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CLONAL VARIABILITY IN THE DISTRIBUTION OF SIEVE TUBES AND COMPANION CELLS IN *HEVEA BRASILIENSIS* BARK TISSUE

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In *Hevea brasiliensis,* latex is produced in the laticiferous tissue present in the secondary phloem or bark. Sieve tubes and companion cells are the other two important components of secondary phloem tissue with the main function of sap conduction. In this context the transportation of metabolites through the sieve elements have great significance in terms of latex synthesis taking place in the laticifers. Ten different clones of *H. brasiliensis* were selected to study the clonal variability in the distribution pattern of sieve tubes, companion cells and dimensional variation of sieve tubes. The sieve elements were identified as enucleated elongated cells placed end to end in the longitudinal axis of the stem. Sieve plates were obliquely arranged at the end of the sieve elements. Occurrence of one or two companion cells, in close association with sieve tubes, implies its functional relationship with the counterpart. Sharing common companion cells among two sieve elements within a row was noteworthy. Considerable clonal variation in sieve tube length was noticed. The maximum length of sieve tube was recorded in the clone PB 235 and minimum in RRIM 703. The maximum diameter of sieve tube was recorded in PB 86. The analysis of variance indicated that the length of sieve tubes exhibited considerable variation between *Hevea* clones but less variability within clones. Characteristically PB clones showed superiority of over other clones under study for the dimension of sieve tubes. The dimensional variation of sieve tubes in the bark tissue can be considered as a marker for the identification of clonal variability and yield potential of *Hevea* clones.

Keywords: Clonal variability, Companion cells, Sieve tubes

INTRODUCTION

Sieve tubes form the most important transporting system in the secondary phloem (Bel *et al*., 2002) mainly related to the assimilation of photosynthates and other substances (Schmitz and Schneid, 1989; Turgeon, 2000; Nakamura *et al*., 2004). Many angiosperms have long sieve tubes with oblique sieve plates (Lu *et al*., 1994; Lotova and Nilova, 1998; Magistris and Castro, 2001; Castro *et al*., 2005). Sieve members do not exhibit a regular development in terms of length but slightly longer in old bark (Trockenbrodt, 1994). Occurrences of short sieve tubes with horizontal simple sieve plates have also been reported as a common feature (Zhang and Gao, 1987; Liu *et al*., 1995; Lotova and

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Timonin, 2003). Hence, the dimensions of sieve elements can be considered as a significant marker in various investigations of secondary phloem (Chavan and Shah, 1983; Costa *et al.*, 1997).

Anisio *et al.* (1998) studied the diameter of sieve tubes in *Hevea* clones and reported significant correlation with rubber production. The relationship between the diameter of sieve tubes and yield has also been well established (Gunnery, 1935; Fernado and Tambiah, 1970). The studies on the influence of ethephon stimulation on tapping by Hao and Wu (1986) revealed the collapse of sieve tubes in the outer conducting phloem in association with the formation of stone cells. Nevertheless, Narayanan and Ho (1970) did not find any relationship between sieve tube and yield. Clonal nursery studies in *H. brasiliensis* conducted by Narayanan *et al*. (1974) revealed the mean diameter of sieve tube as 19 µm. Companion cells are strongly associated with each sieve tube. Chavan, *et al*. (2000) reported that in dicotyledonous species two or more compsanion cells are attached to long sieve tubes. A comparative investigation on the distribution of sieve elements and companion cells in the bark tissue of *Hevea* clones has not been studied in detail so far. Hence the present study aimed at the understanding of the distribution pattern of sieve tubes and companion cells and clonal variability in *Hevea brasiliensis*.

MATERIALS AND METHODS

Ten domesticated clones (Wickham clones) of *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg., *viz*. Gl 1, GT 1, PB 235, PB 28/59, PB 86, RRII 105, RRII 300, RRIM 600, RRIM 703 and Tjir 1 were selected from the Germplasm gardens I, II and III, at the Central Experimental Station of Rubber Research Institute of India, Chethackal, Ranni, Kerala. The gardens were planted in randomised block design (RBD) with three replications and three trees per plot. The trees were under regular tapping and had an age of 17-21 years. Nine mature trees from each clone (three trees per replication) were used for bark sample collection. Virgin bark samples were collected from the selected trees at 150 cm height from the ground. The samples collected were fixed in formalin-acetic -alcohol (FAA) for anatomical and histochemical investigations. Sections were taken using Leica Rotary microtome at 5 -10 µm thickness at cross sectional (CS), tangential longitudinal (TLS) and radial longitudinal (RLS) planes and stained with safranin- fast green for microscopic observations. For the histochemical localization of total polysaccharides, Periodic acid-Schiff's reagent (Ruzin, 1999) was used. The parameters studied were the distribution pattern of sieve tubes and companion cells and the variation in the length and diameter of sieve tubes among *Hevea* clones.

The data were subjected to statistical analysis. Coefficient of variation (CV) was calculated to ascertain the tree-to-tree variation within clones. The mean values were pooled to find out the CV values. Analysis of variation (ANOVA) was worked out to measure the clonal variation. Photomicrographs were taken in Leitz Aristoplan Research microscope and quantitative image analysis was done using Leica Q Win V.2.1 Image analysis software.

RESULTS AND DISCUSSION

Sieve tubes are long enucleated cells (Fig. 1 a and b at arrows) arranged end-to-end with well separated end walls made up of long oblique perforated sieve plates (Fig. 1 c). Even though sieve tubes are enucleated

cells (van Bel, 2003) its role in the transportation of sugars was well documented (Schrier *et al*., 2000; van Bel and Hess, 2008; Liu *et al*., 2012). The companion cells were relatively small with well defined nucleus. In majority of cases two companion cells were attached to the sieve tubes (Fig. 1 d–at arrows). However rare occurrence of sieve tubes with one companion cell, very close to the sieve plate and two sieve tubes sharing a common companion cell were also noticed (Fig. 1e). It has been reported that the sieve elements are incapable for protein synthesis due to the lack of nucleus and ribosomes (Cronshaw, 1981), but the abundant presence of proteins in the translocation stream of sieve tubes presumably from the companion cells which have effective cytoplasmic communication with sieve tubes (Fisher, 1990). The movement of macro molecules symplastically carried out through plasmodesmata (Maule, 2008; Lucas *et al*., 2009; Xu and Jackson, 2010). The vital of sieve elements and companion cells in the phloem translocation was also well studied by different authors (Zhang *et al*. 2010; Tumbull, 2011).

Histological staining revealed the presence of polysaccharides in the sieve tubes especially on sieve plates as granules (Fig. 1 h & i –at arrows). Generally, sucrose is considered as the main carbohydrate component of sieve tube sap (van Bel and Hess, 2008), additionally galactosy-oligosaccharides and sugar alcohols also had presence in the sieve tube sap (Zimmermann and Ziegler, 1975). So the present occurrence of carbohydrates may belong to polysaccharides category.

Considerable varitions in the length of sieve tubes were noticed (Table 1). The length of sieve tube was maximum (875.02 μm) in PB 235 and minimum (329.02 μm) in RRIM 703. In all the clones, the CV values were very low reflecting low tree-to-tree variation within clones. Analysis of variance (Table 1) revealed that PB 235 was superior over eight clones, *viz*. RRII 300, PB 28/59, RRIM 600, RRII 105, Tjir 1, Gl 1, GT 1 and RRIM 703. Similarly PB 86 was superior to seven clones *viz*. PB 28/59, RRIM 600, RRII 105, Tjir 1, Gl 1, GT 1 and RRIM 703. RRII 300 was superior to Tjir 1, Gl 1, GT 1 and RRIM 703; RRIM 600 was superior to GT 1 and RRIM 703; and RRII 105, Tjir 1 and Gl 1 were superior to RRIM 703. The length of sieve tube was higher in PB clones with very low tree-to-tree variation than that of the other clones studied.

Table 1. **Length and diameter of sieve tubes**

Clone	Sieve tube length (μm)		Sieve tube diameter (μm)	
	Mean	CV(%)	Mean	CV(%)
Gl 1	531.22	14	33.36	14
GT 1	441.90	14	31.97	12
PB 235	875.02	15	35.48	12
PB 28/59	588.23	11	34.70	7
PB 86	773.40	13	45.17	17
RRII 105	555.88	16	32.54	20
RRII 300	666.75	9	28.91	12
RRIM 600	555.92	7	37.70	13
RRIM 703	329.02	18	27.08	7
Tjir I	549.95	11	30.50	7
V R (F)	16.5**		10.92**	
C D (5%)	113.83		4.58	

* Significant for $p < 0.05$ ** Significant for $p < 0.01$

The diameter of sieve tubes was maximum (45.17 μm) in PB 86 (Fig.1 f- at arrow) and minimum (27.08 μm) in RRIM 703 (Fig.1 g – at arrow). The low CV values explain the absence of tree-to-tree variations within clones. ANOVA indicated significant clonal variation where PB 86 was statistically superior to Gl 1, RRII 105, GT 1, Tjir 1, RRII 300 and RRIM 703 (Table 1). RRIM 600 was

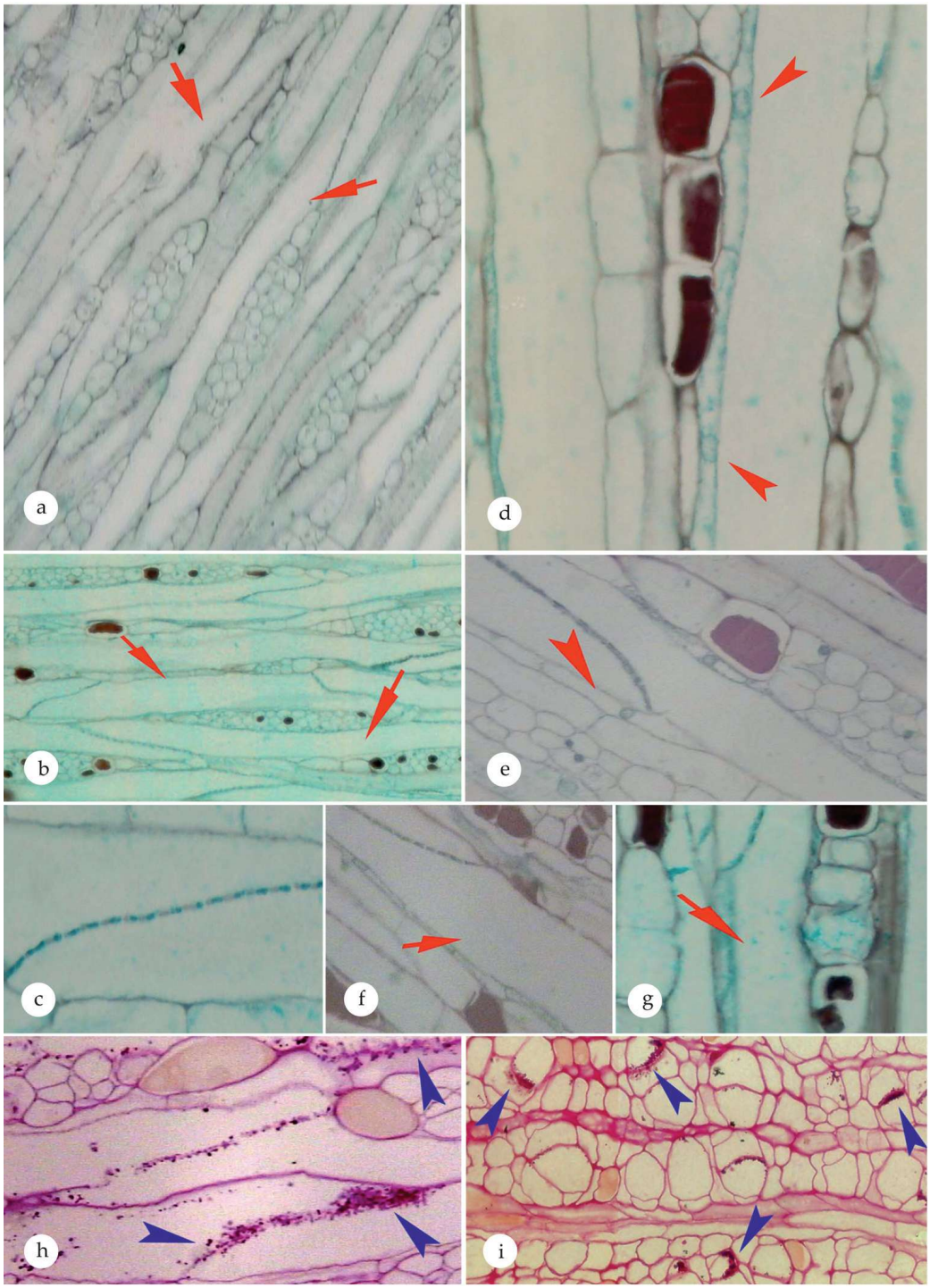

Fig. 1. a to g - TLS of bark stained with safranin - fast green showing morphology of sieve tubes. a- PB 235 very long sieve tubes (arrows). B- RRIM 703 short sieve tubes (arrow). c- long and oblique sieve plate. d- sieve tube with two companion cells (arrow). e- companion cells very close to sieve plate shared by two sieve elements (arrow). f - PB 86 sieve tube diameter (maximum). g- RRIM 703 sieve tube diameter (minimum). h and i - accumulation of polysaccharides around sieve plates, TLS and CS sections respectively (note arrow heads). a & b - X75; c&d- X300; e.f. & g- X200; i-X75 & i-X200

superior to RRII 105, GT 1, Tjir 1, RRII 300 and RRIM 703. Similarly PB 235 observed superiority over three clones *viz.* Tjir 1, RRII 300 and RRIM 703; and PB 28/59 was superior to RRII 300 and RRIM 703. The clones Gl 1, RRII 105 and GT 1 were superior to RRIM 703. The diameter of the sieve tubes has great significance as it meant for the major phloem transport and significantly correlated with rubber production in *Hevea* as reported by Anisio *et al*. (1998).

CONCLUSION

The present study mainly focuses on the nature of sieve tube elements in *Hevea* clones and also the association of companion cells. Sieve tubes were appeared as enucleated long cells predominantly distributed in the soft bark region of the secondary phloem of *Hevea* with obliquely placed sieve plates. Mostly two companion cells observed in association with sieve tubes and even they share companion cells among adjacent sieve tubes in a line. Sieve tubes exhibits considerable variation in its length among clones with less variation within clones. Characteristically, PB clones showed superiority over other clones for the length and diameter of sieve tubes. The present study revealed that the dimensional variability of sieve rubes in the bark tissue can be used as a reliable marker for the identification of the yield potential of *Hevea* clones.

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