

## COMPARATIVE BARK ANATOMY OF THREE INDUCED POLYPLOIDS OF *HEVEA BRASILIENSIS* (WILLD. EX. A. JUSS) MUELL. ARG. AND THEIR DIPLOIDS

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### ABSTRACT

Comparative bark anatomy of the mature trees of three induced polyploids of *Hevea brasiliensis* and their respective diploids (GT 1, Tjir 1 and RR11 105) was studied to explore the feasibility of polyploidization as a tool for crop improvement in *Hevea*.

Two of the clones studied, GT 1 and Tjir 1 showed considerable reduction in bark thickness, number of latex vessel rows and the density of laticifer rows along with highly increased height and width of the phloem rays. The difference between the tetraploids and the diploids was not pronounced for RR11 105 with respect to bark thickness and number of latex vessel rows. The diameter of laticifers and the extent of anastomosing were also high for the polyploids of GT 1 and Tjir 1 compared to their diploids while this difference also was negligible in the case of RR11 105. However, a tendency for higher size and reduced number of cells for the polyploids was indicated that the response was clone-specific. The possible effect of these characteristics are discussed.

### INTRODUCTION

*Hevea brasiliensis* has a chromosome complement of  $2n = 36$ . In earlier years several workers (Markose, 1975; Mendes, 1969; Shepherd, 1969) attempted to produce induced polyploids in this species. In India autotetraploids were evolved both from seeds and vegetative buds using aqueous solution of colchicine. Quantitative studies on certain leaf characters like leaf thickness, leaf weight, lateral vein divergence, stomatal characters and the stem and foliar indices of the induced tetraploids have also been made at the immature age (Markose, 1975).

*Hevea* being a commercial crop and its economic product being obtained from the latex produced in laticiferous tissue of the bark, the variability induced in the bark characters is very important. Reports on the characters of mature trees of *Hevea* at higher ploidy level, especially on the bark

anatomical characters are scanty. Quantitative estimations of certain bark anatomical traits of three induced tetraploids in comparison to their respective diploids of *Hevea brasiliensis* were made. The results and their implications are discussed.

### MATERIALS AND METHODS

An induced seedling tetraploid of Tjir 1, obtained by treating seeds with 0.75% aqueous solution of colchicine and cloned later, a tetraploid of GT 1 and another of RR11 105, both obtained by treating the vegetative buds of the respective clones, were taken for this study along with their diploids at comparable ages. Tjir 1 and GT 1 were available from a trial laid out in 1971 and RR11 105 from an observational trial planted in 1977 at the Central Experiment Station of RR11 at Chethackal.

Three trees at random per clone of the

tetraploids and the respective diploids were selected. Samples of virgin bark at a height of 150 cm from the bud union were collected and fixed in formalin acetic alcohol. Radial longitudinal sections of 120  $\mu\text{m}$  thickness and tangential longitudinal sections 80  $\mu\text{m}$  thickness were cut using a sledge microtome. The sections were stained with Sudan III and observed under the microscope. The following characters were recorded from three sections per bark sample.

- (1) Thickness of soft bast and hard bast;
- (2) Number of laticifer rows;
- (3) Height and width of phloic rays in the laticifer layer;
- (4) Laticifer density (number of laticifers per unit circumference of the tree);
- (5) Diameter of laticifers;
- (6) Number of connections within 0.5 mm height of the laticifers.

For characters 3–6, five observations per section have been taken.

### RESULTS

The thickness of bark and number of latex vessel rows are shown in Tables I and II respectively. At the tenth year of planting GT 1 tetraploid had a mean thickness of only 1.77 millimetre. At the same age the respective diploid had attained 7.63 mm thickness of bark. A similar trend was noticed for Tjir 1 also in which the mean bark thickness of the tetraploid and its diploid were 4.04 mm and 6.93 mm respectively. In the case of RR11 105, four year old tetraploid was comparable to the diploid for bark thickness, the mean values being 4.11 and 4.44.

The retarded growth of polyploids was evident with regard to the girth also. At the ninth year of planting the average girth of GT 1 tetraploid in the field was only 16 cm and that of Tjir 1 tetraploid was 52.5 cm, when the respective diploids had attained 80 cms and 79 cms respectively (personal communication from late V. C. Markose). The mature trees of these two polyploid clones

also showed comparatively lesser tree height on visual observation.

The tetraploids of GT 1 and Tjir 1 recorded very low number of latex vessel rows (Table II) the mean values being 3.89 and 6.89 respectively against the corresponding values 16.22 and 19.00 recorded for the respective diploids. RR11 105 did not show such a wide difference between the two ploidy levels with respect to this character.

On the contrary the laticifer diameter was higher for the tetraploids of GT 1 and Tjir 1 (Table IV), the mean values being 24.24  $\mu\text{m}$  and 25.16  $\mu\text{m}$  respectively against 21.51  $\mu\text{m}$  and 21.87  $\mu\text{m}$  for their respective diploids. Practically there was no difference between the two ploidy levels in the case of RR11 105 in which the tetraploid recorded a mean laticifer diameter of 22.93  $\mu\text{m}$  against 22.60  $\mu\text{m}$  for the diploid.

The density of laticifers/row and the density of connection between latex vessels are also shown in Table IV. In all the three clones, laticifer density was low and density of connections between laticifers was high for the tetraploids than their respective diploids. The mean laticifer density recorded for the tetraploids of GT 1, Tjir 1 and RR11 105 were 20.83, 21.39 and 22.19 against the corresponding values for the respective diploids 26.33, 25.83 and 26.13. With respect to the density of connection between laticifers, the mean values recorded for the tetraploids of the three clones in the order mentioned above were 11.44, 11.51 and 9.07, the corresponding values for the respective diploids being 10.80, 10.44 and 8.87.

Very wide phloic rays was a characteristic feature of the tetraploids in all the three clones under study. The mean values recorded for the tetraploids of GT 1, Tjir 1 and RR11 105, regarding this trait, were 74.80  $\mu\text{m}$ , 70.93  $\mu\text{m}$  and 62.03  $\mu\text{m}$  respectively

Table I. Bark thickness of the tetraploids and their diploids (Mean  $\pm$  S.D.)

Clone	Soft bast (mm)		Hard bast (mm)		Total (mm)	
	Tetraploid	Diploid	Tetraploid	Diploid	Tetraploid	Diploid
GT 1 (10 years)	0.63 $\pm$ 0.40	1.56 $\pm$ 0.21	1.14 $\pm$ 0.48	5.24 $\pm$ 0.60	1.77 $\pm$ 0.46	7.63 $\pm$ 0.97
Tjir 1 (10 years)	1.73 $\pm$ 0.28	2.12 $\pm$ 0.57	2.30 $\pm$ 0.69	4.75 $\pm$ 1.54	4.04 $\pm$ 0.64	6.93 $\pm$ 1.23
RRII 105 (4 years)	1.55 $\pm$ 0.19	1.45 $\pm$ 0.23	2.55 $\pm$ 0.55	3.08 $\pm$ 0.38	4.11 $\pm$ 0.48	4.44 $\pm$ 0.48

Figures in parenthesis indicate age of trees.

Table II. Number of latex vessel rows in the tetraploids and the diploids (Mean  $\pm$  S.D.)

Clone	Soft bast		Hard bast		Total	
	Tetraploid	Diploid	Tetraploid	Diploid	Tetraploid	Diploid
GT 1 (10 years)	3.89 $\pm$ 0.35	11.44 $\pm$ 3.24	—	4.78 $\pm$ 1.99	3.89 $\pm$ 0.35	16.22 $\pm$ 3.03
Tjir 1 (10 years)	5.67 $\pm$ 1.5	12.22 $\pm$ 1.20	1.11 $\pm$ 0.33	6.78 $\pm$ 3.31	6.89 $\pm$ 1.27	19.00 $\pm$ 3.00
RRII 105 (4 years)	3.78 $\pm$ 0.67	3.11 $\pm$ 0.60	1.22 $\pm$ 0.44	2.33 $\pm$ 1.12	5.00 $\pm$ 0.50	5.44 $\pm$ 1.51

Figures in parenthesis indicate age of trees.



Table III. Phloic rays in the tetraploids and their diploids (mean  $\pm$  S.D.)

Clone	Mean height $\mu\text{m}$		Mean width $\mu\text{m}$	
	Tetraploid	Diploid	Tetraploid	Diploid
GT 1 (10 years)	736.67 $\pm$ 130.15	472.6 $\pm$ 67.91	74.80 $\pm$ 3.39	52.51 $\pm$ 3.00
Tjir 1 (10 years)	548.53 $\pm$ 66.3	392.5 $\pm$ 47.23	70.93 $\pm$ 5.13	52.89 $\pm$ 8.69
RRII 105 (4 years)	445.13 $\pm$ 68.14	462.4 $\pm$ 73.32	62.03 $\pm$ 5.06	50.58 $\pm$ 2.84

Figures in parenthesis indicate age of trees.

Table IV. Density, diameter and the intensity of anastomosing of laticifers in tetraploids and their diploids (Mean  $\pm$  S.D.)

Clone	Vessel density per 1 mm		Vessel diameter ( $\mu\text{m}$ )		No. of connection per 0.5 mm height	
	Tetraploid	Diploid	Tetraploid	Diploid	Tetraploid	Diploid
GT 1 (10 years)	20.83 $\pm$ 1.44	26.33 $\pm$ 3.67	24.24 $\pm$ 1.22	21.51 $\pm$ 1.23	11.44 $\pm$ 0.53	10.80 $\pm$ 0.82
Tjir 1 (10 years)	21.39 $\pm$ 2.20	25.83 $\pm$ 2.50	25.16 $\pm$ 1.69	21.87 $\pm$ 1.55	11.51 $\pm$ 0.11	10.44 $\pm$ 0.76
RRII 105 (4 years)	22.19 $\pm$ 1.60	26.13 $\pm$ 1.81	22.93 $\pm$ 0.63	22.60 $\pm$ 0.71	9.07 $\pm$ 0.15	8.87 $\pm$ 0.43

Figures in parenthesis indicate age of trees.

against the corresponding values for their diploids 52.51  $\mu\text{m}$ , 52.89  $\mu\text{m}$  and 50.58  $\mu\text{m}$ . Ray height also showed the same trend with respect to GT 1 and Tjir 1 while the data of RR11 105 did not show such a difference between the two ploidy levels (Table III).

#### DISCUSSION

One of the effects of polyploidy is an increase in cell size. This need not increase the size of the plant as a whole because polyploidy often leads to reduction in the number of cell division during development (Stebbins, 1971; Indira and Abraham Susan, 1977). Retarded initial growth of buds after colchicine treatment has been reported in many plants and also in *Hevea* (Shepherd, 1969). Earlier reports on reduced number and larger size of stomata, associated with tetraploidy in *Hevea* (Mendes, 1969; Mendes and Mendes, 1963) already indicated a tendency for reduction in cell numbers and increase in cell size at higher ploidy level. An increase in leaf blade thickness and leaf weight can be attributed to the increased size of mesophyll tissue. However, *Hevea* is reported to be a tetraploid adapted to the environment while most other species in the family are diploids having chromosome complement of  $2n = 18$  (Majumdar, 1964 and Shepherd, 1969).

In the present study, reduction in stem girth, bark thickness, number of laticifer rows, laticifer density and tree height was noticed in the mature trees of GT 1 and Tjir 1. There was no improvement of any of these traits due to high ploidy level in RR11 105 also although the differences between the tetraploid and the diploid were not so pronounced as in the case of the other two clones. An increase in diameter of laticifers as well as broader and longer phloic rays were the characters of tetraploids. Although this effect was only less pronounced in the case of RR11 105.

In this context it is relevant to say that RR11 105, available for this study had attained only four years growth *i.e.*, just covered the stage of canopy closure. The growth retardation effect due to higher cell size is generally affected at a stage when the plants face competition for space, food or interference of the environment. Earlier reports on increased vigour of RR11 105 (Saraswathy Amma *et al.*, 1984) and IAN 45, 873 (Mendes, 1969) associated with autotetraploidy were based on the observations of young plants, *i.e.*, after two years of growth in the case of RR11 105 and only after nine months growth in the case of IAN 45,873. There was no further report on the latter. Markose (1975) observed higher stem index for young plants of GT 1 tetraploids. However, the clone showed reduced growth in the field as observed in the present study.

Anatomical traits like bark thickness and number of laticifer rows are clonal characteristics (Gomez and Chen, 1967; Gomez, Narayanan and Chen, 1973; Narayanan, Ho and Chen, 1974), influencing the yield of *Hevea* clones (Narayanan, Gomez and Chen, 1973; Ho, Narayanan and Chen, 1973). Positive linear relationship between the number of laticifer rows and initial rate of flow has also been advocated (Sethuraj, Sulochana and George, 1974).

Significant clonal variations of the height and width of phloic rays and the effect of these traits on the orientation of laticifers in *Hevea* clones have been reported (Premakumari, Panikkar and Joseph, 1985). Further studies have indicated the bearing of laticifer orientation and laticifer quantity, in terms of cross sectional area, on the yield of *Hevea* clones (Premakumari, Panikkar and Joseph, 1988). Considerable reductions in the number of laticifer rows and laticifer density along with low girthing in the tetraploids assure a steep fall in the total cross

sectional area of laticifers in the tetraploids. A slight increase in laticifer diameter cannot be the least adequate compensation for this effect.

Width of phloic rays have significant negative association with the density of laticifers at genotypic and phenotypic levels (Premakumari et al, 1984). Waviness of laticifers due to broad rays may affect the rate of latex flow also. Hence the broad phloic rays of tetraploids is not an appreciable trait as far as the yield is concerned.

The technique of polyploidy is usually employed to induce genetic variability and exploit the useful traits directly or by further breeding and selection. As far as *Hevea* is concerned, the anatomical traits and the general tendency discussed above do not promise improvement of yield or growth by this technique although the possibility of inducing other desirable traits cannot be ruled out.

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