



Cytopalynological investigations in tapping panel dryness affected trees of *Hevea brasiliensis* (Muell. Arg.)

L. Sankariammal and C. K. Saraswathyamma

Rubber Research Institute of India, Rubber Board, Kottayam 686 009

(Received: December 2003; Revised: August 2004; Accepted: August 2004)

The para rubber tree *Hevea brasiliensis* belonging to the family Euphorbiaceae, contributes to 99% of the world's natural rubber. It is one of the major tree crops having great impact in the economy of Kerala. A major problem encountered in rubber plantation is the occurrence of Tapping Panel Dryness (TPD) commonly known as brown bast. The symptoms of TPD are spontaneous drying up of the tapping cut and cessation of latex flow followed by thickening, cracking, peeling of the bark and abnormal trunk growth. Rubber biosynthesis is not taking place in the laticiferous cells of the fully affected trees and as a result the yield becomes very low. So this syndrome has been the subject of numerous studies. Even though studies had been conducted so far in morphological, physiological, anatomical and pathological point of view [1-4] reports are lacking in cytopalynological aspects. Hence a study based on this was undertaken in some selected clones and the results are discussed here in comparison with normal clones.

The clones selected for the study were RR11 105, RR11 600 and PB 235. For cytological studies the method adopted was the same as reported by Saraswathyamma *et al.*, [5]. Pollen size index was calculated by the method proposed by Tseng and Ting [6]. Percentage of stainable pollen was assessed using acetocarmine: glycerin mixture (1:1). Flower buds of these clones were collected from three locations viz., Kottayam, Changanacherry and Pathanamthitta. The study was repeated for three consecutive years.

Cytological studies of the flower buds collected from TPD affected trees showed great abnormalities during cell division. Meiotic studies of the pollen mother cells of normal trees showed 18 well separated bivalents at metaphase 1 (Fig. 1) while in TPD affected trees the bivalents were clumping together forming different groups in the equatorial plate (Fig. 2). Abnormal dyads were formed at telophase 1 (Fig. 3). The tetrad in unaffected tree was normal (Fig. 4), while in affected trees most of the tetrads were abnormal (Fig. 5). Triads

and pentads were formed in plenty in addition to tetrads (Figs. 6 & 7). As a result, numerous sterile pollen grains were formed in affected trees when compared to normal (Figs. 8 & 9).

An in-depth study of the pollen size and stainability of the pollen grains of the above clones were done. Percentages of pollen sterility in brown bast affected trees were very high. Sterility percentage increased with the increase in the intensity of the syndrome and there was significant difference observed in normal and severely affected trees (Table 1). Average size of pollen Table 1. Pollen sterility percentage in normal and TPD affected trees

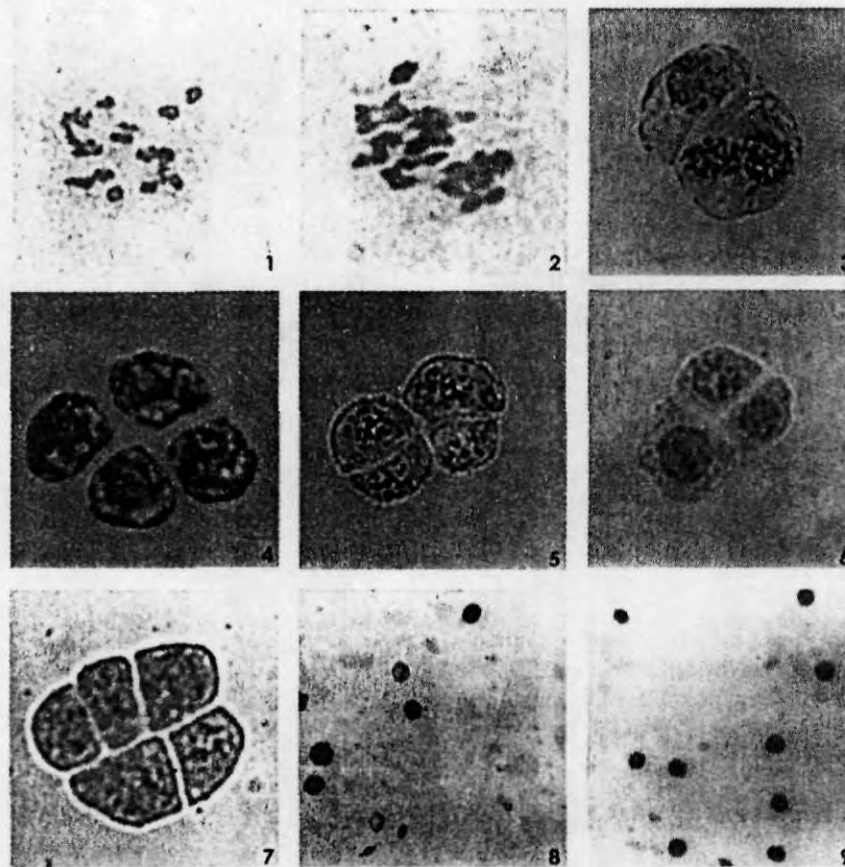
Clone	Pollen sterility in normal trees (T)	Pollen sterility in TPD affected trees according to intensity of syndrome (%)		
		Initial	Intermediate	Severe
RR11 105	7.23	9.59	23.28	41.83
RR11 600	10.62	12.82	20.53	34.76
PB 235	9.77	11.57	18.46	30.42
C. D. (0.05)	0.87	0.98	1.32	1.48

in TPD affected trees were smaller when compared to the pollen of unaffected trees. Pollen size in normal trees of RR11 105, RR11 600 and PR 107 were $40.63 \times 32.25\mu\text{m}$, $39.34 \times 30.38\mu\text{m}$ and $39.19 \times 30.73\mu\text{m}$ respectively while that in affected trees were $38.12 \times 31.35\mu\text{m}$, $37.64 \times 28.28\mu\text{m}$ and $38.23 \times 28.47\mu\text{m}$. The difference was not statistically significant. It was also noticed that the weight and germination percentage of

Table 2. Seed weight and germination percent of normal and TPD trees

Clone	Treatment	Seed weight (gm)	t test	Seed germination (%)	
				Seed germination (%)	t test
RR11 105	Normal	4.85	**	90.23	**
	TPD	3.74		71.81	
RR11 600	Normal	4.62	**	88.54	**
	TPD	3.55		68.43	
PB 235	Normal	4.38	**	86.23	**
	TPD	3.32		65.54	

**P < 0.01



Figs. 1-9. *Hevea brasiliensis*. Different stages of cell division and pollen grains of normal and TPD affected trees. (Figs. 1&2 \times 1300, Figs. 3-7 \times 2500, Figs. 8&9 \times 400). 1. Metaphase 1 of normal cell showing 18 bivalents, 2. Metaphase 1 of TPD cell showing clumping of chromosomes, 3. Abnormal dyad of TPD cell, 4. Tetrad of normal cell, 5. Tetrad of TPD cell, 6. Triad of TPD cell, 7. Pentad of TPD cell, 8. Pollen grains of TPD tree, 9. Pollen grains of normal tree.

seeds in TPD affected trees were significantly low when compared to unaffected trees (Table 2).

A cell's life during cell division is known as "physiological reproduction". During cell division the biosynthetic capacity of a cell or its growth potential is doubled. Here abnormalities seen during cell division may be due to physiological disturbance in the cells caused by TPD syndrome. The reason if any for the clumping of chromosomes in the somatic cells of affected trees has to be investigated further. There is no increase or decrease of chromosome number in the ePMCs, but abnormalities were observed during cell division in affected trees leading to high pollen sterility. Pollen sterility studies carried out in different clones of *Hevea* ranged from 7 to 12% [7], but in severely affected trees it was very high (30-40%). Significant difference was observed in seed weight and seed germination percentage of normal and affected trees. Low seed weight may be the reason for reduced seed germination of seeds collected from affected trees. Sensitivity to bark dryness varies greatly from one clone to another, showing the importance of the genetic factors. Hence rubber being a perennial tree crop it is worthwhile to

select clones/trees devoid of TPD for breeding programmes and further multiplication.

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