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Short Communication

Cytopalynological investigations in induced tetraploid of para rubber tree [*Hevea brasiliensis* (Muell. Arg.)]

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T ara rubber tree *Hevea brasiliensis* is one of the main crops of Kerala's economy. The genetic base of this plant is very limited. For creating genetic variability induction of polyploidy has been tried in this crop by many workers [1-4]. The clone PR 107 is an Indonesian primary clone. Polyploidy was successfully induced in this clone by the application of colchicine and the results of the study are presented here.

Aqueous solution of colchicine (0.75%) was applied to young bud sprouts of the clone PR 107 continuously for seven days (cotton swab method). The basal buds of those which showed morphological variations were utilized for vegetative multiplication. Subsequent vegetative generations (up to VM 10) were raised employing the basal buds of the respective shoots. Tetraploidy was confirmed by meiotic and mitotic studies using flower buds and shoot tips. For this ethyl alcohol: acetic acid: chloroform mixture (3:1:1) was used as ive and 2% acetocarmine solution was used for ing. Meiotic data was recorded by observing 50 pollen mother cells. For pollen stainability studies acetocarmine: glycerin mixture (1:1) was used.

The induced tetraploid showed wide variation in cytology and palynology when compared to its diploid counterpart. Meiosis in the diploid was normal showing 18 bivalents at metaphase I (Fig. 1) and 36 chromosomes in the shoot tip cells (Fig. 2). In the tetraploid, meiosis was highly irregular showing univalents, bivalents and quadrivalents at metaphase I (Fig. 3) and 72 chromosomes in the shoot tip cells (Fig. 4) as against 36 in the diploid. Anaphase showed anomalies such as unequal segregation, formation of laggards and micronuclei. The chromosome association of induced polyploid at metaphase I was as follows. Out of 50 PMCs analysed, the number of cells showing univalents, bivalents, trivalents and quadrivalents ranged between 2-6, 24-27, 2-3 and 2-4 respectively (Table 1).

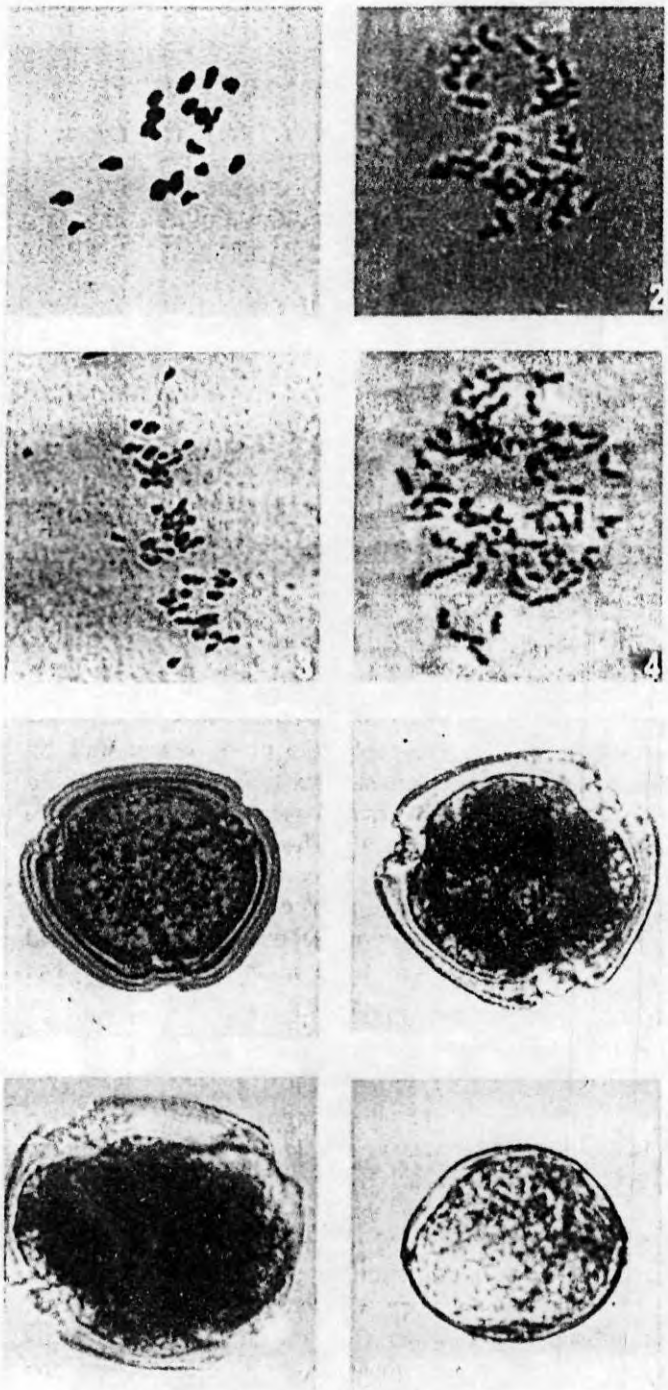
Table 1. Chromosome association at metaphase 1 in PR 107 tetraploid (2n = 4x = 72)

Chromosome association	Total cells analyzed	Range	Mean
Univalents	50	2-6	3.5±0.27
Bivalents	50	24-27	25.3±0.21
Trivalents	50	2-3	2.4±0.23
Quadrivalents	50	2-4	2.7±0.28

Pollen stainability of the diploid was 90.43% and it contains only 3-zonocolporate pollen grains (Fig. 5). In the teraploid, pollen stainability was 70.24%. Three types of pollen grain *viz.*, 3-zonocolporate, 4-zonocolporate and micro pollen grains were observed in the tetraploid (Figs. 6, 7 and 8). Among the fertile pollen 24.55% were 4-zonocolporate and 45.69% were 3- zonocolporate. Average pollen size of the diploid was 39.72 × 30.88µm while in the tetraploid it was 45.84 × 36.72µm.

The PR 107 colchiploid exhibited a predominance of bivalents (50%). Quadrivalents observed was only 4-8%. Occurrence of multivalent association is a common feature in autotetraploids due to the presence of more than two homologues. The homologues are expected to form multivalent associations, but practically it does not happen due to many reasons. Kostoff [5] suggested that smaller chromosomes tend to produce less quarivalents than those with longer ones. The reduced number of multivalents observed in the present study may be due to the smaller size of chromosomes and decreased rate of pairing. Reduced number of quarivalents in induced tetraploid was reported in crops such as *Sesamum* [6] and *Cajanus cajan* [7]. The pollen mother cells of the induced tetraploid showed abnormalities such as clumping of chromosomes, formation of chromosome bridges and unequal distribution of chromosomes during cell division. The chromosomal irregularities lead to low pollen fertility in the tetraploid.

Pollen size index is a reliable parameter for



Figs. 1-8. Clone PR 107 of *Hevea brasiliensis* (Figs. 1-4 × 1250, Figs. 5-8 × 1500). 1. Metaphase 1 of diploid showing 18 bivalents, 2. Somatic cell of diploid showing 36 chromosomes at metaphase, 3. Metaphase 1 of tetraploid showing Is, IIs, IIIs & IVs, 4. Somatic cell of tetraploid showing 72 chromosomes, 5. 3-Zonocolporate pollen grain of diploid, 6. 3-Zonocolporate pollen grain of tetraploid, 7. 4-Zonocolporate pollen grain of tetraploid, 8. Micro pollen grain of tetraploid

detecting tetraploids in rubber. Here the pollen size of the diploid was smaller (39.72 × 30.88µm) when compared to the tetraploid (45.84 × 36.72µm). Large pollen size in induced tetraploid clone of rubber (RRII 105) was reported earlier by Saraswathyamma and Sethuraj [8]. Moreover, 4-zonocolporate and micro pollen grains are characteristic features of the tetraploid. The diploid showed a better pollen fertility when compared to the tetraploid where the meiotic abnormalities adversely affected pollen fertility. The induced tetraploid serves to enrich the genetic stock of *Hevea brasiliensis*.

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