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Meiotic Abnormalities in a Sterile Clone of *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg.

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The genus *Hevea*, belonging to the family Euphorbiaceae, comprises of 10 species. Among these, *H. brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. is commercially exploited for natural rubber. Literature pertaining to detailed cytological studies in this crop is meagre because the species is not easily amenable to cytological techniques. The economic life span of the tree is about 30–35 years and ortet selection is one of the important methods of tree improvement. From among a population of ortet selections maintained at the Experiment Station of the Rubber Research Institute of India, two clones were found to exhibit male sterility (Annamma *et al.* 1980). This paper presents results of detailed investigations on the meiotic abnormalities of one of these clones.

Materials and methods

Young male flower buds, at the appropriate stages, were collected from RRII 17, a male sterile clone and from a fertile clone (RRII 105). The samples were fixed in modified Carnoy's fluid 3: 1: 1 (ethyl alcohol: acetic acid: chloroform). After 24 hrs the materials were transferred to 70 per cent alcohol. The anther columns were dissected out and kept overnight in 45 per cent acetic acid before preparation of squashes. A total of 1000 meiotic cells selecting 20 each from 50 slides were analysed from squash preparation of randomly selected male flowers. Female flowers at different stages of development were fixed in 1: 3 acetic alcohol and preserved in 70 per cent alcohol. These were processed and serial sections (8–10 μm thickness) were taken, stained in safranine and fast green (Johansen 1940) and made permanent. Artificial pollinations were carried out incorporating the pollen from three fertile clones (RRII 105, RRII 118 and RRIM 600), for three consecutive flowering seasons, for assessing fruit set.

Results

Flowers of the male sterile clone were apparently normal in their morphology, though less in size compared to flowers of male fertile clones. In mature male flowers there was full development of perianth lobes but it contained only sterile pollen grains. Only 10 per cent of the male flowers attained full size, whereas in the others the anther column remained as shrivelled black pin heads.

Meiotic studies of the normal fertile clone RRII 105 had shown that there was no abnormalities. In metaphase I, 18 bivalents were seen (Fig. 1) and the stainability of pollen was 95 per cent. Meiotic studies of male flower buds from the sterile clone had shown that there was a wide spectrum of abnormalities. A normal meiotic behaviour of 18 II was never observed in the sterile clone. It was quite difficult to get metaphase I and also to detect bivalents at this stage. There was predominant formation of univalents (Fig. 3) ranging from

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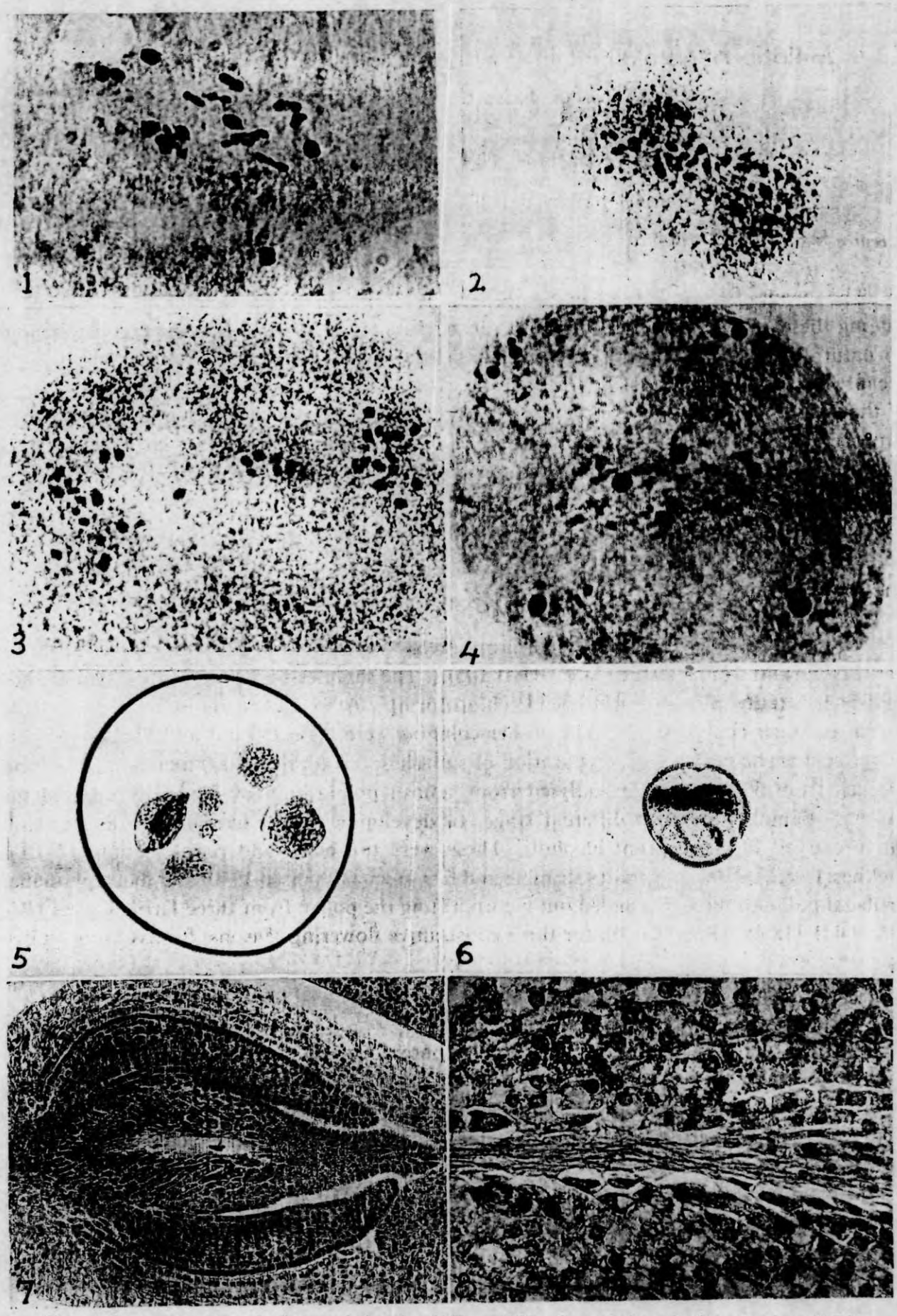

Figs. 1–8. 1, metaphase I of control showing 18II. ×4000. 2, metaphase I of sterile plant (RRII 17) showing bivalents. ×3000. 3, anaphase I of RRII 17 showing laggards. ×3000. 4, telophase II of RRII 17. ×3000. 5, six microspores in a tetrad. ×1200. 6, degenerating pollen grain. ×1200. 7, L. S. of ovule of control showing embryosac. ×480. 8, L. S. of ovule of RRII 17 showing shrivelled embryosac. ×1200.

8 to 32 and bivalents (Fig. 2) 2 to 14 (Table 1). There was poor spindle formation, non-orientation of the univalents and clumping of chromosomes at the equatorial plate. Anaphase I was highly irregular with unequal distribution of chromosomes, absence of active polar movement and presence of varying number of laggards (Fig. 3). The distribution of microspores during tetrad stage varied from 3 to 9 (Fig. 4). The highest frequency (44%) was noted for six microspores (Fig. 5). Cells showing five and seven microspores was 17 and 16 per cent, respectively. 14 per cent of cells exhibited four and three microspores were noted in 5 per cent of the cells. The occurrence of eight and nine microspores were observed in three and one per cent of cells. However, after the microspore formation there was complete degeneration of cytoplasm and nuclei resulting in the formation of sterile pollen grains (Fig. 6) having varying size and shapes. The smallest pollen grains had an average size of 21.38×18.93 μm and the largest had 55.33×49.23 μm. There were about 41 per cent of sterile pollen grains having an average size of 31.23×28.48 μm and 38.5 per cent had 41.88×37.50 μm mean size. The normal size of pollen grain in the fertile control clone was 39.00×36.00 μm.

It is difficult to trace the development of megaspore in *Hevea*. The normal embryosac (Fig. 7) as in the case of the fertile clone, could never be observed. It showed shrivelled embryosac (Fig. 8) with disintegrated cytoplasm and nuclei. In natural pollination as well as in artificial hand pollination no fruit set was noted on the clone RRII 17.

Table 1. Chromosome association at metaphase I of sterile clone of *Hevea brasiliensis*

Association	Frequency	Per cent	Association	Frequency	Per cent
14 II+ 8 I	20	2	7 II+22 I	30	3
13 II+10 I	90	9	6 II+24 I	130	13
12 II+12 I	80	8	5 II+26 I	40	4
11 II+14 I	30	3	4 II+28 I	30	3
10 II+16 I	150	15	3 II+30 I	120	12
9 II+18 I	30	3	2 II+32 I	60	6
8 II+20 I	190	19			

Discussion

The phenomenon in which the homologous chromosomes pair normally at pachytene but fail to remain associated as bivalents at diakinesis and metaphase I due to lack of chaisma formation was termed as desynapsis (Desi *et al.* 1973). Since there is formation of variable number of bivalents and univalents at metaphase I, the male sterility apparently is a case of desynapsis. The congression of chromosomes was imperfect and the chromosomes rarely oriented themselves on metaphase plate. Frequently, the unpaired chromosomes tended to stay near the pole. In some cells the univalents were distributed randomly throughout the pollen mother cell. The distribution of univalents at metaphase I may be either polar or more or less equatorial (Riley and Law 1965). The unequal distribution of chromosomes at anaphase had resulted in the production of gametes with varying degrees of chromosomal imbalance. Consequently gametes with complete genome are not produced. At the end of meiosis, the pollen mother cells produced varying number of microspores, which also differed in size.

The present study is a first report of desynapsis in *Hevea*. Asynapsis was already reported in this crop (Ramaer 1935). A large number of meiotic abnormalities causing sterility had been described and discussed by Darlington 1937. In *Hevea*, partial and complete spontaneous male sterility had been reported (Ramaer 1935, Majumder 1964 and Saraswathy Amma *et al.* 1988). Induced male sterility had also reported (Saraswathy Amma *et al.* 1985, 1989)

in *H. brasiliensis*. Formation of polyspores in the male sterile clone might have resulted from irregular division during the first anaphase and fromation of more than two groups. Such abnormalities were reported in other desynaptic species (Ahloowalia 1969, Misra and Shastry 1969, Singh and Gupta 1981, and Karihaloo and Koul 1983).

As in the case of PMCs, meiosis apparently did not appear to be normal in the megaspore mother cell and disintegrating embryosac was noted in the mature female flower. Absence of fruit set both under natural condition and artificial hybridisation indicates the possibility of desynapsis in the megaspores. A similar situation was reported in *Allium* (Koul 1975).

Since the plant is showing tolerance to abnormal leaf fall disease (Markose 1984) this can be utilised for crown modification in *Hevea*. Desynaptic plant may provide a valuable tool for experimental approach to the problems of chromosome pairing and chiasma formation.

Summary

Meiotic abnormalities in a spontaneous sterile clone of *Hevea brasiliensis* were investigated. During metaphase I varying degrees of bivalents and univalents were noted indicating desynapsis. This is the first report of desynapsis in *Hevea*. Anaphase I was typified by unequal disjunction, absence of active polar movement and varying degree of laggards. At telophase II, instead of normal tetrad formation, 3–9 microspores were found. There is total degeneration of cytoplasm and nuclei resulting in complete sterility. Megaspores also showed sterility.

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