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COMPARATIVE CYTOMORPHOLOGICAL STUDIES ON A DIPLOID, A TRIPLOID AND A TETRAPLOID CLONE OF *HEVEA BRASILIENSIS* (WILLD. EX ADR. DE JUSS) MUELL. ARG.

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Cytomorphological observations were made on an induced tetraploid clone, an evolved triploid clone and a diploid clone of *Hevea brasiliensis* (Willd. ex. Adr. de Juss.) Muell. Arg. Both the tetraploid and the triploid had varied number of leaflets, large floral parts and stomata and thicker leaflets compared to those of the diploid. In growth and morphological features at the early stage of growth, the triploid appeared to be intermediate between the diploid and the tetraploid. Meiotic studies of the tetraploid showed large number of bivalents, while frequency of univalents, trivalents and quadrivalents was comparatively low. The triploid exhibited univalents, bivalents and trivalents. Anaphase I showed unequal segregation and formation of laggards and micronuclei in the tetraploid and the triploid, the frequency of their occurrence being more in the triploid. Pollen stainability was very low in the triploid.

Key words – Hevea brasiliensis, Diploid, Triploid, Tetraploid, Meiosis, Amphididiploid.

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INTRODUCTION

Cytogenetical studies on *Hevea brasiliensis* (the Para rubber tree) and the other eight species of the genus are limited. Detailed knowledge on the cytogenetics of *H. brasiliensis,* are however, important in inducing genetic variability by artificial means as the genetic base available within the species in the East is very limited.

With a view to broadening the genetic base, induction of genetic variability by the application of mutagenic agents had been attempted (Shepherd, 1969; Markose, 1975; Zheng Xuequin et al, 1980). The Rubber Research Institute of India has succeeded in inducing tetraploidy by the application of colchicine in a diploid clone RRII 105 and also in evolving a triploid subsequently by crossing the diploid as female with the induced tetraploid as the pollen donor (Markose, 1975; Saraswathyamma et al, 1980). This communication deals with the comparative cytomorphology of the diploid, the triploid and the tetraploid, and is the first report on the meiotic observations of a triploid evolved in *H. brasiliensis.*

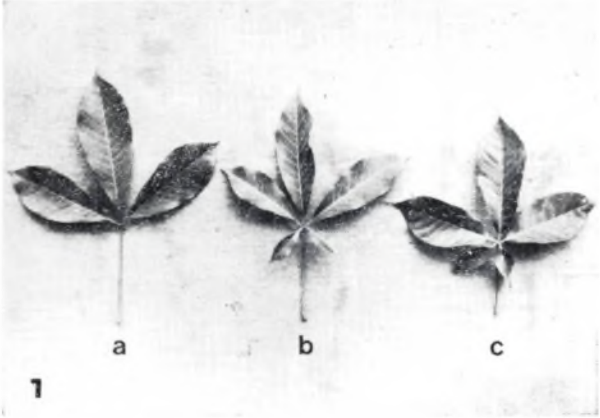
MATERIALS AND METHODS

Vegetative progenies of the diploid (clone RRII 105), the tetraploid (RRII 105) and the triploid [Gl 1 (2x) x RRII 105 (4x)] were established through budgrafting. The budgrafts were raised in polybags and transplanted in the field. Morphological observations were recorded from twenty plants of each group at the age of 30 months. Foliage characters were recorded from the middle leaflet of twenty mature leaves selected at random from each cytotype. Stem index, petiolar index and leaf index (Mendes, 1969) and specific leaf weight (Chatterton et al, 1972) were estimated. For stomatal study the middle leaflets of three leaves at comparable position were taken from five plants at random. Epidermal peelings were prepared by the standard method (Premakumari et al, 1979) and observed under light microscope. The number of stomata in microscopic field was counted and the length and breadth of stomata were measured using eyepiece micrometer. Bark samples were collected from five plants at random and fixed in formalin acetic-alcohol mixture. Radial longitudinal sections were cut using a sledge microtome and observed after staining with Sudan III. Budgrafted plants of the tetraploid and the triploid were induced to flower early, at the age of 30 months by ring barking (Saraswathyamma, 1975). Male flower buds, at the appropriate stage of development, from these as well as from the diploid were collected and fixed in modified Carnoy's fluid. After 24 hours the materials were transferred to 3:1 alcohol acetic acid. Staminal columns were dissected out and stained overnight in 2 per cent acetocarmine. Preparations were made in 45 per cent acetic acid and observations were taken from 50 pollen mother cells from temporary mounts. Pollen stainability was assessed in 1:1 acetocarmine - glycerol mixture.

RESULTS AND DISCUSSION

Both the tetraploid and the triploid showed variations in the number of leaflets, the range being two to five compared to three in the diploid (Fig. 1). Observations on morphological characters of the three cytotypes are summarised in Table 1. The tetraploid plants showed more vigour in terms of height and girth. At 30 months growth, the average girth was 17.90, 14.43 and 13.55 cm for the tetraploid, triploid and diploid, respectively. The veins and veinlets were very prominent in tetraploid. The specific leaf weight was more for the tetraploid. The flowers of the tetraploid and the triploid were bigger compared to those of the diploid. Variation was noted in the size of the pollen grains as well. The size was maximum for those from the tetraploid followed by the triploid and the diploid, the values being 55.0 52.0, 47.0 x 38.0 and 39.0 x 36.0 μm, respectively. In most of the characters studied the triploid was intermediate between the tetraploid and diploid. The petiolar index, however, was maximum for the triploid compared to the other two types, while the stem index was the same for the triploid and the tetraploid. Stomatal density was comparatively more for diploid. Size of the stomata in terms of length and width was highest for tetraploid followed by the triploid and diploid. Thickness of bark and number of latex vessel rows were numerically more for triploid than for tetraploid and diploid (Table 1).

Meiosis in the diploid clone was normal with the formation of 18 bivalents at metaphase I (Fig. 2). Details of chromosome associations at metaphase I in the tetraploid (Fig. 6) and the triploid are given in Tables 2 and 3. The details of tetraploid were reported earlier (Saraswathyamma et al, 1984). The triploid exhibited univalent formation (1–17) followed by bivalents (1–10) and trivalents (7–17) (Fig. 3). The mean occurrence of chromosome associations per cell was 10.36 ± 0.68 for univalents, 6.71 ± 0.23 for bivalents and 10.07 ± 0.24



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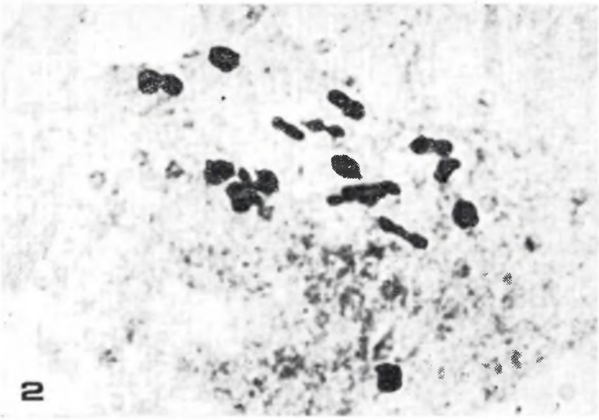

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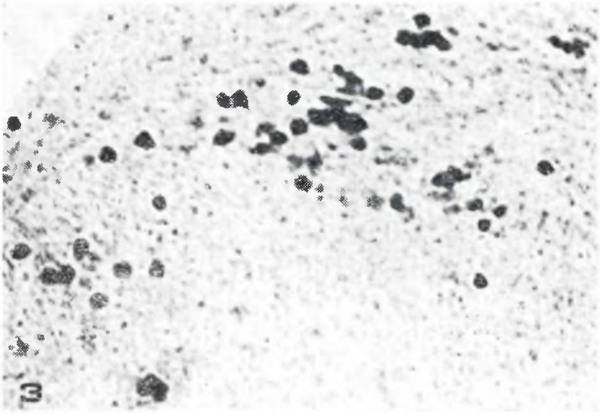

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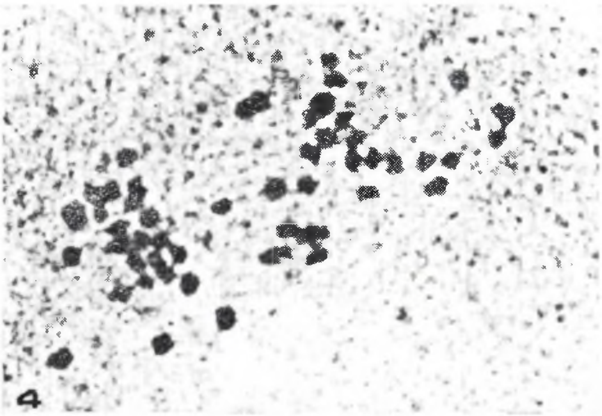

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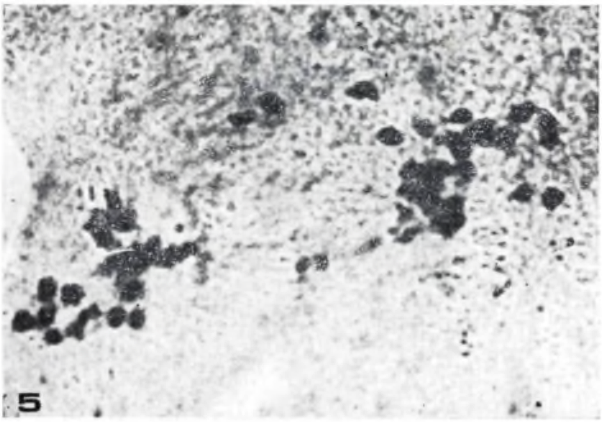

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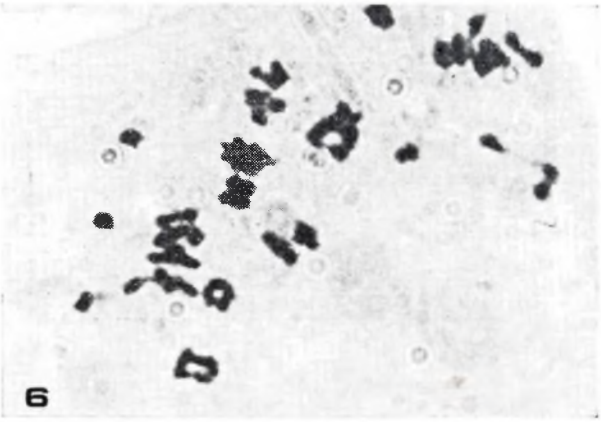

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1 Leaves (a-control, b-tetraploid, c-triploid) X 1/7

2 Metaphase I of the diploid 18 II x 4000

3 Metaphase I of the triploid $2n = 54$ x 3500

4 Anaphase I of the triploid x 3800

5 Laggards of the triploid x 4000

6 Metaphase I of the tetraploid 3 IV + 4 III + 22 II+4 I. Note the sticking of bivalents x 4000

for trivalents. During anaphase I, unequal segregation and formation of laggards (Fig. 4, 5) and micronuclei were also noted. The stainability of pollen was very low for the triploid (5.5 per cent). The pollen from the tetraploid showed 80.0 per cent stainability while those from the diploid recorded 92.8 per cent.

Table 1. **Morphological characteristics of diploid, triploid and tetraploid of *Hevea brasiliensis***

Character	Cytotypes		
	2*x*	3*x*	4*x*
Height (m)	4.97 ± 0.22	5.13 ± 0.23	5.31 ± 0.09
Girth (cm)	13.55 ± 3.67	14.43 ± 0.47	17.90 ± 0.86
Stomatal density	51.32 ± 2.39	34.67 ± 1.53	35.07 ± 5.80
Stomatal length (μm)	30.21 ± 2.78	39.73 ± 3.07	41.29 ± 4.74
Stomatal breadth (μm)	17.89 ± 0.93	30.11 ± 2.33	31.36 ± 2.50
Bark thickness (mm)	2.22 ± 0.20	2.36 ± 0.13	2.25 ± 0.42
Latex vessel rows	3.67 ± 1.35	5.58 ± 1.42	4.67 ± 0.62
Petiolar index	0.014	0.019	0.016
Leaf index	0.004	0.005	0.006
Stem index	0.029	0.036	0.036
Specific leaf weight (g cm^{-2})	0.0073	0.0079	0.0092
Flower size (mm)	4.4 x 2.7	9.6 x 4.0	10.4 x 4.4
Pollen grain size (μm)	39.0 x 36.0	47.0 x 38.0	55.0 x 52.0

Table 2. **Chromosome associations at metaphase I in diploid, triploid and tetraploid *Hevea brasiliensis*.**

Cytotype	Univalents		Bivalents		Trivalents		Quadrivalents		% Stainable pollen
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	
Diploid	—	—	—	18.00	—	—	—	—	92.8
Triploid	1–17	10.36 ± 0.68	1–10	6.71 ± 0.23	7–17	10.07 ± 0.24	—	—	5.5
Tetraploid	1–9	5.90 ± 0.38	21–32	26.50 ± 0.46	0–4	1.70 ± 0.21	0–4	2.00 ± 0.18	80.0

Table 3. **Chromosome association during metaphase I in the triploid and in the tetraploid.**

Sl. No. of cell	TRIPLOID	TETRAPLOID
	Chromosome association	Chromosome association
1	12 III + 6 II + 6 I	3 IV + 1 III + 26 II + 5 I
2	11 III + 7 II + 7 I	1 IV + 1 III + 32 II + 1 I
3	11 III + 5 II + 11 I	2 IV + 1 III + 27 II + 7 I
4	12 III + 5 II + 8 I	2 IV + 30 II + 4 I
5	9 III + 9 II + 9 I	2 IV + 2 III + 26 II + 6 I
6	10 III + 7 II + 10 I	2 IV + 1 III + 27 II + 7 I
7	10 III + 6 II + 12 I	1 IV + 3 III + 26 II + 7 I
8	8 III + 10 II + 10 I	2 IV + 4 III + 24 II + 4 I
9	14 III + 3 II + 6 I	1 IV + 2 III + 27 II + 8 I
10	11 III + 5 II + 11 I	2 IV + 1 III + 26 II + 9 I
11	9 III + 5 II + 17 I	1 IV + 2 III + 28 II + 6 I
12	8 III + 8 II + 14 I	1 IV + 1 III + 29 II + 7 I
13	7 III + 10 II + 13 I	1 III + 30 II + 9 I
14	13 III + 5 II + 5 I	4 IV + 2 III + 22 II + 6 I
15	17 III + 1 II + 1 I	2 IV + 2 III + 28 II + 2 I
16	9 III + 7 II + 13 I	3 IV + 1 III + 26 II + 5 I
17	8 III + 7 II + 16 I	2 IV + 2 III + 24 II + 10 I
18	10 III + 7 II + 10 I	3 IV + 4 III + 21 II + 6 I
19	10 III + 5 II + 14 I	2 IV + 2 III + 26 II + 6 I
20	11 III + 6 II + 9 I	1 IV + 32 II + 4 I

It was reported that the induced tetraploid of RRII 105 showed comparatively more vigour at the early stage of growth (Saraswathyamma et al, 1984). Mendes (1969) had observed increased vigour in a polyploid clone of *Hevea* of origin IAN-873. The leaves of polyploid were reported to be thicker and deep green in colour (Shepherd, 1969; Markose, 1975; Saraswathyamma et. al, 1984). The present observations have shown that growth vigour was more for the tetraploid compared to that of the diploid. Further, most of the morphological characters of the triploid were intermediate compared to those of the diploid and the tetraploid (Table 1). Similar observations were reported with regard to artificial triploids in *Luffa echinata* (Agarwal et al, 1979).

The chromosome complement of diploid *H. brasiliensis* is 2n = 36 (Majumder, 1964). The induced tetraploid showed 2n = 72 (Markose, 1975) and the triploid showed 2n = 54 (Saraswathyamma et al, 1980). Cytogenetic stability is an important aspect for the induction of polyploidy for further exploitation and crop improvement. Maintenance of the induced cytogenetic variability primarily depends on the propagation method of the species concerned. In *Hevea,* vegetative propagation through budgrafting is easy and difficulties have not been experienced in budtake on normal stocks. Even after ten generations of vegetative multiplication the induced tetraploid of RRII 105 maintained its characters (Saraswathyamma et al, 1984). The triploid, evolved from the seedling resultant of cross pollination between diploid and tetraploid, has also maintained this character on vegetative multiplication.

H. brasiliensis is supposed to be of amphidiploid origin (Shepherd, 1969; Ong, 1975) with complete bivalent formation. Meiotic studies of induced tetraploid in *Hevea* showed a large number of bivalents, a few multivalents and univalents. Some of the explanations put forth for low incidence of multivalents in autotetraploids are the small size of chromosomes, preponderance of sub-metacentric, sub-telocentric and acrocentric chromosomes and presence of genes for low chiasma frequency (Darlington, 1965; Stebbins, 1971). Reduction in quadrivalent frequency in autotetraploids were reported in *Cosmos* (Mathew and Rajkumar Thomson, 1984) and *Zinnia* (Gupta and Rajini Koak, 1976). Small size of chromosome is reported to be responsible for low frequency of multivalents in these crops. But it is a matter of controversy as to whether size of chromosome plays any role in the formation of quadrivalents in autotetraploids. Pal and Pandey (1982) reported the influence of genes or gene complexes which suppressed the multivalent association of chromosomes in grain Amaranths.

Meiosis in the evolved triploid exhibited the formation of trivalents, bivalents and univalents. In the early stages of meiosis, trivalent formation was predominant. However, precocious disjunction of the trivalents resulting in the formation of bivalents and univalents was noted at metaphase I. A similar meiotic behaviour was reported in *Vinca rosea* (Sudhakaran, 1971). Although complete trivalency is usual in autotriploids, varying number of univalents, bivalents and trivalents were reported in *Solanum, Zea, Pyrus* and *Tulipa* (Balog, 1984). Low frequency of segregational errors in anaphase I and subsequent division may be responsible for the formation of 80 per cent stainable pollen grains in the tetraploid, whereas, the triploid showed only 5.5 per cent pollen stainability due to apparently high frequency of segregational errors in the meiotic division.

Polyploidy is one of the evolutionary mechanisms in producing radically different and well adapted genotypes (Stebbins, 1968). Polyploids can enrich the germplasm of *Hevea* which can be utilised for future breeding programme.

Triploids are particularly important in cytogenetic research as source material to obtain primary aneuploid series. Such aneuploids will be of value in genetic investigations.

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