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Cytomorphological Studies in an Induced Polyploid of *Hevea brasiliensis* Muell. Arg.

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The Para rubber tree, *Hevea brasiliensis* Muell. Arg. belonging to the family Euphorbiaceae is the most important commercial source of natural rubber. The genus *Hevea* has nine species. Work on the cytogenetical aspects of the genus in general, and that of *H. brasiliensis* in particular, is rather meagre. Since the geneitc base of cultivated *H. brasiliensis* is limited, every effort has to be made to broaden the breeders' stock. An important method to cause genetic variability is the induction of polyploidy by artificial means and this technique has also been tried in *Hevea* (Shepherd 1969, Markose 1975, Zheng Xuequin *et al.* 1980). The Rubber Research Institute of India has succeeded in inducing polyploidy in *Hevea*, by the application of colchicine. This paper deals with the cytomorphological analysis of an induced polyploid in *Hevea brasiliensis*.

Materials and methods

Clone RRII 105, evolved by the Rubber Research Institute of India through hybridisation and selection with Tjir 1 and G1 1 as parents, was used for the present investigation. Plants raised in budwood multiplication nurseries were cut back and at the time of bud break they were swabbed with cotton. 0.75% aqueous colchicine was dropped on to these tender bud sprouts so that the cotton gets wet. The treatment was continued daily for seven days. The shoot developing from the treated buds were regularly observed. The basal buds of those which showed morphological variations were utilised for vegetative multiplication. Subsequent vegetative generations were also raised employing the basal buds of the respective shoots.

Observations on morphological characters were recorded from one year old budgrafts of the VM 10* generation. Normal one year old budgrafts of RRII 105 were also observed for comparative purpose. Assessment of foliage characters was made from the middle leaflet of twenty selected leaves. Mitotic studies were made on tender leaf tips.

Three year old budgrafts were ringbarked for early induction of flowering (Saraswathy Amma 1975). For meiotic studies young male flowers at the appropriate stage of development were fixed in 1 : 3 acetic alcohol mixture. Anther columns were dissected out and kept in 2% acetocarmine, overnight. Meiotic preparations

* Vegetatively multiplicated 10th

were made in 45% acetic acid. The temporary preparations were made permanent in acetic acid-butanol series. The meiotic data were recorded from 50 pollen mother cells. Pollen grains were collected just prior to anthesis, stained in 1 : 1 aceto-carmine-glycerol mixture, for assessing stainability. Meiotic studies of the normal budgrafts were also made for comparison.

Results

Data on morphological characters of the induced polyploid and normal diploid are given in Table 1. The polyploid plant showed more vigour in terms of height and diameter compared to the control. The leaves of the polypoid were thicker and dark green in colour. The leaf size and petiole length were also more for the induced polyploid. The number of leaflets varied from 2–5 whereas the normal plant showed typical trifoliate leaves (Fig. 1). The veins in the leaf of induced polyploid

Table 1. Morphological characters of diploid and induced polyploid clone of *H. brasiliensis*

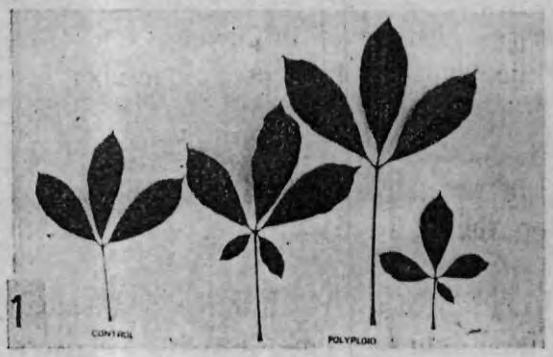
Character	Diploid Mean	Induced polyploid Mean
Height (cm)	133.1 ± 6.48	166.5 ± 6.16
Diameter (mm)	13.4 ± 0.39	16.9 ± 0.69
Whorl length (cm)	15.2 ± 3.01	14.4 ± 2.09
Interwhorl length (cm)	19.0 ± 2.68	21.0 ± 3.01
Leaf L×B (cm)	159.3 ±12.60	208.4 ±20.40
Leaf thickness (mm)	0.12± 0.03	0.22± 0
Petiole length (cm)	19.0 ± 1.01	24.6 ± 1.20
Number of veins	43.9 ± 1.08	36.3 ± 1.55
Flower size (mm)	4.4 × 2.7	10.4 × 4.4
Pollen size (μm)	39×36	55×52
Pollen stainability (%)	92.8	80.0

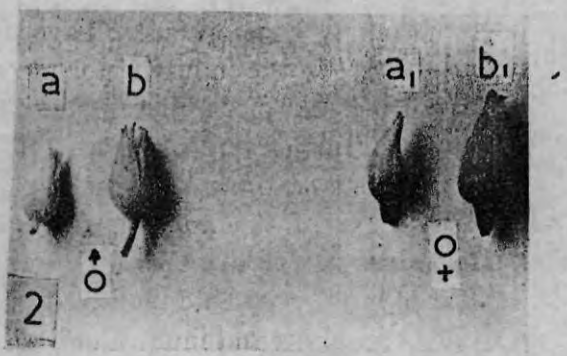
were very prominent. The number of secondary veins were less, compared to that of the normal. The flowers were bigger in size (Fig. 2). The pollen grains were also bigger and 30% of them showed four germpores instead of the usual three in the diploid.

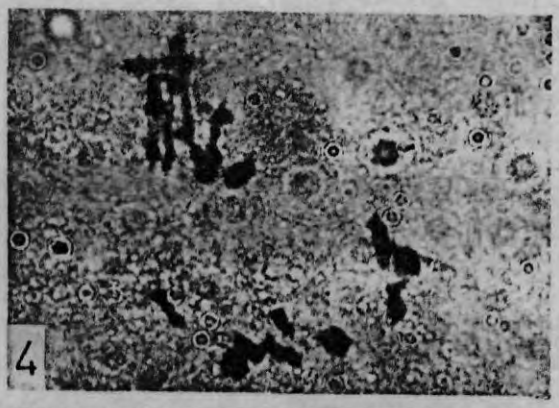
Mitotic studies of the normal plant have shown 36 chromosomes in the somatic cells. Karyotype studies from young leaf tips of colchicine treated budgrafts showed 2n=72 chromosomes confirming its tetraploid nature (Fig. 3). Meiosis in the diploid *Hevea* was normal with the formation of 18 bivalents at metaphase I (Fig. 4). However, the stickiness of chromosome is a common feature. Details of chromosome associations at metaphase I in the induced polyploid are given in Table 2. At metaphase I of the polyploids, besides predominant bivalent formation, univalents, trivalents and quadrivalents were also observed (Figs. 5 and 6). Univalents, bivalents, trivalents and quadrivalents showed a range of 1–9, 21–32, 0–4 and 0–4 respectively. Anaphase I showed unequal segregation, formation of laggards, micronuclei, etc. The subsequent division did not show any abnormalities.

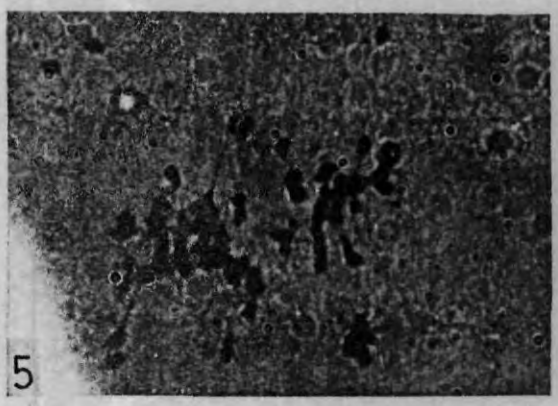
Discussion

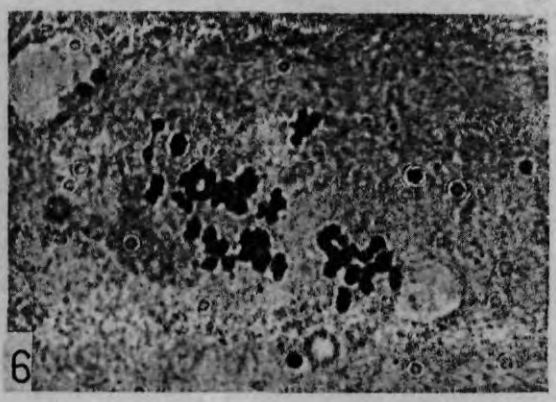
The induced polyploid of RRII 105 showed more vigour associated with autotetraploidy. Mendes (1969) had reported more vigour in a polyploid *Hevea* clone of IAN-873. However, stunted growth had been observed in GT 1 polyploid











Figs. 1–6. 1, leaves of the control (normal diploid) and induced polyploid. 2, flowers of diploid and polyploid. a, a_1: diploid; b, b_1: polyploid. 3, chromosome complement of the induced polyploid 2n=72. ×1500. 4, metaphase I of the diploid 18_{II}. ×1500. 5 and 6, metaphase I of induced polyploid showing univalents, bivalents and multivalents. ×1500.

(Anonymous 1976). The leaves of polyploid were reported to be thicker and deep green in colour (Shepherd 1969, Markose 1975, Zheng Xuequin *et al.* 1980). The veins and veinlets were very prominent and number of leaflets showed variation ranging from 2 to 5. The dark green colour of the polyploid leaves may be due to more thickness as has been reported in *Ipomoea* species (Vijayabai *et al.* 1976). The

length of the petiole of polyploid was comparatively more, especially in the base of the storey, enabling more exposure of the leaves to sunlight, The floral parts were also larger than those of the normal plants as in the case of induced polyploids of *Capsicum* (Indira and Susan Abraham 1977) and *Matricaria* (Arora and Madhusoodanan 1981).

Hevea brasiliensis (2n=36) is believed to be tetraploid since most of the species under the family Euphorbiaceae have a 2n complement of 18 chromosomes (Majumdar 1964). In the induced polyploid, the somatic chromosome number was confirmed to be 2n=72. Cytogenetic stability is an important aspect of induced polyploids for further improvement. It was found that this character can be maintained in *Hevea brasiliensis* by making use of vegetative propagation. Even after ten generations of vegetative multiplication the induced polyploids of RRII 105 maintained its polyploid characters.

The induced polyploid showed the formation of a large number of bivalents and a few univalents and multivalents showing its chromosome diversity. A decrease in number of quadrivalents and increase in bivalents, frequency is a general indication that the genomes are divergent. These support the wiew that *Hevea*

Table 2. Chromosome associations at metaphase I in induced ploypoid (2n=72)

	Total no. of cells analysed	Range	Mean
Univalents	50	1–9	5.9±0.38
Bivalents	50	21–32	26.5±0.46
Trivalents	50	0–4	1.7±0.21
Quadrivalents	50	0–4	2.0±0.18

brasiliensis is an amphidiploid (Shepherd 1969, Ong 1975). Polyploidy is one of the best evolutionary processes in producing radically different and well adapted genotypes (Stebbins 1968).

The induced polyploid has been found to be fertile although the percentage of fruit set was very low. This has enabled the synthesis of a triploid progeny by crossing diploid (2n=36) with tetraploid (2n=72) (Saraswathy Amma *et al.* 1980). The polyploid can thus enrich the genetic stock of *Hevea* and can be used for future breeding programme.

Summary

The cytomorphological investigations of an induced polyploid of *Hevea brasiliensis* are given. The induced polyploid showed morphological variations like more leaf thickness, prominent veins and veinlets, varied number of leaflets and large floral parts. Mitotic studies have confirmed that the plant is a tetraploid with 2n=72 chromosomes. At metaphase I, besides bivalent formation, univalents, trivalents and quadrivalents were also observed. It supports the view of amphidiploid origin of *Hevea brasiliensis*.

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