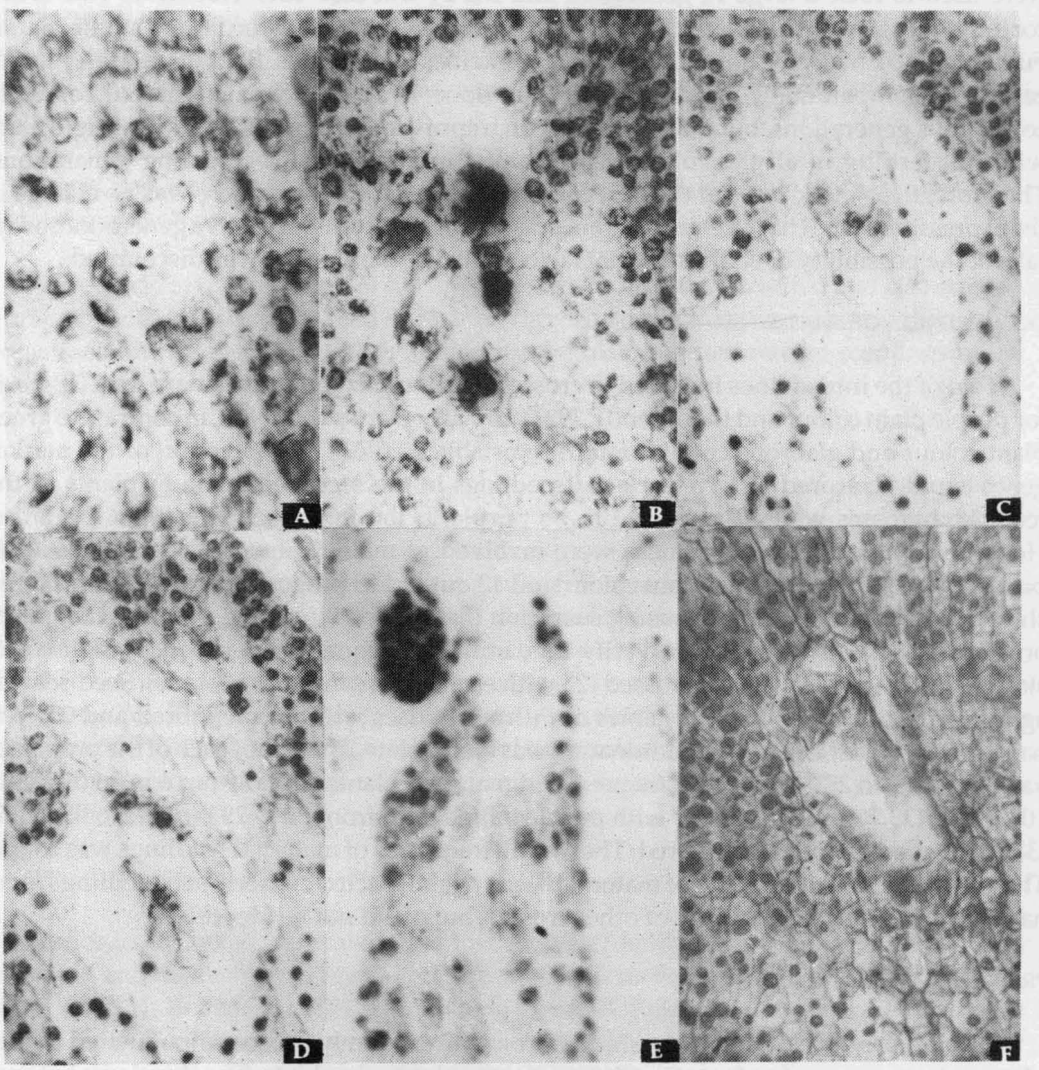

Fig. 1. Development of aposporous embryo and endosperm in pearl millet.
A–F) L.S. of prepollinated ovule: A) enlarged multinucleate nucellar cell (X300), B) three multinucleate aposporous initials (X400), C & D) three-celled proembryo and three free nuclei of endosperm (two sections of the same ovule) (X400), E) precociously developed embryo and endosperm (X200), and F) diminutive embryo sac (X400).

[2], however, were not encountered in this study. Chromosome counts from cells with reasonable spread in embryo and endosperm revealed 2n number in the former and 2n and 4n numbers in the latter, the 4n number arising through endoreduplication.

POST-POLLINATION DEVELOPMENT

Variable sterile plants were crossed as female with fully fertile inbreds (IP 4807, IP 8214) and the spikes after pollination were fixed at specific time intervals (1, 2, 6, 24 h etc.) to study post-pollination development and 270 ovules were studied (Table 1, items 1–8). Failure of fusion of gametic nuclei (egg and sperm nuclei or secondary and sperm nuclei) was observed in some embryo sacs which showed signs of degeneration from 6th day after pollination (Table 2, item 7(iii), Fig. 2B) while the rest showed normal fertilization and development. In one ovule, proembryo and a massive endosperm were found 24 h after pollination (Table 2, item 4(iii), Fig. 2A) instead of a 2-celled proembryo and free nuclear endosperm normally

Table 1. Prepollination development of embryo sac, embryo and endosperm in variable sterile plants

Stage of development	No. of ovules
*Single hypodermal archesporium	41
Enlargement of some nucellar cells (aposporous initials)	8
1 to 6 aposporous initials with multiple nuclei	10
*2-nucleate embryo sac	8
*4-nucleate embryo sac	8
*8-nucleate embryo sac	12
*Organised sexual embryo sac	18
Diminutive 8-nucleate embryo sac	6
Proembryo and endosperm nuclei (apomictic)	1
Globular embryo and endosperm (apomictic)	18

*Normal course of development.

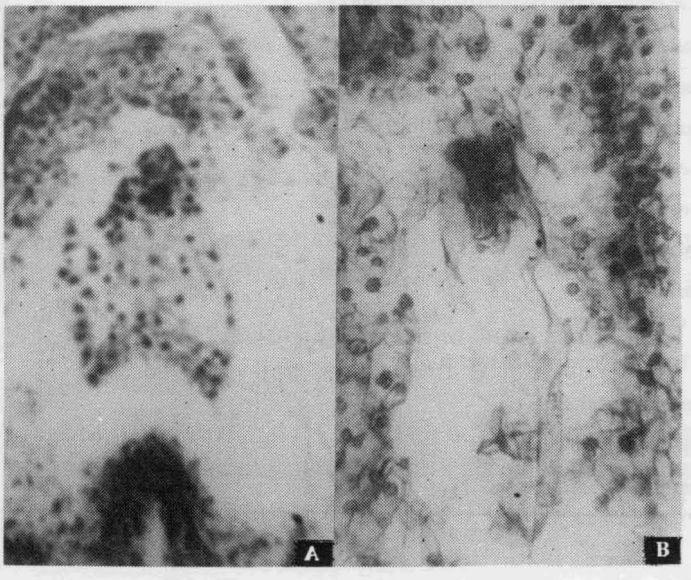

Fig. 2. L.S. of ovule showing post-pollination development.
A) Ovule 24 h after pollination, showing advanced growth of embryo and endosperm compared to normal (X150).
B) Degenerating embryo sac 6 days after pollination (X400).

expected at that stage. This must have been due to precocious development before the ovule was pollinated. Small sized embryos were found in some ovules (Table 2, item 8(ii)) which were about 440 x 283 µm compared to 2130 x 639 µm of normally developed embryos. These small embryos must have led to the inviable small seed on the spikes. The sequence of events in the normally developing embryo and endosperm is in line with the report of Rao and Kumari [9].

Table 2. Post-pollination development of the embryo and endosperm in variable sterile x fertile inbreds (IP 8214, IP 4807) and their reciprocals

Time of fixation after pollination		Stage of development	No. of ovules
	Variable sterile x fertile inbreds		
1 h	*i)	Entry of pollen tube into embryo sac	28
	ii)	Diminutive embryo sac — no entry of pollen tube	5
3 h		*Egg nucleus at fertilization	24
6 h		*Syngamy completed, early endosperm nuclei formed	18
24 h	*i)	2-celled proembryo and free nuclear endosperm	14
	ii)	Unfused gametic nuclei	7
	iii)	Embryo and endosprm showing advanced growth	1
2 days	*i)	Proembryo and endosperm	16
	ii)	Unfused gametic nuclei	4
3 days	*i)	Globular embryo and cellularising endosperm	15
	ii)	Unfused gametic nuclei	4
5th to 7th day	*i)	Differentiation of embryo	66
	ii)	Diminutive embryo sac (degenerating)	2
	iii)	Degenerating embryo sac	12
8th to 12th day	*i)	Well developed embryo	12
	ii)	Small sized embryo	15
	Fertile inbreds x variable sterile		
1h		*Entry of pollen tube	10
24 h to 2 days	*i)	Proembryo and early endosperm	12
	ii)	Unfused gametic nuclei	3
5th to 8th day	*i)	Normal embryo development	9
	ii)	Degeneration of embryo sac	3

*Normal course of development.

Four spikes from two of the fertile inbred lines (IP 4807, IP 8214) were fixed at different times after pollination with pollen from variable sterile plants (Table 2, item 9 to 12) to study the effects in the reciprocal cross. Of the 37 ovules studied, normal fertilization and development were found in several, but in some gametic fusion was not observed. This indicates that the nonseed set in some of the ovules after these crosses was exclusively caused by nonfusion of gametic nuclei.

From the present study, it is evident that the reduced seed set is caused by events like nonfertilization of some of the normally formed sexual embryo sacs, occurrence of diminutive embryo sacs and underdeveloped embryos in a proportion of the ovules. The frequencies of these abnormalities vary from spike to spike contributing to the variation in the extent of seed set. However, the realised seed set could be somewhat higher than the frequencies of abnormalities in a spike would permit, due to the occurrence of functional precociously formed aposporous embryo and endosperm in some cases. Such precocious development is also variable from spike to spike, as revealed by the variation in the frequencies of maternal seedlings recovered in crosses. Pollen from variable steriles, though apparently normal, induces sterility to varying extent on otherwise fertile spikes of inbreds through nonfusion of gametic nulcei in some of the crossed ovules. It also transmits other factors leading to female sterility as mentioned, along with those for aposporous development, which express themselves from F_1 generation onwards.

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