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COMPARATIVE CYTOMORPHOLOGICAL STUDIES ON SPONTANEOUS AND SYNTHESIZED TRIPLOIDS OF *HEVEA BRASILIENSIS* (WILLD. EX ADR. DE JUSS.) MUELL. ARG.

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The genus *Hevea* belonging to the family Euphorbiaceae comprises of ten species Among these, the para rubber tree, *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. i the only species commercially exploited for natural rubber, which provides the livelihoo for over thirty million people in the world. The economic life span of the tree is about 30 t 35 years. The tree is diploid (2n = 2x = 36) with a basic number of x = 18 [1–5]. Triploids i this species were reported for the first time from the Rubber Research Institute of Indi [6–7]. This communication deals with the comparative cytomorphological investigation on a spontaneous triploid and a synthesized one.

MATERIALS AND METHODS

The spontaneous triploid is a seedling selection from the Rubber Research Institute o India. Tetraploidy was induced in a diploid by colchicine treatment [5] and the tetraploi thus obtained was incorporated in breeding programme as the male counterpart and diploid (GI 1) as the female. From each of the triploids 15 budgrafted plants were raised i polybags and growth attributes were recorded at 24 months of growth. Foliage character were scored from the middle leaflet of twenty mature leaves, selected at random, from each cytotype. Stem index, petiolar index and leaf index [8] and specific leaf weight [9] were estimated. Leaf chlorophyll content was estimated as per method suggested by Arnon [10] Mitotic studies were made on tender leaftips after pretreatment with saturated para-dichloro benzene following conventional techniques. Budgrafted plants of both the triploids were induced to flower at the age of 30 months by ringbarking [11]. Male flowe buds at the appropriate stage of development were collected and fixed in Carnoy's fluid. Anthers were stained with acetocarmine and squashed in 45 per cent acetic acid. Observations were recorded in 100 pollen mother cells selecting, at random, 10 cells each from 10 slides. Pollen stainability was assessed in acetocarmine-glycerol mixture (1 : 1) and pollen size was measured after acetolysis [12]. Photomicrographs were taken from temporary mounts. The mean values of the growth parameters studied as well as the chromosome associations from 100 cells were compared employing t test.

RESULTS AND DISCUSSION

Wide range of morphological variations was noted between the spontaneous and the synthesized triploids. The synthesized triploid showed variation in the number of leaflets, the range being two to five (Fig. 1: 1) and also exhibited semi dwarf stature due to closer internodes. The spontaneous triploid showed dark green colour for leaves but was indistinguishable from the diploid in other respects. The growth attributes of these two triploids are given in Table 1. Significant differences ($P < 0.01$) were observed for plant diameter, number of leaves per flush, length of female flower, leaf area, stem index, petiolar index and foliar index. Significance at 5% level was noted for the number of flushes. There was no significant difference for total chlorophyll content and length of male flower.

In the spontaneous triploid only a very low (10%) percentage of male flowers attained full size and maturity. The anther columns were found shrivelled and dropped even before maturity. On the other hand, there was normal development of male flowers in the synthesized triploid. In both the triploids, however, no abnormalities were recorded for the female flower and its development. Moreover, no fruitset was noted in both.

Table 1. Growth attributes of synthesized and spontaneous triploid

Parameter	Synthesized	Spontaneous	t value
Plant height (cm)	169.80 ± 2.230	216.80 ± 7.300*	8.54
Stem diameter (mm)	15.68 ± 0.287	20.83 ± 0.329**	14.47
Number of flushes	4.73 ± 0.066	6.20 ± 0.200**	9.53
Number of leaves per flush	11.20 ± 0.305	16.40 ± 0.305	14.74
Female flower length (mm)	11.02 ± 0.145	1.99 ± 0.067**	20.63
Male flower length (mm)	7.80 ± 0.054	7.81 ± 0.064	0.19
Total chlorophyll (mg/g fresh weight of leaf)	3.39 ± 0.275	4.07 ± 471	1.58
Leaf area (mm^2)	75.33 ± 0.605	86.77 ± 0.870**	12.
Stem index	0.0654 ± 0.00034	0.0703 ± 0.00035**	12.
Petiolar index	0.0168 ± 0.00026	0.0181 ± 0.00021**	4.62
Foliar index	0.0056 ± 0.00004	0.0068 ± 0.00006**	20.70

*,**Significant at $P < 0.05$ and $P < 0.01$, respectively.

The chromosome complement of the somatic cell was found to be 2n = 3x = 54 in both the triploids (Fig. 1: 2, 3). Meiotic division exhibited a wide range of abnormalities in both the triploids. At metaphase I (Fig. 1: 4, 5), various chromosome associations like trivalents, bivalents and univalents were observed (Tables 2, 3). In the spontaneous triploid, the range of trivalents was 9–18, bivalents 0–10, and univalents 0–10. The average occurrence of chromosome associations per cell was 12.44 for trivalents, 5.76 for bivalents and 6.22 for univalents. There was formation of 18 trivalents in 5% meiocytes and 15% recorded a maximum of 10 univalents and 2% exhibited 10 bivalents which was the maximum.

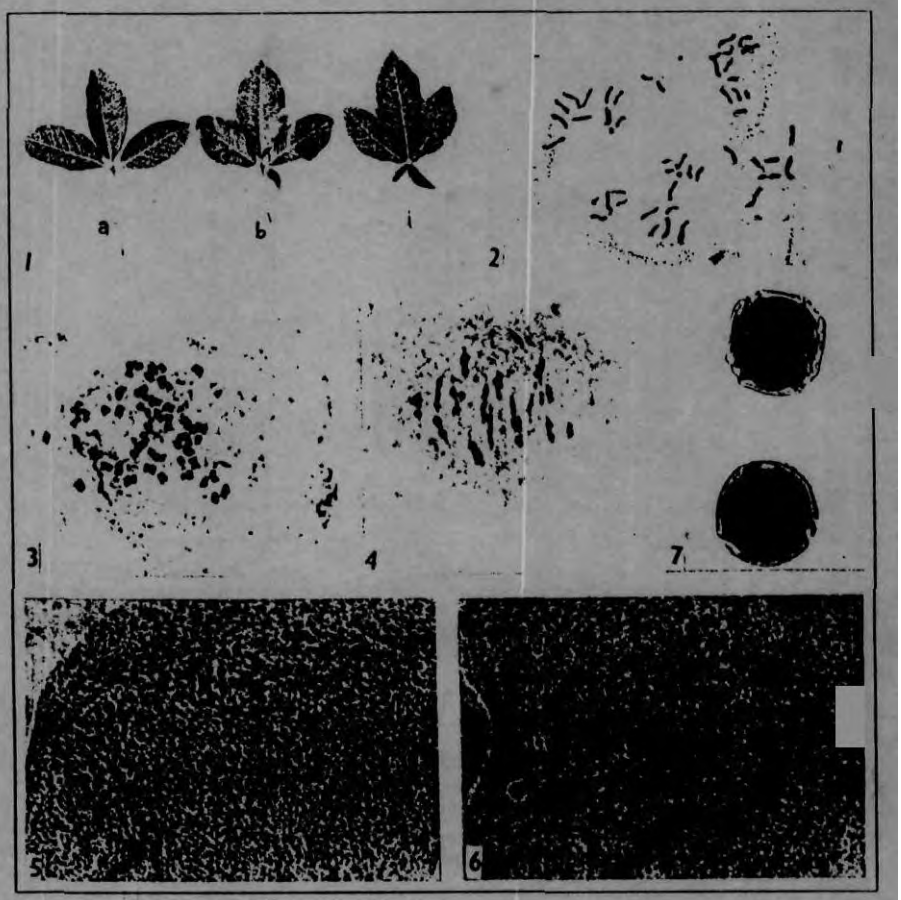

Fig. 1. Characteristics of triploid *Hevea brasiliensis*.

1) Leaves of triploids, x1/7; a) spontaneous, b) synthesized; 2) somatic metaphase of synthesized triploid (2n = 3x = 54), x2500; 3) somatic metaphase of spontaneous triploid (2n = 3x = 54), x2800; metaphase I of spontaneous triploid with predominant trivalents, x3000; 5) metaphase I of synthesiz triploid, x3500; 6) anaphase I of synthesized triploid showing laggards, x4000; and 7) pollen grains .. synthesized triploid show 3 and 4 pores, x190.

In the synthesized triploid the range of trivalents was 7 to 17, bivalents 1 to 10 and univalents 1 to 17. The mean occurrence of chromosome association per cell was 11.06 for

trivalents, 6.11 for bivalents and 8.72 for univalents. In both the triploids, during anaphase I (Fig. 1: 6), unequal segregation and formation of laggards were observed.

The t test for the mean number of trivalents, bivalents and univalents (Table 4) in 100 cells showed that there was significant difference between the two triploids. High frequency of trivalents was noted for the spontaneous triploid and high univalent association for the synthesized triploid. The pollen grains in the synthesized triploid showed increase in size. There were pollen grains having three and four pores (Fig. 1: 7) and the size of pollen grains ranged from 10 μm to 55 μm.

Table 2. Chromosome association during metaphase I in the spontaneous triploid

Chromosome association	Percentage of cells	Chromosome association	Percentage of cells
$9_{III} + 9_{II} + 9_I$	11	$13_{III} + 6_{II} + 3_I$	4
$10_{III} + 10_{II} + 4_I$	2	$13_{III} + 5_{II} + 5_I$	14
$10_{III} + 8_{II} + 8_I$	1	$14_{III} + 1_{II} + 10_I$	1
$10_{III} + 9_{II} + 6_I$	3	$14_{III} + 5_{II} + 2_I$	5
$10_{III} + 7_{II} + 10_I$	9	$15_{III} + 3_{II} + 2_I$	4
$11_{III} + 6_{II} + 9_I$	6	$15_{III} + 2_{II} + 5_I$	2
$11_{II} + 7_{II} + 7_I$	2	$16_{III} + 2_{II} + 2_I$	3
$12_{III} + 6_{II} + 6_I$	10	$17_{III} + 1_{II} + 1_I$	3
$12_{III} + 5_{II} + 8_I$	7	$17_{III} + - + 3_I$	2
$12_{III} + 9_{II} + -$	1	$18_{III} + - -$	5
$12_{II} + 4_{II} + 10_I$	5		

Table 3. Chromosome association during metaphase I in the synthesized triploid

Chromosome association	Percentage of cells	Chromosome association	Percentage of cells
$7_{III} + 10_{II} + 13_I$	2	$11_{III} + 7_{II} + 7_I$	7
$8_{III} + 9_{II} + 12_I$	2	$11_{III} + 4_{II} + 13_I$	1
$8_{III} + 8_{II} + 14_I$	3	$12_{III} + 5_{II} + 8_I$	3
$8_{III} + 7_{II} + 16_I$	3	$12_{III} + 6_{II} + 6_I$	9
$8_{III} + 10_{II} + 10_I$	2	$12_{III} + 4_{II} + 10_I$	1
$9_{III} + 7_{II} + 13_I$	4	$13_{III} + 5_{II} + 5_I$	9
$9_{III} + 8_{II} + 11_I$	1	13III + 6II + 3I	2
$9_{III} + 6_{II} + 15_I$	2	$14_{III} + 3_{II} + 6_I$	2
$9_{III} + 5_{II} + 17_I$	2	$14_{III} + 1_{II} + 10_I$	1
$9_{III} + 9_{II} + 9_I$	5	$14_{III} + 5_{II} + 2_I$	6
$10_{III} + 7_{II} + 10_I$	6	$15_{III} + 2_{II} + 5_I$	2
$10_{III} + 8_{II} + 8_I$	3	$15_{III} + 3_{II} + 3_I$	2
$10_{III} + 6_{II} + 12_I$	4	$16_{III} + 2_{II} + 2_I$	1
$10_{III} + 10_{II} + 4_I$	1	$17_{III} + 1_{II} + 1_I$	3
$11_{III} + 6_{II} + 9_I$	6	$17_{III} + - + 3_I$	3
$11_{III} + 5_{II} + 11_I$	2		

The distinguishing features of the synthesized triploid are the variations in the number and size of leaflets and semidwarf stature. The dark green colour for the leaves of spontaneous triploid may be due to greater thickness and chlorophyll content. Similar results had been reported in *Luffa* [13] and *Capsicum* [14, 15]. Triploids are reported to be vigorous in sugarcane and sweet potato [16]. In the present investigation the spontaneous triploid was found to be more vigorous compared to the induced triploid during early stage of growth. This may be due to the possible effect of back crossing [17]. Synthesized triploid had been evolved by crossing diploid (Gl 1) and induced tetraploid of RRII 105. RRII 105 is a hybrid clone evolved by the Rubber Research Institute of India by crossing Tjir 1 and Gl 1.

In the spontaneous triploid 5 per cent of meiocytes showed complete trivalents (18 III). From the cytological observations it is clear that the spontaneous triploid is an autotriploid characterised by the presence of a maximum number of 18 trivalents. In the synthesized triploid a maximum of only 17 trivalents was observed at metaphase I. In other words 18 trivalents, which is the maximum possible, was not observed in any of the microsporocytes. This may likely be due to the genetic differences between the two genomes involved in its origin or the precocious disjunction of trivalents.

Table 4. Comparison of chromosome association in the synthesized and spotaneous triploid

Chromosome association	Synthesized	Spontaneous	t value
Trivalent	11.06 ± 0.236	12.44 ± 0.245**	4.05
Bivalent	6.11 ± 0.205	5.76 ± 0.226	1.14
Univalent	8..72 ± 0.415	6.22 ± 0.297**	4.90

**Significant at $P < 0.01$.

In nature, triploids may originate either due to crossing between tetraploids and diploids or by fusion of reduced egg cells with two male nuclei or fertilization of unreduced egg cell and a male nucleus. Since spontaneous tetraploids are not reported to occur in *Hevea*, a cross between tetraploids and diploids is practically impossible. Hence it can reasonably be assumed that fertilization of functional 2n egg by haploid sperm or of haploid egg by a 2n sperm nucleus has occurred. Spontaneous triploids are reported by the fertilization of reduced and unreduced gametes in *Allium* [16] and *Cynodon* [18]. Induced triploids are reported in other crops like *Pyrus* [19] and *Luffa* [13]. But triploids are rare in tree crops. Among the 10 species of *Hevea* a triploid clone is reported only in *Hevea guianensis* [20]. Lack of fruitset in the triploids *H. brasiliensis* is of special advantage as fruits are reported to act as a major source of inoculum causing abnormal leaf fall disease [21].

To conclude, two triploids showed distinct morphological variations. Cytologically, the spontaneous triploid shows the autotriploid nature. Moreover, in this triploid complete male sterility is observed. But in the synthesized triploid 5.5% stainable pollen grains along with sterile pollen grains are noted. Both the triploids are found to be fruitless. Triploids are valuable for detailed genetic investigations and enrichment of genetic resources. They are also important as source material to obtain primary aneuploid series.

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REFERENCES

1. H. H. Ramaer. 1935. Cytology in *Hevea*. Genetica, **17**: 193–235.

2. S. K. Majumder. 1964. Chromosome studies of some species of *Hevea*. J. Rubb. Res. Inst. of Malaya, **18**: 269–275.

3. H. Ong. 1981. Cytology of *Hevea*. *Hevea* breeding course. Lecture notes, RRIM. Malaysia: 1–21.

4. C. K. Saraswathy Amma, V. C. Markose, J. Licy and A. O. N. Panikkar. 1984. Cytomorphological studies in an induced polyploid of *Hevea brasiliensis* Muell. Arg. Cytologia, **49**: 725–729.

5. N. W. Simmonds. 1989. Rubber breeding. *In*: Rubber (eds. C. C. Webster and W. J. Baulkwill). Tropical Agricultural Series.

6. C. K. Saraswathy Amma, V. C. Markose, J. Licy, Y. Annamma, A. O. N. Panikkar and V. K. B. Nair. 1980. Triploidy in *Hevea brasiliensis* Muell. Arg. Abstr. Intern. Rubb. Conf. India, 1980.

7. M. A. Nazeer and C. K. Saraswathy Amma. 1987. Spontaneous triploidy in *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muel. Arg. J. Plantations Crops, **15**: 69–71.

8. L. O. T. Mendes. 1969. Chromosome doubling of *Hevea* rubber tree. Paper presented to the session of Brazilian Academy of Sciences, Rio de Janeiro, G. B. Brazil.

9. N. J. Chatterton, D. R. Lee and Hungerford. 1972. Diurnal changes in specific leaf weight of *Medicago sativa* L. and *Zea mays* L. Crop Sci., **12**: 576–578.

10. D. J. Arnon. 1949. Copper enzymes in isolated chloroplast polyphenol oxidase in *Beta vulgaris*. Plant Physiol., 24: 1–15.

11. C. K. Saraswathy Amma. 1975. Induction of early flowering in *Hevea*. Rubb. Bd. Bull., **12**: 6.

12. G. Erdtman. 1952. Pollen morphology and plant taxonomy. Angiosperm. Waltham, Mass, USA.

13. P. K. Agarwal, R. P. Roy and D. P. Mishra. 1979. Artificial triploid in *Luffa echinata*. Cytologia, **44**: 739–745.

14. M. S. Chennaveeraiah and A. F. Habib. 1973. Cytomorphological studies in a spontaneous triploid of *Capsicum annum* L. Cytologia, 38: 677–685.

15. K. J. Frey. 1967. Plant breeding. Proc. Symposium, Iowa State University. Iowa State University Press, Ames, Iowa: 65–138.

16. T. N. Khoshoo and V. B. Sharma. 1959. Cytology of the autotriploid *Allium rubellum*. Chromosoma, 10: 136–143.

17. C. K. Saraswathy Amma. 1990. Morphological, Cytological and Genetical Investigations of Induced and Spontaneous Male Sterile Clones of Para Rubber Tree—*Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. Ph.D. Thesis. University of Kerala.

18. P. K. Gupta and A. K. Srivastava. 1970. Natural triploidy in *Cynodon* (L.) Pers. Caryologia, 23: 29–35.

19. C. D. Darlington and A. A. Moffett. 1930. Preliminary and secondary chromosome balance in *Pyrus*. J. Genet., 22: 130–151.

20. J. T. Baldwin. 1947. *Hevea*: a first interpretation. J. Heredity, 38: 54–64.

21. P. N. Radhakrishna Pillai, M. K. George and V. K. Rajalakshmi. 1980. Leaf and shoot diseases. *In*: Handbook of Natural Rubber Production in India (ed. P. N. Radhakrishna Pillai). RRII, Kottayam: 249–273.