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Radiation Induced Male Sterility in *Hevea brasiliensis* (Willd. ex Adr. De Juss.) Muell. Arg.

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Hevea brasiliensis is a perennial tree belonging to the family Euphorbiaceae and natural rubber produced in the world almost exclusively comes from this species. The tree is monoecious. Genetic variability in this crop is limited. Hence induction of genetic variability, by special techniques like mutation and polyploidy is being tried (Mendes and Mendes 1963, Shepherd 1969, Huat and Subramaniam 1973) for the last two decades by research institutions in various rubber growing countries. This approach was also tried at the Rubber Research Institute of India (Markose *et al.* 1974) and germinated seeds were treated with gamma rays, 10 to 40 Gys, during 1972 at the Bhaba Atomic Research Centre, Trombay. From the resultant progenies, plants showing morphological variations were multiplied vegetatively and the characters were stabilized in subsequent generations. Those plants exhibiting persistent variations upto VM_7 were again budgrafted and planted at the Central Experiment Station, Chethackal, Ranni during 1976. From the progenies of 30 Gy-exposed population a plant showing dwarf stature was identified. This paper presents the cytomorphological observations on this clone.

Materials and methods

Budgrafted plants of the radiation-induced clone and control (RRII 105) were raised in polybags. The growth attributes of these plants were recorded at 10 months' age. Height, diameter of the scion at the base, number of flushes and total number of leaves in a flush were recorded. The leaf characters were recorded from thirty middle leaflets, selecting three leaves from bottom, middle and top of each flush of growth. The plants were induced to flower by ring barking. Flower size was measured from thirty flowers selected at random. Male flowers at the appropriate stages of development were collected, along with those from the control, at random and fixed in Carnoy's fluid 3: 1: 1 (ethyl alcohol: acetic acid: chloroform). After 24 hours they were preserved in 70% alcohol. Anther columns were disected out and kept overnight in 1% acetocarmine solution. Meiotic preparations were made in 45% acetic acid. A total of 1000 meiotic cells were observed and photomicrographs were taken from permanent preparations.

Results and discussion

The morphological characters of the budgrafted plants are depicted in Table 1. The induced mutant showed reduction in height and girth. At the age of ten months, the mean height of the induced mutant was only 41.23 cm while the control plant recorded 101.07 cm. Mean leaf size ($L \times B = 39.06$ cm) and petiole length (8.73 cm) were comparatively less. The

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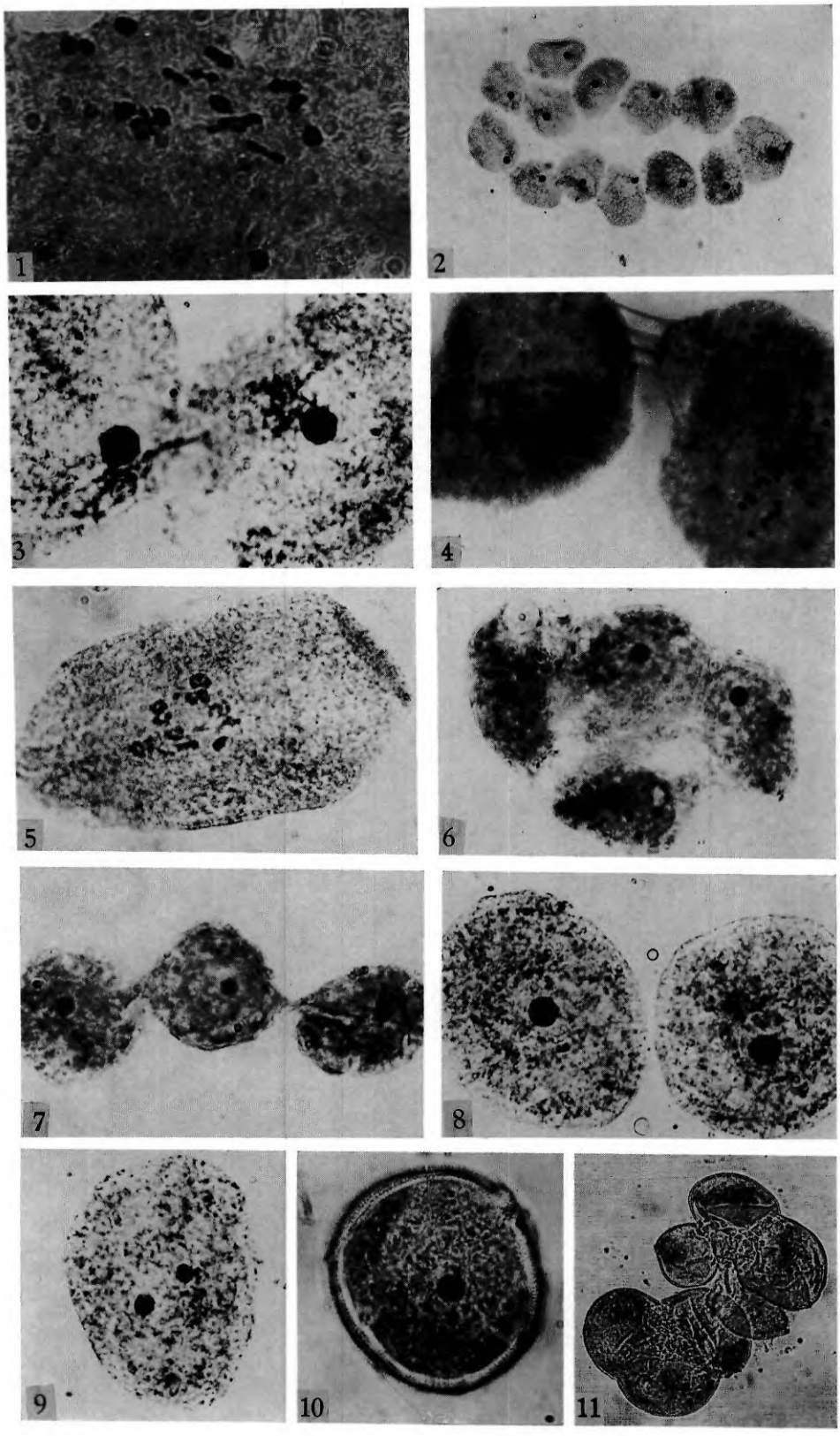
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control plant showed more leaf size (147.27 cm) and petiole length (16.20 cm). The interflush length was also reduced for the induced mutant. The male and female flowers were small compared to those of the control. The mature male flowers were completely devoid of stainable pollen. Eventhough the plant is showing total male sterility fruit set was noted.

The somatic number of *Hevea* is 2n=36. Meiotic studies showed 18 bivalents (Fig. 1). There was no meiotic abnormalities in the control (RRII 105). Meiotic studies of young male flower buds from the induced mutant had shown that 15% of flowers showed cytomixis. Cytoplasmic connections were noted between the PMCs at all stages of meiosis (Fig. 4). Chromatin materials were also seen passing from one cell to the other from early prophase (Fig. 3) to telophase II. The frequency of cells showing cytomixis is given in Table 2. At telophase II, 55% of cells showed cytoplasmic connections. In some cases most of the pollen mother

Table 1. Growth attributes of 10 month old budgrafted radiation induced and control plants of *Hevea brasiliensis*

Parameters	Induced mutant	Control
Height (cm)	41.23±16.58	101.07±4.56
Diameter (mm)	8.41± 2.25	11.08±1.57
Interflush length (cm)	5.23± 0.50	13.56±1.95
Petiole length (cm)	8.73± 3.42	16.20±0.98
Leaf size (cm)	39.06± 4.16	147.27±6.08
Flower size ♂ (mm)	3.70× 1.93	4.40±2.70
Flower size ♀ (mm)	5.94× 2.80	10.67±3.28
Pollen stainability	—	92.8%

Table 2. Frequency of cells showing cytomixis/cytoplasmic connections

Meiotic stage	Total cells observed	Percentage
Prophase I	713	36.0
Metaphase I	100	28.0
Anaphase I	100	10.0
Telophase I	100	8.0
Telophase II	547	55.0
Microspore	100	2.0

cells of a flower were found to be involved in cytomixis while the adjacent flowers were normal without any aberrations. During prophase I, 1–15 cells, at metaphase I, 2–4 cells, and at telophase II, 2–8 cells in field were observed to show cytomixis or cytoplasmic connections. Direct fusion as well as connecting cytoplasmic strands were observed among the pollen mother cells (Fig. 2). Due to the transfer of chromatin materials, pollen mother cells with reduced number from the model number n=18 was observed (Fig. 5). At anaphase I bridge formation with and without laggard was seen in 3% cells.

After telophase II aberrant cytokinesis was noted resulting in total sterility. Due to cytokinetic aberrations the distribution of nuclei showed wide variations. There were different types of abnormalities in the cleavage of cytoplasm. The nuclei were distributed in

Figs. 1–11. 1, Metaphase I of control showing 18 bivalents, ×4000. 2, Prophase I. row of cells showing cell fusion as well as cytoplasmic connections, ×190. 3, Prophase I. showing migration of chromatin materials, ×3000. 4, Late metaphase showing cytoplasmic strands, ×3000. 5, Cell showing reduced chromosome number, ×3000. 6, Tetrad showing central cleavage of cytoplasm, ×1200. 7, Microspores connected with cytoplasmic strands, ×1200. 8, Microspores, ×3000. 9, Microspore with divided nucleus, ×3000. 10, Megapollen, ×3000. 11, Pollen conglomerates, ×480.

2+1+1, 2+2 and the details are given in Table 3. In the first type, four microspores were formed as in the case of normal cells. In the second case, there was a central cleavage of cytoplasm resulting in the union of four microspores (Fig. 6). In the third instance, there was no cleavage of cytoplasm and the nuclei were distributed in a common cytoplasm. There were microspores connected with cytoplasmic strands (Fig. 7). Normal microspores (Fig. 8) as well as microspores with divided nuclei (Fig. 9) were also observed. Megapollen were noted in 0.5% cells (Fig. 10). Pollen conglomerates were also recorded (Fig. 11). Whatever may be the type of abnormalities, all ultimately resulted in the production of sterile pollen. The pollen grains were of different shape and size (Table 4). Due to radiation effect, the morphology of the plant was also altered. It showed short stature and reduced vigour which was not observed in the control plant. Boertjes and Dejong (1984) had reported a sterile plant resultant of radiation in *Chrysanthemum* which showed reduced plant height and altered form and size.

The induced plant showed delay in flowering compared to the control. Even after ten years of planting it did not flower and hence artificial flowering was attempted. Flowers were apparently normal in their development.

Table 3. Details of cells showing cytokinetic aberrations

Distribution of nuclei in the tetrad stage	Percentage
2+1+1	20
2+2	15
1+3	10
4	5
1+1+1+1 (Normal)	50

Table 4. Measurements of sterile pollen grains

Size of pollen (μm)	Percentage
35.42×30.42	18.50
39.52×36.45	37.00
51.10×44.02	22.00
71.50×60.00	12.50

This is the first report of radiation induced male sterility in *Hevea*. The occurrence of cytomixis was reported in *Hevea* (Saraswathy Amma and Panikkar, 1988). In the induced mutant cytolplasmic connections were seen from early prophase to microspore stage. Cytoplasmic connections were observed between groups of cells i.e. 1–15 cells in prophase. A similar case in which cytomixis was observed in all the meiotic stages was reported in *Mentha piperita* (Kundu and Sharma 1988). Passage of nuclear materials was also reported in telophase II in *Vigna* (Sen and Bhattacharya 1988). The origin and evolutionary significance of cytomixis are not precisely understood. Several suggestions had been put forward to explain the cause and probable origin of cytomixis (Maheswari 1950, Heslop-Harrison 1966, Whealan 1974, Bauchan *et al.* 1987). In the present study cytomixis may be due to the effect of gamma radiation resulting in unbalanced genetic system. Cells with reduced chromsome number were also observed. Details of chromosome aberrations induced by radiations were described by Evan (1962). Failure of completion of quadripartition of the cytoplasm leads to the formation of unusual forms of microspores. Due to cleavage aberration the megapollen was also formed. Wide spectrum of aberrations associated with male sterility were reported in *Alopecurus* (Johnsson 1944) and in *Impatiens sultani* (Tara and Namboodiri 1974, 1976). These aberrations led to the formation of sterile pollen. In those flowers where there was no meiotic abnormalities, after microspore formation there was complete degeneration of cytoplasm resulting in total male sterility. Pollen grains showed varying size and shape which might be due to aberrant cytokinesis. The pollen conglomerates were noted in the mature anther. Formation of pollen conglomerates was also reported in *Alnus* (Bensimon 1985).

The sterility in this induced plant is due to the genetic imbalance caused by gamma radia-

tion. Cytomixis and wide spectrum of cytokinetic aberrations are also observed which also lead to sterility. Eventhough the induced mutant is showing total male sterility, fruit set is noted in this clone indicating that this is female fertile. Hence this can be utilized for hybrid seed production in *Hevea* if found otherwise suitable. Further work is necessary to explore the possibilities, of which the altered stature of the plant is a useful attribute in breeding programme of *Hevea*.

Summary

A plant exhibiting semi-dwarf stature was identified from a gamma ray induced VM_7 population. Cytomixis was observed in 30% of the meiocytes. Cytoplasmic connections were observed in all stages of PMCs ranging from early prophase stage to microspore stage. Movement of chromatin materials was also observed. A wide spectrum of cytokinetic aberrations was also noted. As a result of these abnormalities the plant showed total male sterility. In *Hevea*, male sterility can be exploited for the production of hybrid seeds and dwarf stature in breeding programme.

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