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Apomictic symptoms in the aspect of embryology in a sorghum line 296B

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Summary The presence of facultative apomixis in line 296B was proved by the embryological study. The twin embryosacs were observed at florescence. The autonomous development of embryo was confirmed by multiple cell proembryo existing with undeveloped polar nuclei in one embryosac. Cell structure and size of apomictic proembryo were different from sexual proembryo. The structural feature of proembryo can be used to distinguish apomictic proembryo with developed endosperm from sexual embryo. The apomictic development in this line is attributed to aposporous type. The frequency of apomixis is at least 16－21%. The apomictic characters and the potential for fixing heterosis in line 296B was discussed in the present paper.

Key words *Sorghum bicolor*(L.)Moench,apomixis,aposporous,296B

Introduction

Apomixis,asexual seed formation,has been reported in some lines of *Sorghum bicolor*(L.) Moench. Rao et al(1968)first reported obligate apomixis in line R473. Rao et al(1972)concluded that the mechanism of apomixis in this line was apospory,with autonomous development of endosperm and embryo from unreduced egg. Murty et al(1979)provided the characteristics and frequency of multiple embryosac in line R473. However,Hanna et al (1970)argued that the obligate apomixis could not be concluded in line R473 and they reported another apomictic sorghum line with polygynaceous character in which the maternal-type offspring in the testcross progeny was up to 25% at the same time. Marshall et al (1977)studied the variation in malate dehydrogenase locus(Mdh 1) of R473 with starch gel electrophoresis. They concluded that no evidence for obligate apomictic reproduction was obtained and their studies did not rule out the possibility of a low level of apomixis in R473. Reddy et al(1980)reported that a male-sterile mutant of R473 as females and normal R473 as male plants produced F_1 hybrids sexually,thus indicating that they were not obligate apomicts. Furthermore,Tang et al(1980)observed sorghum lines and their F_1 progenies for characteristics indicating apomixis. They concluded that these lines including R473 had a low level of apomictic seed formation and the highest apomictic frequency line was R473. Henceforth,Murty et al(1981,1983,1984,1985a,1985b)developed a concept of 'vybrids' defined as the progenies obtained from crossing two facultative apomicts,which reproduce through facultative apomixis. Niu et al(1991)reported breeding vybrids through crossing facultative apomictic line R473 with 296B. Their results were that vybrids could be produced only in combinations between two different derived facultative apomicts.

Zhang et al(1991) conducted a study on the ability of fixing heterosis in sorghum line

296B, found this line is similar to R473 in cross incompability and regarded 296B as a facultative apomict. The present report deals with the apomictic symptoms in the view of embryology in line 296B.

Materials and methods

Sorghum line 296B with the ability of fixing heterosis and studied by Zhang et al(1991), was used for the present study. The specimens were collected in Sorghum Research Institute, Yuci, in 1992. Fixation was conducted every day before florescence. The panicles were emasculated artificially and fixed almost every 4 hrs from 6 hrs to 4 days after pollination, then were fixed every 1—2 days up to the stage of seed maturity. Whole ovary staining technique was employed and sections were cut at a thickness of 8—10 μ. Observations were made under OLYMPUS VANOX microscope.

Results

The morphological characters of ovary and ovule of line 296B are similar to the description of the same species, presented by Artschwager et al(1949)and Rao et al(1968). We intend to describe the critical developing procedures of apomixis and to compare the differences between apomictic and sexual reproduction in this line.

Megasporogenesis and female gametophyte development

1. Sexual development The archesporium is differentiated just below the single layer of nucellar epidermis at the micropyle end(Fig. 2), which develops directly into megaspore mother cell later (Fig. 3). The megaspore mother cell expands in size, then carries out meiosis to form a tetrad(Fig. 4). The chalazal end cell acts as functional megaspore, which enlarges to become the uni-nucleus embryosac(Fig. 5) and the other three megaspores degenerate. Nuclear mitosis takes place three times in the embryosac followed by the formation of an 8-nuclei embryosac(Fig. 6 to 9). It is corresponding to polygonum type embryosac.
2. Apomictic embryosac development At the early stage the megasporogenesis is the same as the sexual reproductive process. At the tetrad stage, an obstacle to sexual development was observed with the character of whole tetrad degeneration(Fig. 16). Sometimes, we observed twin mature embryosac with egg apparatus, two polar nuclei and antipodal seperately, arranging up and down from micropyle to chalazal end or obliquely(Fig. 17 to 19). This is considered to be the characteristic of apomixis in line 296B.

Embryo and endosperm development

1. Sexual development After fertilization, the zygote(Fig. 11)usually divides periclinally, resulting in a larger basal cell and a smaller terminal cell(Fig. 12), when two synergids have degenerated. Further cell divisions of these two cells result in formation of proembryo with a long suspensor(Fig. 13 to 15). Cells with dense cytoplasm in embryo proper are small and are arranged in a regular fashion. Cells in the suspensor appear to be in a state of differentiation, however. Following this differentiation, the development of embryo conforms to the type of Graminaceae. The endosperm mother cells produced by fertilization of two polar nuclei(Fig. 10)divides rapidly to form a free nucleus endosperm, generally with 1—2 nucleulus/i, distributed around the periphery of embryosac. More free muclei are in the micropyle end than in another region. At the stage of about 250 nuclei, the endosperm starts to become cellulization, extending from micropyle to chalazal end and from out to in-

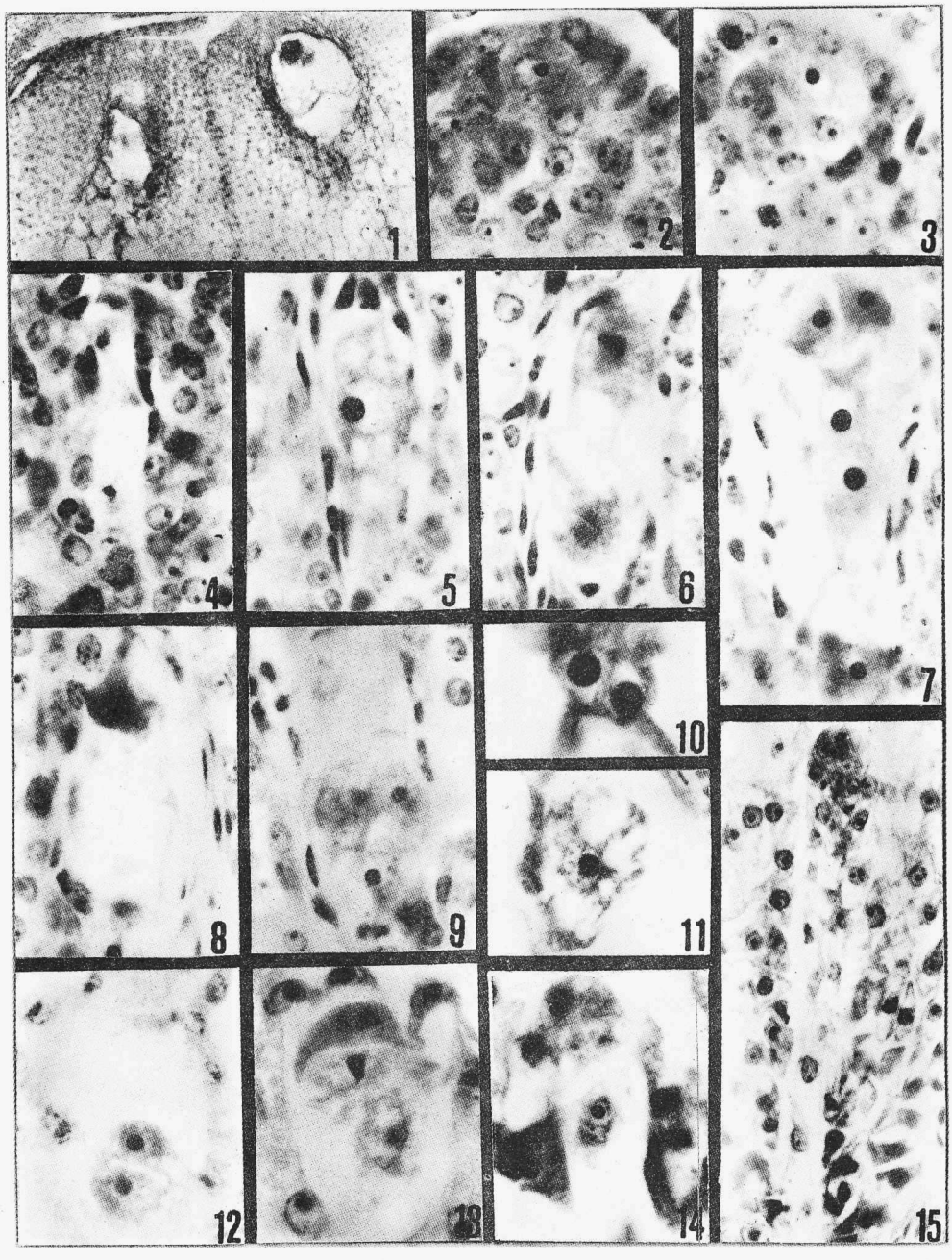
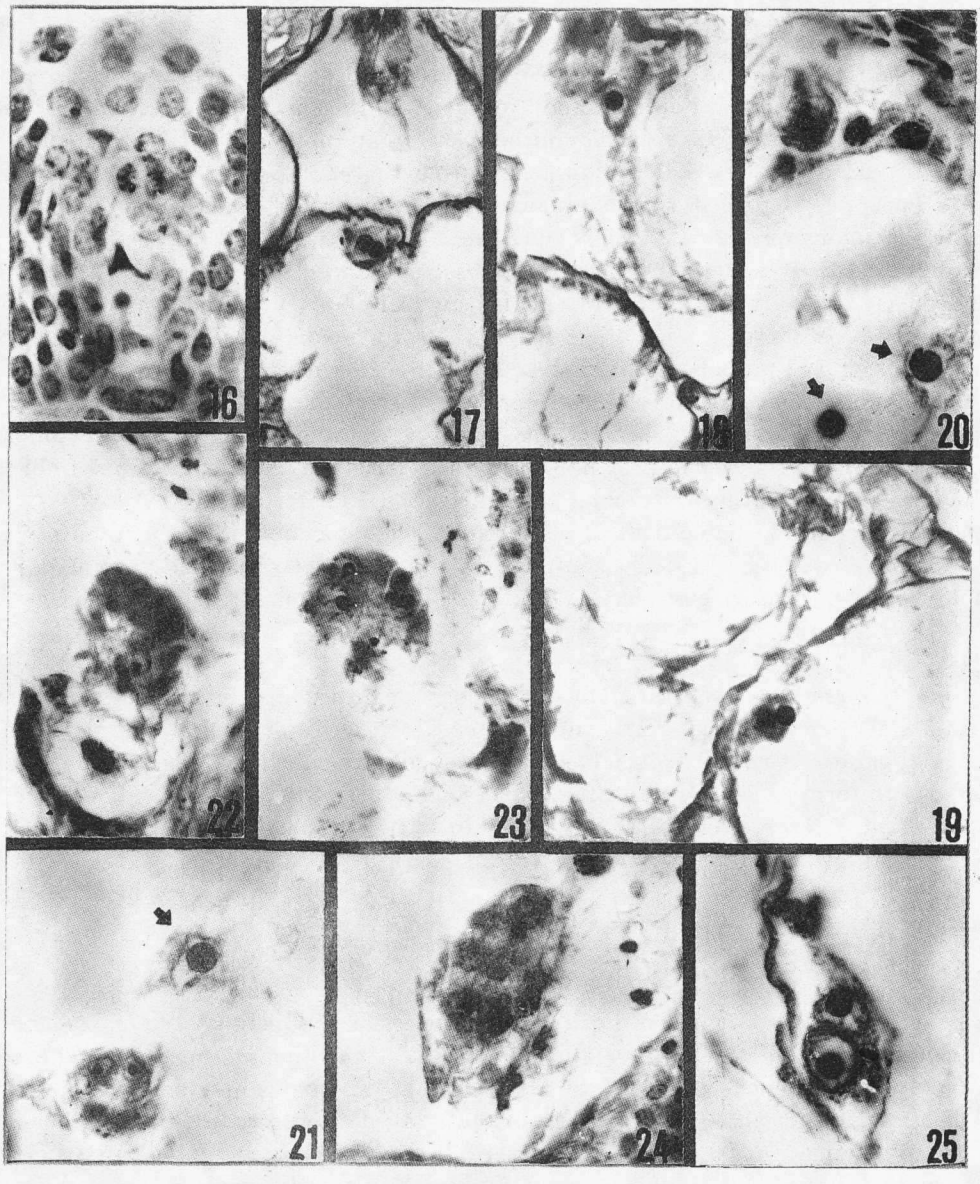


Fig. Megasporogenesis, female gametophyte, embryo and endosperm development
1. Double ovule in one ovary. ×120 2. Archesporious cell. ×750 3. Megasopre mother cell. ×750 4. Dyad at metaphase of meiosis. ×750 5. Uninucleus embryosac. ×750 6. 2-nuclei embryosac at metaphase of mitosis. ×750 7—9. Continuous sections of mature embyosac. ×370 10. Two polar nuclei. ×750 11. Zygote. ×750 12. 2-celled proembryo. ×750 13. 4-celled proembryo, showing two suspensor cells. ×900 14. 8-celled proembryo. ×750 15. Multiple celled proembryo with a long suspensor. ×370



16. Whole tetrad degeneration. ×800 17—19. Twin embryosac. 17. 18. ×370, 19. ×750 20. An embryosac containing proembryo, endosperm and two undeveloped polar nuclei. ×500 21. 2-celled proembryo and an undeveloped polar nucleus. ×750 22&23. Apomictic proembryo with a large suspensor cell. ×500 24. Apomictic proembryo lacking a long suspensor (which sexual proembryo has). ×900 25. Another side of 24, showing two undeveloped polar nuclei. ×900

ner layer, at this time the proembryo is at the 10 cells stage. So the endosperm develops more rapidly than the proembryo. That is a character of the sexual embryosac in line 296B as well as in other sexual species.

2. Apomictic development We observed that the two polar nuclei existed still from 12 hrs to 72 hrs after anthesis and kept as they were before anthesis (Fig. 25), when the embryo

developed to 2 to 20 cells (Fig. 21 to 24). It is obviously different from the sexual embryosac, which has numerous free endosperm nuclei or endosperm cells (Fig. 12 to 15). This observation is conclusive evidence of autonomous development of egg to embryo. Formation of endosperm is still problematic. We infered that the endosperm came from triple fusion also, and the case we observed above occurred in the condition of constrained un-fertilization. At the same time, we found another case where proembryo, two polar nuclei and free nucleus endosperm coexisted in one embryosac (Fig. 20). We considered that twin embryosacs fused, accompanied with earlier development of the sexual polar nuclei and the egg from the apomictic embryosac developed more rapidly than from the sexual one. Therefore we concluded that the embryo came from an unreduced and unfertilized egg cell, but the endosperm came from triple fusion in this kind of embryo. Moreover, the structure of the apomictic proembryo was not the same as that of a sexual proembryo. The cells of the apomictic proembryo proper appeared to be large in size, with more vacuole (Fig. 22 & 23) than the cells of sexual proembryo. At the earlier stage of apomictic proembryo, the cells of the suspensor were obviously large and the nuclei were prominent (Fig. 22), in comparison with smaller cells in the sexual proembryo. At the later stage, the apomictic proembryo had not the long suspensor that the sexual proembryo had (Fig. 24 & 15).

3. The phenomenon of double ovules The unusual phenomenon of double ovules was observed in single ovary (Fig. 1). The multiple ovules per floret suggested possible apomixis (Hanna et al, 1987), so we considered this to be another evidence of apomixis in line 296B.

The procedure of apomixis

According the results described above, the possible procedure of apomixis is that the apomictic embryosac derives from a nucellar cell. Before florescence, the embryosac contains egg apparatus, two-polar nuclei and a cluster of antipodals. The unreduced egg directly develops to form proembryo, and two-polar nuclei fuse with a sperm to develop into endosperm in most cases. In view of this procedure, the reproductive pattern is attributed to apospory.

Discussion

Apomictic characters in line 296B

296A/B is an excellent male sterile line of commercial production in India. Zhang et al (1991) considered 296B had some relations with R473, which may be related to the property of facultative apomixis. In their studies, the ability of fixing heterosis was confirmed. Further study (Niu et al, 1991) proved that 296B played an important role in breeding 'vybrids' as an independent facultative apomict. Preliminary observations indicated a close but as yet not understood association between incompability, male sterility and apomixis (Rao & Murty 1972). We found the sterile uninucleus or trinuclei pollen at lower level in some anthers, which may be associated with apomixis. The embryological observations showed that 296B has facultative apomixis in accordance with apospory. Twin embryosacs and autonomous embryo development with undeveloped polar nuclei were the evidence of apomixis in line 296B. Twin embryosacs reported in many papers (Rao et al, 1972; Tang et al, 1980) were concluded as the result of a nucellular cell differentiation. A multiple cell proembryo with short suspensor coexists with undeveloped polar nuclei in one embryosac has not been reported in Graminaceae. That means the sperm did not get to embryosac and both polar nuclei and egg did not fuse with sperms at all. We concluded the egg can autonomously divide and the embryo can be derived from 2N egg.

The freqency of apomixis in line 296*B*

Because apomixis is a deviation from sexual reproduction and the several steps which only lasting very short time differ from normal sexual development, it was difficult to catch the different periods between apomixis and sexual reproduction. Furthermore, it was impossible to detect the apomictic sections from sexual sections during the same developmental procedures. In view of these problems, the frequency of apomixis can not be assessed exactly by calculating the proportion of apomictic sections in whole sections we made. In spite of this, we compared the sections at same stages and found three twin embryosacs out of 14 sections and 8 apomictic embryos out of 51 sections. The probable frequency of apomixis was 16—21% at least. Additional precise study is needed in the testcross.

The potentiality of fixing heterosis by line 296*B*

The apomictic feature of 296B has been determined embryologically in the present study. According to Murty et al(1981), facultative apomicts can be used as a tool of breeding 'vybrids'. Previous studies (Zhang et al, 1991; Niu et al, 1991) have proved 296B combined with R473 can fix heterosis almost entirely. On the basis of these findings, they advance the procedure of breeding vybrids. We suggest that 296B would be used for fixing heterosis with other facultative apomicts in sorghum. Further studies must be carried out to determine the gene number and dominance or recessiveness.

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