Studies on the structure of bark of *Hevea brasiliensis* with special reference to alignment of phloic elements and clonal variability

Thesis

Submitted to

Mahatma Gandhi University

Kottayam

for the fulfilment of the requirement for the award of the Degree of

Doctor of Philosophy

in

Botany

Under the Faculty of Science

By

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October

2005

Dedicated to my Parents

Gifted to my wife Pinky
and
sons Haby and Harry

Declaration

*I, do hereby declare that this thesis entitled "**Studies on the structure of bark of Hevea brasiliensis with special reference to alignment of phloic elements and clonal variability**" is a bonafide record of the research work done by me under the guidance of Dr. C. P. Reghu, Botanist, Germplasm Division, Rubber Research Institute of India, Kottayam and that no part of this work has been submitted earlier for the award of any degree or diploma in any other University.*

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Certificate

This is to certify that the thesis entitled **"Studies on the structure of bark of *Hevea brasiliensis* with special reference to alignment of phloic elements and clonal variability"** is an authentic record of original research work carried out by Shri. Philipose Omman, Lecturer in Botany, Catholicate College, Pathanamthitta, at the Rubber Research Institute of India, Kottayam, Kerala, under my supervision and guidance during the period - January 1998 to August 2005, for the award of the degree of Doctor of Philosophy in Botany in the faculty of Science, Mahatma Gandhi University, Kottayam, Kerala.

The work presented in this thesis has not been submitted for the award of any other degree or diploma earlier. It is also certify that Shri. Philipose Omman has fulfilled all the requirements and has passed the qualifying examination for Ph.D. of Mahatma Gandhi University, Kottayam.

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ACKNOWLEDGEMENT

I wish to place on record my indebtedness to all the persons who have extended their support, cooperation and well wishes for the completion of this thesis. First I would like to offer my sincere gratitude to my guide Dr. C. P. Reghu, Botanist, Germplasm Division for his scholastic guidance, timely suggestions, critical discussions and sustained support.

I am grateful to the Chairman, Rubber Board and Dr. N.M. Mathew, Director, Rubber Research Institute of India, Kottayam for giving permission and providing necessary facilities to carry out this work.

I am deeply obliged to Dr. Y. Annamma, Deputy Director, Germplasm Division for her constant encouragement and Dr. Jacob Pothen, former Deputy Director, CES, Chethackal, for permitting me to collect sample materials for the study.

I wish to express my deep sense of gratitude to Mr. Ramesh B Nair, Asst. Director, Statistics, RRII, for his valuable suggestions and help rendered in conducting the statistical analysis.

I owe a special word of thanks to Dr. Saji T Abraham, Scientist, for critically reading the statistical results, Dr. Francis Mathew, Research Scholar, for his unstinted help, and all other scientists and staff of the Germplasm division, for their sincere cooperation.

My special thanks to the entire Library staff, members of library, computer section, instrumentation and other office staff of RRII, Kottayam, for providing timely help.

I am greatly indebted to the University Grants Commission for granting me FIP and to the Government of Kerala for granting deputation to complete the thesis.

I express my heartfelt gratitude to His Holiness Moran Mar Baselius Marthoma Mathews II, the education agency, H. G. Philipose Mar Eusebius and H.G.Dr. Yakoob Mar Iraneus, Manager, MOC, Colleges, Prof. V.I. Joseph, Secretary, MOC, Colleges, former Principals and the present Principal Prof. Prasad Thomas, for granting permission and providing timely official help.

I wish to thank Dr. George Koshy, H.O.D of Botany, Catholicate College, and other colleagues of the department, for their encouragement.

This thesis has been made possible only due to the support and prayers offered by my parents, the boundless cooperation and help of my wife Pinky and beloved sons Haby and Harry.

Last but not the least, I wish to thank God Almighty, without whose blessing nothing is possible.

Philipose Omman

CONTENTS

Chapter 3

Chapter 4

Chapter 1

Introduction

Euphorbiaceae is one of the most diverse and largest dicot family with about 326 genera and over 8935 species (Govaerts, *et al.*, 2000). This family is having a significant position among the other taxa as many of its members possess the most important plant product 'latex'. The occurrence of latex has been reported in various plants belonging to dicots, monocots and even pteridophytes (Bras, 1957; Metcalfe, 1967; Romberger, *et al.*, 1995). The milky latex of *H. brasiliensis* (para rubber) is the sole source of Natural Rubber (NR) which almost satisfies the needs of Rubber Industry. World NR output has reached up to 8.4 million tons in 2004 (Malaysian Rubber Review, 2005).

Hevea brasiliensis, a perennial tree species belonging to the family Euphorbiaceae, is the major contributor of NR. Even though laticiferous species account for several thousands, only about 2000 species contain rubber hydrocarbon in their latex. Among these, 500 species have been experimented as the source of NR (Bonner and Galston, 1947). Latex vessels or laticiferous tissues are specialized cells or tissues in which latex is synthesized and stored. Economically, the rubber content in the latex is an important criterion which differentiate many species, as source of NR. In this regard, *H. brasiliensis* stands top as it possesses very high NR content compared to other rubber yielding plants (Raghavendra, 1991).

1.1 The genus *Hevea*

The primary centre of origin of the genus *Hevea* is the Amazon basin of South America and the surrounding regions of Manas, Mato Gross and Acre. Natural habitat of different species of *Hevea* are also found in Brazil, Bolivia, Colombia, Ecuador, French Guiana, Guyana, Peru, Surinam and Venezuela (Wycherley, 1992).

Under the genus *Hevea*, 10 species have been recognized so far *viz. H.guianensis, H. brasiliensis, H pauciflora, H. spruceana, H.rigidifolia, H. benthamiana, H .nitida, H. microphylla, H. camporum* and *H.camargoana* in the order of first descriptions of the concepts (Schultes, 1970; 1977; 1987; Wycherley, 1992; Annamma and Abraham, 2005).

1.2 Structural organization of bark and distribution of laticifers in *Hevea*

Bobilioff (1923) and Gomez (1982) have described different cell types and organization of *Hevea* bark. During the course of secondary growth, cambial derivatives divide the wood elements and phloem elements towards the inside and outside, respectively. The whole phloem tissue formed exterior to the cambium is termed as bark.

Anatomically *Hevea* bark consists of two distinct zones, the inner soft bark and the outer hard bark (Bryce and Campbell, 1917). Laticifers are differentiated from the fusiform initials of the cambium, in the form of concentric rings, alternating with other phloic elements such as sieve tubes, companion cells, phloem fibres, axial parenchyma and ray parenchyma. Due to the continued activity of the vascular cambium new laticifers are differentiated and the older ones are pushed outwards. Outer zone of bark is hard due to the occurrence of copious amount of sclerified stone cells.

In cross section of bark, latex vessels appear as more or less circular in shape and remain almost parallel to the cambium. In radial longitudinal plane, latex vessels look like tubular structure in different rows. The rows are arranged as straight tubes running in between other phloic tissues. Even though rare connections have been reported in between latex vessel rows (Arisz, 1918; 1919), most of the other researchers reported the absence of such radial connections between laticifer rows (Arens, 1911; Meunier, 1912; Kaimal, 1951). In tangential longitudinal sections, laticifers resembled anastomosing network of tubes, weaving round the phloic rays.

1.3 Alignment of phloic elements in the bark

Petch (1911) made the first observation about the orientation of wood elements, when bark was stripped off. Out of 25 trees observed, wood elements were oriented vertically in seven trees and towards the right in 18 trees. Later, De Jong (1916) studied the angle of inclination of latex vessels in 93 trees and reported an average angle of inclination of 3.7° to the right from the vertical.

An authentic investigation to determine the angle of wood elements in *H. brasiliensis,* was that of Gomez and Chen (1967). Of the 28 clones studied, all of them showed an average rightward inclination of laticifers ranging from 2.1° to 7.1°. Out of this, three clones *viz.* RRIM 600, BD 5 and RRIM 618 had groups of trees with leftward inclination of latex vessels within the range of 3.22° to 3.84°. They also noticed the inclination of laticifers in seedling trees with a rightward inclination of 4.2° to 5.1°.

1.4 Tapping

Tapping is the process of controlled wounding of the bark for latex extraction. The evolution and development of modern tapping system resulted in the economic exploitation of the rubber crop (Ridley, 1897; Abraham and Tayler, 1967). It has been demonstrated that rubber trees can be regularly exploited by periodic excision of shavings of bark along a tapping cut made on the tree trunk in a spiral fashion. The present system of tapping was formulated based on various experiments conducted on the anatomy and physiology of *Hevea* (De Jong 1916; Bobilioff, 1923; Mass, 1925). Tapping process is carried out by using tapping knives (Wright, 1912). Generally, tapping is performed on rubber tree by means of half spiral cut from upper left to lower right at a specific angle of 25° for seedlings and 30° for budded trees (Vijayakumar *et al.*, 2000). To avoid confusion and difficulty regarding the notations while dealing with different tapping systems, International Rubber Research and Development Board (IRRDB) has formulated a tapping notation for *Hevea* which was later revised by Lukman (1983).

1.5 Slope of tapping cut and tissue alignment

During the early evolution of modern tapping system, one of the most intriguing question was that whether the slope of the spiral cut should be to the left or to the right. Petch (1911) recommended left hand cut for more yield than right hand cut based on the finding of rightward inclination of wood elements in *Hevea*.

De Jong (1916) calculated the extra yield of left hand slope over the right, for various angles of cut, with a deviation of 3.5° of the latex vessels to the right. These theoreti-

cal results proved to be important while, comparing with the practical results on seedling trees (Mass, 1925) and budded trees (Rubber Research Institute of Malaya, 1940). Similar studies conducted by Dijkman (1951) also proved the extra yield on tapping in relation to the inclination of latex vessels.

Gomez and Chen (1967) also thoroughly discussed the advantages and disadvantages of steepening the slope of cut in buddings from the recommended 30° for buddings to 45° considering the latex vessel inclination at 3-4° towards the right and observed an yield increase upto 2-3% whereas the length of tapping cut has been increased by 22%. Similar result was also observed in seedling trees. Thus steepening of the tapping cut resulted in higher bark consumption which is considered as a serious disadvantage. In this context Gomez and Chen (1967) opined that a thorough knowledge of the inclination of latex vessels in *Hevea* should be essential before adopting the system of tapping. The survey of literature revealed that detailed investigation in this line has not been conducted so far.

1.6 Relevance of the present study

In the above circumstances, understanding the actual alignment, orientation and angle of inclination of laticifers and other phloic elements in the bark of rubber tree, pertaining to the exploitation of this perennial crop for latex yield is of utmost significance. This would enable to categorize different clones which are having specific pattern of inclination and orientation of laticifers. Similarly studies on the interrelationship with various bark characters as well as factors influencing the orientation of latex vessel in the bark tissue also helps to derive appropriate clonal specific exploitation systems.

In this context a detailed investigation on the structure of bark of *Hevea brasiliensis* with special reference to alignment of phloic elements and clonal variability has been carried out in ten clones of *Hevea brasiliensis* in the mature phase. Attempt was also made to understand the inclination pattern of laticifers in the juvenile growth phase of *H. brasiliensis*. The present investigation was carried out with the following objectives:

1. Structure of bark of *Hevea*
2. Variation of different structural characters within clones and between clones
3. The alignment and angle of inclination of laticiferous tissue and phloic elements
4. Angle of inclination of laticifers in seedling and budded trees at the juvenile stage
5. Structural factors affecting inclination of latex vessels in the bark
6. Association and interrelationship of various structural characters of bark
7. Histochemical status of different reserve metabolites in the bark

Chapter 2

Review of Literature

2.1 Laticifers

The origin of the term laticifers or laticiferous system is still obscure. Numerous classical observations and citation were available about the occurrence of coloured milky substance in plants. Grew (1682) noted the presence of lactiferous vessels in many plants. Grew's illustration of lactiferous substance was analogous to milk in animals, both in colour and coagulability. The term latex (means fluid or liquid) was common among English physician as early as 1662 (Chandler, 1933). The usage of the term latex was encountered while describing the medicinal properties of plants (Schultz, 1839). The term laticifers have appeared in many of the scientific literature (Jackson, 1928; Esau 1953) and is the most ideal term than laticiferous vessels or laticiferous structure.

2.1.1 Ontogeny of laticifers

Concepts regarding the formation of laticifers are based on the recognition of lactiferous vessels (Grew, 1682) and vasa propria (Malpighi, 1901) *viz.* intercellular space concept and cellular concept, respectively.

Many plant anatomists have overwhelmly supported the occurrence of coloured, resinous or mucilaginous liquid in the vessels or intercellular spaces of the plant tissue (Bernhardi, 1805; Mirbel, 1815; Sprengel, 1817; Treviranus, 1835; Schultz, 1839; Mohl, 1844; Anonymous, 1846). Laticiferous structures were also considered as intercellular secretory cavities (Mirbel, 1815; Link, 1837; Anonymous 1846).

The preponderant of cellular concept was Moldenhauer (1812) who did the demonstration of laticifers on cells with maceration technique on plants like Musa, Asclepias and Chelidonium. The existence of very long laticifers in plant systems were described and shown by many authors (Schacht, 1851; Hartig, 1862; Hanstein, 1864; Faivre, 1868).

2.1.2 General classification of laticifers

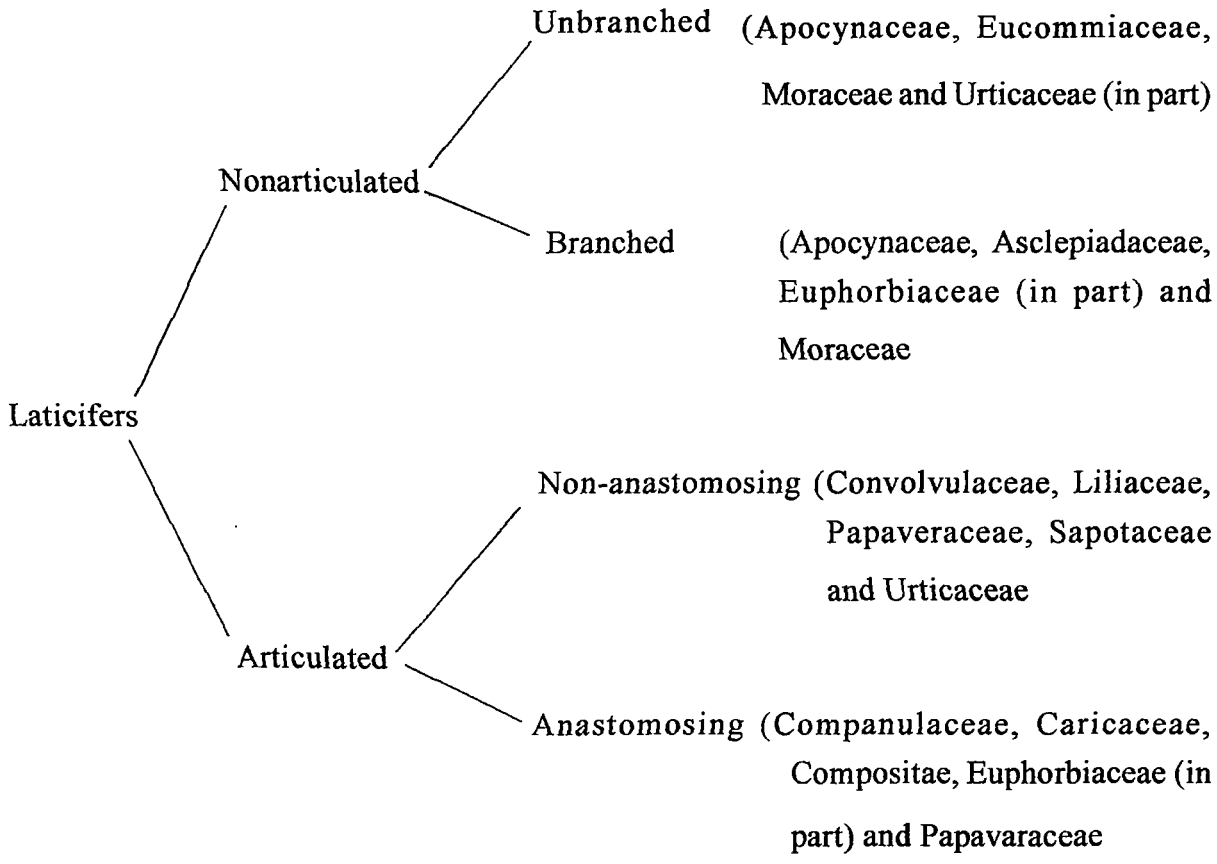
Several authors have classified laticifers based on the structural differences existing among the laticifer bearing plants (Unger, 1847; Hartig, 1862; Hanstein 1864; David 1872; Mayus, 1905).

Hartig (1862) made an initial attempt to classify latex systems based on anatomical progress made at that time as articulated tubes and non-articulated tubes. The latex tubes were seen as composed of rows of superimposed cells where the cross wall of the cell of the groups were perforated. Even before his findings, non-articulated latex vessels consisting of elongated cells with no detectable cross walls along the entire length of the latex vessels were reported by Unger (1847). Hanstein (1864) observed adjacent articulated vessels with anastomous in Cichoriaceae, Campanulaceae, Lobeliaceae and Caricaceae. Chauveaud (1891) classified the different forms of laticifers encountered in various plant species regardless of their taxonomic position.

De Bary (1884) categorised laticiferous tubes into articulated and non-articulated type based on their origin and nature. The division and distinction was greatly accepted in the field of laticifer anatomy (Tschrich, 1889; Sperlich, 1939; Foster, 1949).

Easau's (1953) classification of laticifers is the most recent classification as it includes the various forms of laticifers (Table 1). In some cases both articulated and non-articulated laticifers occur in the same family Euphorbiaceae (Schaffstein, 1932). Both types of laticifers were present in some plants like Stapelia and Trichocaulon (Asclepiadaceae) (Shaffestein, 1932).

Table 1. Classification of laticifers in plants (Easu, 1953).



2.2 Laticiferous System in *Hevea brasiliensis*

2.2.1 Nature and ontogeny

Presence of laticifers in *Hevea* was reported by Scott (1886) and Calvert (1887). Extensive work has been conducted during the 20th century in the anatomy of laticiferous system in *Hevea* (Bryce and Campbell, 1917; Keuchenius, 1918; Bobilioff 1918; 1920; Vischer, 1920; La Rue, 1921; Bryce and Gadd, 1923; Bally, 1922; Taylor, 1926; Ashplant, 1928a; 1928 b; 1928c; Sanderson and Sutcliffe, 1929; Frey-Wyssling 1930;). All the above studies showed that the laticifers in *Hevea* are articulated, anastomosing and coenocytic in nature.

Scott (1882) investigated the ontogeny of laticiferous system in *Hevea.* During the initial formation, laticifers could be recognized as elongated cells with smaller cross sectional area. He further noticed the presence of cross walls even at the stage when the latex is distinguishable and dissolution of cross walls takes place when the root growth reaches 3-4 cm length in the seedlings. Calvert (1887) identified three systems of laticifers in the stem of *Hevea.* Extensive studies have also been made on the ontogeny of latex vessels and other associated components of the bark (Arisz, 1918; Bobiliof, 1918; 1923). Milanez (1946; 1948; 1951) studied in detail the ontogeny of laticifers in *Hevea.* Initially the prolaticifers formed from the cambium undergo unequal nuclear division. Several such cells formed in the procambial vicinity form anastomoses.

Electron microscopic studies carried out by Gomez (1966) disproved the existence of medullary and hypodermal origin of laticifers. He suggested that the principal laticiferous

system observed in the procambial region belongs to phloem proper. Several such cells showed specific stainability with specific dyes and safaranin (Gomez, 1966). Many of these cells showed transverse and longitudinal anastomoses with neighbouring cells. These cells can be called as prolaticifers and later formed the anastomous laticiferous system.

Eventhough articulated latex vessels are the principal types of laticifers in the secondary phloem tissues of *Hevea*, non-articulated laticifers have also been reported in the primary tissues of young trees (Quian, 1987). Induction and differentiation of laticifers could be achieved by the external application of Jasmonic and Linolenic acids (Wu *et al.*, 2002).

2.2.2 Staining behaviour of laticifers

Non-polar lipid stains like Sudan III and Sudan IV (Pearse, 1968; Wigglesworth, 1988) are commonly used for staining of laticifer tissues in *H. brasiliensis* (Gomez *et al.*, 1972; Qian, 1987; Abraham *et al.*, 1992; Premakumari *et al.*, 1992; Reghu *et al.*, 1996). Some other stains like aqueous safaranin and malachite have also been tried earlier (Wimalaratna, 1973). A new staining procedure for staining laticifers in the bark of *H. brasiliensis* have been developed recently by Omman and Reghu (2003).

2.3 Quantitative factors influencing the structure of bark

Direct or indirect relationship of various factors with laticiferous system in *H. brasiliensis* and their prominent role in determining yield have already been established. These factors are described below under different heads.

2.3.1 Girth

Tree girth has been identified as one of the most important character pertaining to latex yield in *H. brasiliensis* (Ho *et al.*, 1973; Narayanan *et al.*, 1973; Premakumari *et al.*, 1997; Koshy, 1997). The tapping process was reported to be retarding the girth and biomass production (Abraham and Tayler, 1967; Templeton, 1969; Sethuraj, 1981; George *et al.*, 1984). Studies conducted in rubber tree proved that tree girth was a highly significant clonal character (Sethuraj, 1981; Nazeer *et al.*, 1986; Premakumari *et al.*, 1986; Premakumari *et al.*, 1991; Licy *et al.*, 2003).

Premakumari *et al.*, (1997) reported that girth increment on tapping was negatively correlated with density of laticifers and phloic ray characteristics. Narayanan *et al.*, (1974) made extensive investigation on interrelationships of various structural characters and observed linear correlation of girth with bark thickness, number of laticifer rows and yield. Preliminary evaluation studies in wild *Hevea* germplasm indicated low level of variation in tree girth (Abraham *et al.*, 1992). Costa *et al.*, (2000) reported significant genetic variability in the girth of three year old plant of *Hevea.* Girth increment over 4 years of tapping recorded broad sense of heritability estimates (Goncalves *et al.*, 1995).

Girth has been considered as an important factor influencing the yield in *Hevea.* High correlation of girth with yield and bark thickness has been noticed in high yielding clones during early selection (Lavorentic *et al.*, 1990). The relationship of yield and girth has been confirmed in mature trees (Narayanan and Ho, 1970) and in nursery clones (Narayanan and Ho, 1973). Hence girth has been considered as a stable character for the location specific selection of *Hevea* clones in different environments (Goncalves, 2004). Accord-

ing to Goncalves *et al.*, (1989) girth had no correlation with plugging index , but positive correlation with yield and bark thickness. Gomez *et al.*, (1972) used girth as an important variable to workout the laticifer area index, the most important parameter to assess the efficiency of tapping.

2.3.2 Bark thickness

Latex is produced within the laticiferous tissue of the bark and exploited by the process of tapping. All the tissue systems of the bark are functionally related with laticifers. Thus the variability accounted for the bark characters are very important.

Total bark thickness comprises the thickness of whole bark tissue that surrounds the wood externally in *Hevea.* It has been identified as clonal characteristics and was related to laticifer rows. (Gomez and Chen, 1967; Gomez *et al.*, 1972; Narayanan *et al.*, 1974) The thickness of bark also influenced the yield of *Hevea* clones (Narayanan *et al.*, 1973; Ho *et al.*, 1973; Gottardi, 1995 and Goncalves *et al.*, 2004). Also in hybrid clones, the thickness of virgin and renewed bark were very often considered as important characters for yield determination (Licy *et al.*, 2003). Bark thickness has also been reported as an influential factor in drought tolerance in *Hevea* clones (Premakumari *et al.*, 1993a).

The relationship between traits like bark thickness and laticifer rows have been reported by various workers (Bobilioff, 1923; Gomez *et al.*, 1972; Narayanan *et al.*, 1973). Studies carried out by Narayanan *et al.*, (1974) proved that the thickness of bark has been related with girth, number of latex vessel rows and distance between laticifer rows. Lavorentic *et al.*, (1990) estimated about 42% variation in bark thickness on tree girth.

The principal layer of tissue close to the cambium is usually termed as soft bark. Functionally the soft bark primarily meant for passage of nutrients (Hao and Wu , 1986). During tapping care should be given to protect this soft tissue from damage (Hebant and Fay, 1980; Auzac and Jacob, 1984). Wu and Hao, (1986) studied the importance and occurrence of sieve tubes in the soft bark region. The structure and thickness of conducting phloem of rubber tree has been carefully studied by Hao *et al.*, (1980) and Reghu *et al.*, (1996) reported the variation of bark structure in wild germplasm.

Studies conducted by Premakumari *et al.*, (1993b) in six clones of RRIM recorded significant clonal variation in the thickness of soft bark. High proportion of soft bark region was recorded in the virgin bark of *H. brasiliensis* (Premakumari *et al.*, 1992). A considerable portion of the bark tissue lying close to the soft bark zone externally has been designated as hard bark, while describing the anatomical features (Riches and Gooding 1952; Gomez, 1982).

2.3.3 Laticifer rows

Latex vessels are cylindrical tubes distributed in the form of rows or rings in the secondary phloem. Laticiferous system has been considered as the site of rubber synthesis in *H. brasiliensis* (Dickerson, 1965; Southorn, 1966; Gomez, 1966). Latex is exploited from these latex vessels by a process of controlled wounding called tapping.

The number of laticifer rows has been reported as a quantitative anatomical parameter pertaining to latex yield in *H. brasiliensis* (Bobilioff, 1923; Gomez, 1966). The correlation of this trait with yield has been proved by many workers in *Hevea* (Bobilioff 1920;

Larue, 1921; Taylor, 1926; Rubber research institute, Malaya, 1963, 1964, 1966, 1968; Narayanan *et al.*, 1973; Narayanan *et al.*, 1974).The number of laticifer rows has been identified as a clonal character (Vischer 1921; Sanderson and Sutcliffe 1929; Gottardi *et al.*, 1995) which varies considerably with age (Bryce and Campbell, 1917; Gomez *et al.*, 1972) and height (Vischer, 1920; Bryce and Campbell, 1917; Sanderson and Sutcliffe, 1929; Gomez *et al., 1*972) of the tree. But at young stages the variability is not significant (Costa *et al.*, 2000).

The number of laticifer rows in seedling trees at the age of 10 years were ranged from 9-13 (Bobilioff, 1920; Bryce and Gadd, 1923; Sanderson and Sutcliffe, 1929) whereas in budded trees at the age of 8.5 years it is even up to 26 rings (Gomez *et al.*, 1972). About 40% of the laticifer rows are situated within the distance of 2 mm from cambium and the number further declines over a distance of 5-8 mm (Gomez, *et al.*, 1972).

Premakumari *et al.* (1981) studied variations of cambial activity and number of laticifer rows in clone Gl 1and noted an increase in the number of laticifer rows with an increase in the rate of cambial activity. Positive correlation between number of laticifer rows and initial flow rate has been reported earlier (Sethuraj *et al.*, 1974). Reghu *et al.*, (1996) carried out a detailed investigation on the structure of bark in wild *Hevea* germplasm and reported the variation in the number of laticifer rows in different zones of bark. Hamzah *et al.*, (1975) reported negative correlation between number of laticifer rows and inter row distance

Premakumari *et al.*, (1993a) recorded significant reduction in the number of laticifer rows in the soft bark compared to that of the hard bark. Due to the high correlation of yield with number of laticifer rows, considerable emphasis has been given for the selection of high yielding clones based on the number of laticifer rows in *Hevea* (Rubber Research Institute Malaya, 1966; Wycherly, 1969). Variation in the number of laticifer rows between virgin bark and renewed bark has been reported in *Hevea* clones at the age of 11 years (Premakumari *et al.*, 1992).

2.3.4 Inter row distance between laticifers

The distance between laticifer rows has been considered as an yield determining character in *Hevea* (Paiva *et al.*, 1982). Gomez *et al.*, (1972) noted considerable variation in the average distance between laticifer rows in different clones. Goncalves *et al.*, (1995) also reported the variability and repeatability for this character in 76 trees. Narayanan *et al.*, (1974) observed positive correlation between girth and average distance between laticifer rows. Gottardi (1995) noted significant genotypic and phenotypic correlations among different bark characters including distance between consecutive rows of laticifers.

2.3.5 Latex vessel density

The number of latex vessels within a row in unit distance is termed as the density of latex vessels. Gomez *et al.*, (1972) reported higher density in the soft bark than that of hard bark and this trait has been identified as a potential trait for crop improvement programs (Abraham *et al.*, 1992). The density of latex vessels in the virgin and renewed bark varies considerably in RRII 105 and Tjir 1 (Premakumari *et al.*, 1992). Reghu *et al.*, (1996)

reported wide range of variability in many structural characters of the bark including density of latex vessels in wild *Hevea* germplasm. Significant genotypic and phenotypic variation existed in the density of latex vessels has also been reported (Gottardi, 1995). The relationship between the density of latex vessels and width of phloic rays has also been reported earlier (Premakumari *et al.*, 1984).

2.3.6 Frequency of interconnections

Clonal variability in the frequency of interconnections between latex vessels has been reported by Premakumari *et al.*, (1984; 1991) and opined that this trait had only low or moderate genetic advance along with high heritability estimates. The authors further pointed out that the number of inter connections per unit distance within the laticifer rows depend on the density and diameter of latex vessels.

2.3.7 Latex vessel diameter

Latex vessel diameter has been reported as an important factor which determines the latex yield (Asplant, 1927; 1928a; 1928b; 1928c). Simple correlations among yield, girth, bark thickness, number of laticifer rows and diameter of latex vessels have been reported earlier by various researchers. (Gomez *et al.*, 1972; Ho *et al.*, 1973; Narayanan *et al.*, 1973; Ho, 1972, 1976; Sethuraj *et al.*, 1981; Premakumari and Panikkar, 1989). Moreover the radius of latex vessels has been used as an important variable to ascertain the laticifer area index, the potential quantitative anatomical parameter being used for breeding and selection programmes (Gomez *et al.*, 1972).

Studies conducted by Frey-Wyssling (1930) and Riches and Goodding (1952) related the influence of the diameter of latex vessels on the rate of flow of latex during

tapping and stressed that the volume of latex is directly proportional to the radius of latex vessels. In nine year old *Hevea* clones, Premakumari *et al.*, (1985) recorded the diameter of latex vessels which range from 16.6 μm to 26.87 μm, whereas Gomez (1982) recorded the diameter with in the range of 21.60 - 29.90 μm in mature trees of eight Malaysian clones.

2.3.8 Laticifer area index

Considering various factors pertaining to tapping, Gomez *et al.*, (1972) worked out an index called 'laticifer area index' using the formula $nfG\ \pi r^2$, where 'n' is the number of laticifer rows; 'f' is density of laticifers; 'G' is tree girth; and 'r' is the radius of latex vessels. Laticifer area index has been used as an important parameter to find out the total cross sectional area of laticifers cut open during tapping. Premakumari *et al.*, (1993b) noticed significant clonal variability in the laticifer area index in *Hevea* clones. Reghu *et al.*, (1996) recorded higher laticifer area index in wild *Hevea* germplasm than that of RRII 105 and GT 1. Premakumari *et al.*, (1993a) also reported the positive relationship of laticifer area index with yield.

2.3.9 Phloic rays

Premakumari *et al.*, (1984) reported negative correlation of ray width with latex vessel density. Significant increase in ray height in drought tolerant trees has been reported by Premakumari *et al.*, (1993 a). Ray height has been identified as a distinguishable character in various anatomical investigations, especially for the classification of different species within the genera (Magistris, 2001). In certain Oak species, Trockenbrodt (1994) observed a positive relation between the age of tree and ray height. Significant clonal variability in the height/width ratio of phloic rays between virgin bark and renewed bark has been reported earlier (Premakumari *et al.*, 1992).

2.3.10 Sieve tube

Sieve tubes are the most important transporting system in the secondary phloem (Bel *et al.*, 2002) mainly related to the assimilation of photosynthates and other substances (Turgeon, 2000; Schmitz and Schneid, 1989; Nakamura *et al.*, 2004). Many angiosperms have long sieve tubes with oblique sieve plate (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). Occurrence 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 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 15 *Hevea* clones and reported significant correlation with rubber production. The relationship between the diameter of sieve tubes and yield has also been well established (Fernado and Tambiah, 1970; Gunnery, 1935). 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 tubes. Chavan, *et al.*, (2000) reported that in dicotyledonous species two or more companion cells are attached to long sieve tubes.

2.3.11 Stone cells

In the early development of the virgin bark, the phloem fibres coalesce to form lignified stone cells. Group of highly lignified parenchyma distributed in various zones of bark is also termed as stone cells. The hardness of the bark depends on the quantity of stone cells present (Gomez, 1982). In *Hevea* bark, the formation of stone cells has been reported as a clonal character (Premakumari *et al.*, 1993b).

2.4 Histochemistry

The chemical constituents of wood and bark tissues are extremely complex due to the fact that the respective tissue systems are made up of many chemical constituents which are not distributed in uniform pattern. Hence histochemical methods help to obtain some insight in the chemical process and metabolic status within the tissue (Stevens, 1975). The distribution of metabolites such as starch, lipids, proteins and conversion of these reserve metabolites in to extraneous materials like polyphenols, tannins etc. in bark tissue certainly have some influence on the structural development of the secondary phloem.

Insoluble polysaccharides are mostly cell wall deposits and lignins which are polymeric compounds deposited in the matrix of cellulose microfibrils of the cell wall which give mechanical strength, increased sap conduction, defence mechanism and imperviousness to bio degradation (Cote, 1977). It has been reported that the phloem tissues in conifers accumulates polyphenols in response to mechanical wounding, fungal infection and insect attack (Franceschi *et al.*, 1998; 2000; Nagy *et al.*, 2000).

Studies on the anatomical and histochemical aspects of bark regeneration in *H. brasiliensis* (Thomas *et al.*, (1995) reported the occurrence of phenolics and tannin in

phloic rays and axial parenchyma, especially in the outer region of both virgin and renewed bark. Fay *et al.*, (1989) reported that bark regeneration involves the replacement of new tissue at the site of injury which modifies the initial structure. Thomas *et al.*, (1995) also reported the occurrence of starch in certain parenchymatous tissue adjacent to the cambial zone and lignification in all types of phloic elements including laticifers. Except these limited reports the survey of literature revealed that the information on the histochemical status of *Hevea* bark is very scanty.

Chapter 3

Materials and Methods

3.1. Materials

Ten Wickham clones of *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg., were selected from the Germplasm gardens I, II and III, and were planted during 1977, 1979 and 1981, respectively, at the Central Experimental Station of Rubber Research Institute of India, Chethekal, Ranni, Kerala. The experimental station is situated at 9° 22' N latitude and 76° 50' E longitude with an altitude of 80m above the MSL. These germplasm gardens comprised of 102 Wickham clones, planted in Randomised Block Design (RBD) with three replicates and three trees per plot. The trees were under regular tapping and had an age of 17-21 years.

In addition to this, seedling trees from two cross combinations of Wickham clones Vs Wild Brazilian germplasm accessions and budded clones of RRII 105 and RRIM 600 were also selected from the progeny evaluation trial, of age 4 years established at the Rubber Research Institute of India, Kottayam. The details of the materials selected for the present study are described in Table-2.

Table 1: Details of materials selected

Sl. No	Wickham clones	Age (in years)	Origin/Parentage
1	Tjir 1	21	Primary clone evolved by Tjirandji Estate, Indonesia
2	Gl 1	21	Primary clone evolved by Glenshiel Estate, Malaysia
3	PB 86	21	Primary clone evolved by Prang Besar Estate, Malaysia
4	GT 1	21	Primary clone evolved by Gondang Tapen Estate, Indonesia
5	PB 28/59	21	Primary clone evolved by Prang Besar Estate, Malaysia
6	RRII 105	19	Hybrid clone (Tjir 1 x Gl 1) evolved by Rubber Research Institute of India
7	RRIM 600	19	Hybrid clone (Tjir 1 x PB 86) evolved by Rubber Research Institute of Malaysia
8	RRIM 703	19	Hybrid clone (RRIM 600xRRIM 500) evolved by Rubber Research Institute of Malaysia
9	PB 235	21	Hybrid clone (PB 5/51x PB 5/78) evolved by Prang Besar Estate
10	RRII 300	17	Hybrid clone (Tjir I x PR 107) evolved by Rubber Research Institute of India
Seedling plants (Wickham x Brazilian germplasm)			
1	Seedling Progeny	4	Hybrid progeny, (RRII-105 x MT 1005)
2	Seedling Progeny	4	Hybrid progeny, (RRIM –600 x AC 495)
Budded plants (Wickham clones)			
1	RRII 105	4	Hybrid clone (Tjir 1 x Gl 1) evolved by Rubber Research Institute of India
2	RRIM 600	4	Hybrid clone (Tjir 1 x PB 86) evolved by Rubber Research Institute of Malaysia

2. Methodology

2.1 Selection of trees

Nine mature trees from each clone (three trees per replication) and eight plants from seedling progenies and budded plants (four plants from each progenies) in the juvenile phase were selected, to study the structure of bark.

Three mature trees from each clone (one tree per replicate) were selected to study histochemical parameters.

3.2.2 Collection and processing of bark samples

To study the orientation and inclination of laticifers / phloic elements, virgin bark samples were collected from the selected trees at 150 cm height (for mature trees) and 20-30 cm height (for seedling plants) from the ground. The sampling method reported by Gomez (1967) with certain modifications was adopted as described in Fig.1. A vertical line was drawn on the tree trunk along the longitudinal axis of the tree (Fig. 1 a). One of the cutting edges of the bark sampler was placed parallel along the vertical line (Fig 1 b) and the bark samples (Fig. 1 c) of the size 2 x 2 cm and 2 x 3 cm were collected. Immediately after sampling, a marking was made on the sampled bark by cutting on the right top corner (Fig. 1 d) to maintain the orientation of the bark sample on the tree. The samples collected were fixed in formalin-acetic -alcohol (FAA) and were sectioned at 30 – 60 µm thickness at different planes *viz.* cross sectional (CS), tangential longitudinal (TLS) and radial longitudinal (RLS) plane, using Reichert Jung sledge microtome. Sections were stained with Oil Red O (Omman and Reghu, 2003) and mounted in 50% glycerin and the micro slides (Fig. 1 e) were prepared by maintaining the orientation of the tissues as in the tree.

3.2.3 Method of observation

The bark sections were observed under Leitz Aristoplan Research microscope attached to Leica Q 5000 I W Image Analysis System. The images of the bark sections documented in the Image Analysis System were used to measure the inclination of laticifers / phloic rays and other anatomical traits by means of Leica Q Win V.2.1 Image analysis software.

The TLS of the bark were used to measure the inclination, density and diameter of laticifers and frequency of interconnections. Cross section and RLS were used to count

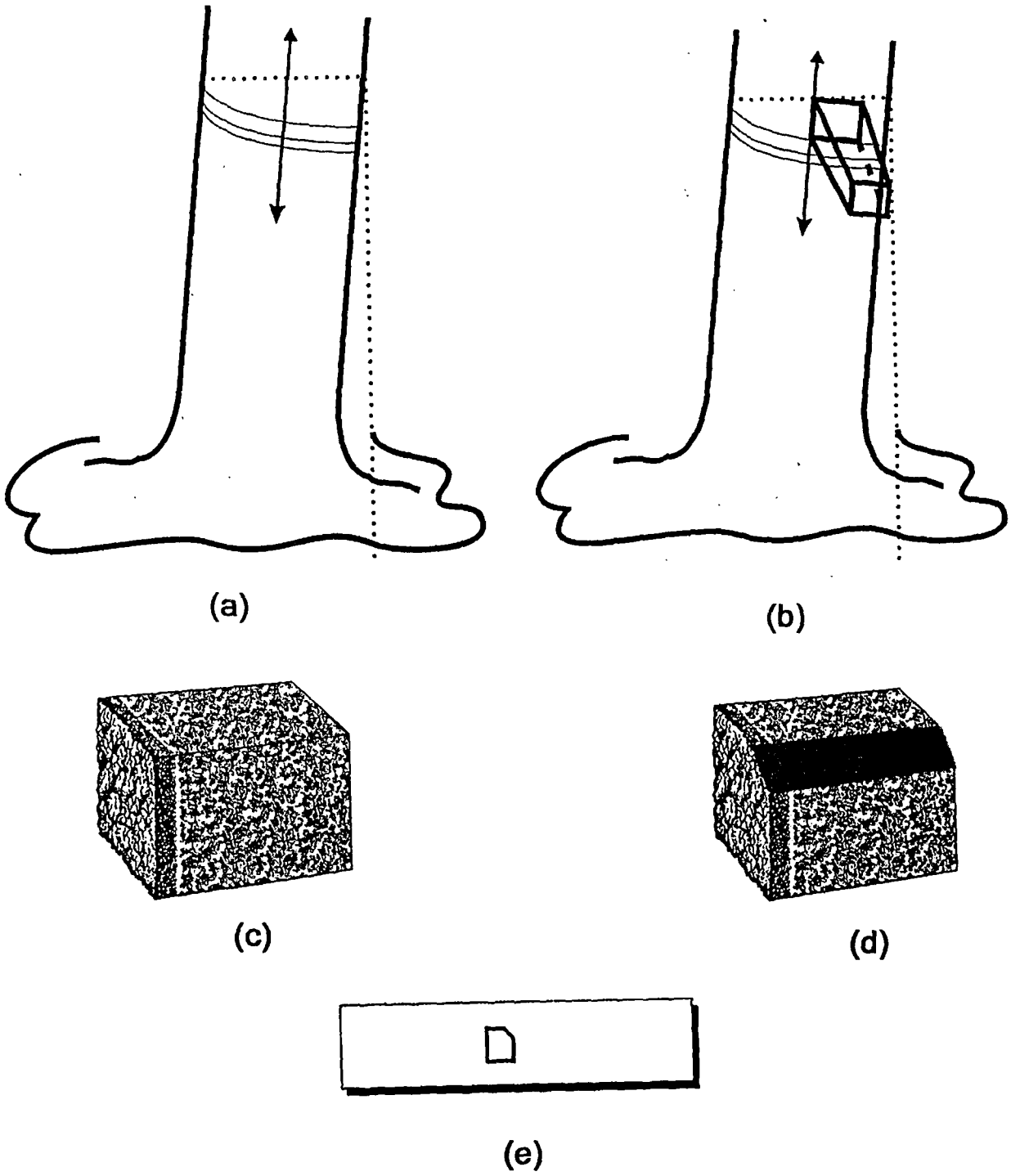


Figure 1. Method of bark sampling and mounting of sections. a. vertical line drawn on tree trunk along the longitudinal axis. b. bark sampler placed parellel along the vertical line. *c.* collected bark sample *d.* a cutting made on the corner of the bark sample. e. Mounting of sections on the slides maintaining the orientation of the tissue.

the number of laticifer rows, inter row distances, area occupied by stone cells and thickness of soft / hard bark. For each anatomical parameter, observations from ten microscopic fields were taken per plant.

3.3. Characters studied

3.3.1 Leaning angle of trees

3.3.2 Tree girth : Measured at 150 cm height from the ground.

3.3.3 Total bark thickness (mm): The sum of soft bark and hard bark thickness measured at 150 cm height. The thickness of the hard bark was further sub divided into the inner hard bark and outer hard bark thickness:

3.3.3.1 Soft bark (SB) thickness: The distance from the cambial zone outward upto the zone of initiation of stone cells

3.3.3.2 Inner hard bark (IHB) thickness: The distance from the inner most layer of stone cells to the inner most row of functional latex vessel.

3.3.3.3 Outer hard bark (OHB) thickness: The distance from the innermost functional laticifers to the remaining outermost hard bark zone.

3.3.4 Number of latex vessel rows in the soft bark and hard bark

3.3.5 Average distance between adjacent laticifers rows in SB and IHB (mm)

3.3.6 Average distance between the cambium to the 1st row of latex vessel (mm)

3.3.7 Total density of latex vessels per row per 1mm distance

3.3.7.1 Density of latex vessel contiguous to rays

3.3.7.2 Density of latex vessels non-contiguous to rays

3.3.8 Frequency of interconnections between laticifers ($5x10^{-2}$ mm^2 area)

3.3.9 Diameter of latex vessels (μm)

3.3.10 Total cross sectional area of latex vessels (Laticifer area index): The total cross sectional area of the latex vessels at a given CS of the bark (Laticifer Area Index) was computed as per the following formula (Gomez *et al.*, 1972).

Total cross sectional area of latex vessels = nfG (πr^2)

Where n is the total number of latex vessel rows

f = density of latex vessels per row per 1mm circumference of the tree

G = girth of the tree (cm)

r = radius of latex vessel

3.3.11.1 Angle of inclination of laticifers in SB

3.3.11.2 Angle of inclination of laticifers in IHB

3.3.12.1 Angle of inclination of phloic rays in SB

3.3.12.2 Angle of inclination of phloic rays in IHB

3.3.13.1 Frequency of phloic rays contiguous to latex vessels per unit distance (765 µm) in TLS of SB and IHB

3.3.13.2 Frequency of uni-, bi- and multiseriate rays contiguous to latex vessels per unit distance (765 µm) in TLS of SB and IHB

3.3.14.1 Frequency of phloic rays in latex vessel free zone per unit distance (765 µm) in TLS of SB and IHB

3.3.14.2 Frequency of uni-, bi- and multiseriate rays in latex vessel free zone per unit distance (765 µm) in TLS of SB and IHB

3.3.15.1 Height and width of phloic rays (µm) contiguous to latex vessels in TLS of SB and IHB.

3.3.15.2 Height and width of phloic rays (µm) in latex vessel free zone in TLS of SB and IHB.

3.3.16.1 Height / width ratio of phloic rays contiguous to latex vessels in TLS of SB and IHB.

3.3.16.2 Height / width ratio of phloic rays in TLS of SB and IHB

3.3.17 Length and diameter of sieve tubes (µm)

3.3.18 Number of stone cell rows in IHB

3.3.19 Area occupied by stone cells per 255 x 10^{-3}mm^2 CS area in IHB and OHB..

3.4 Histochemical studies

The following staining methods and histochemical tests were employed using sledge microtome sections of the bark at 30 – 60 µm thickness.

3.4.1 **Starch**: Iodine-Potassium iodide (Johansen, 1940)

3.4.2 **Total polysaccharides**: Periodic acid – Schiff's (PAS) reagent (Ruzin, 1999).

3.4.3 **Lipids**: Sudan Black B (Ruzin, 1999).

3.4.4 **Total protein**: Mercuric-Bromophenol (Mazia *et al.*, 1953)

3.4.5 **Phenols**: Tannin acid-ferric chloride (Mace, 1963)

3.4.6 **Tannin**: Ferric sulphate (Rawlins and Takahashi, 1952).

3.4.7 **Lignin**: Phloroglucin -HCl. (Purvis *et al.*, 1964; Ruzin, 1999)

3.5 Statistical analysis

The following statistical analysis were carried out (Gomez and Gomez, 1983; Panse and Sukhatme, 1985):

3.5.1 Coefficient of variation (CV) was calculated to ascertain the tree-to-tree variation within clones. Mean values were pooled to find out the CV values. The CV was not calculated wherever the data was absent / insignificant. The variation within trees was taken as low, medium and high with respect to the CV values. For example 0 to 30 was taken as low, 31 to 50 as medium and 51 and above as high.

3.5.2 Correlation : Simple correlation was worked out to findout the relationship among themselves and also between different characters.

3.5.3 Analysis of variation (ANOVA) was estimated to measure the extend of clonal variation between different clones.

3.5.4 Regression analysis was done to find out the effect of various independent variables and their associated influence on a dependent variable (the latex vessels inclination)

For statistical analysis of data ,softwares of excel (MS office) and SPSS 10 were used.

3.6 Photomicrography

Photomicrographs were taken in Leitz Aristoplan Research microscope attached to Wild MPS 46 Photo Automat using Kodak Gold 35mm colour film.

3.7 Image analysis: Quantitative image analysis was done using Leica Q Win V.2.1 Image analysis software.

Chapter 4

Results

4.1 Anatomy of bark

The commercial exploitation of natural rubber *H. brasiliensis* (Para Rubber) is being carried out by the systematic excision of the bark tissues. *Hevea* bark is composed of concentric layers of sieve tubes, companion cells, phloem fibers, parenchymatous tissues and network of latex vessels. The latex is formed in latex vessels, which are oriented in the bark tissue in a specific pattern. Structurally the bark consists of three zones *viz.* (i) the inner soft bark region contiguous to cambium, (ii) the middle hard bark zone consisting of functional latex vessels termed as inner hard bark (iii) the outer hard bark zone. (Fig. 2).

Laticiferous system is formed as concentric ring of tubes differentiated from the fusiform initials of the cambium. In each ring, the individual vessels are arranged in the form of cylindrical meshwork of tubes. At many points the adjacent latex vessels within a row are interconnected to form the anastomous network like structure. During the growth period of the plant, the successive rows of latex vessels are differentiated from the cambium along with other phloic elements. The meristematic activity of the cork cambium (phellogen) and the formation of sclerified stone cells also exerted pressure leading to the disorganization of laticifers in the hard bark to a great extend.

4.2 Leaning of trees

The leaning angle of trees (slope) of all the clones showed considerable variation within the range of 0.08 to 0.22. The mean value for this character was the highest in RRIM 703 (0.22), followed by RRIM 600 (0.17); RRII 105 (0.13); Gl 1, PB 28/59, PB 86 (0.12); Tjir 1 (0.11) and lowest in GT 1, PB 235 and RRII 300 (0.08). Of the ten clones studied, three clones *viz.* RRIM 703, RRIM 600 and PB 28/59, showed medium tree to tree variation, whereas the rest of them had high tree to tree variation as revealed from the CV values. The analysis of variation indicated that the clonal variation for this trait was not statistically significant (Table 2).

4.3 Tree girth

The mean tree girth was the highest in PB 235 (128.22 cm) and lowest in RRII 105 (79.11 cm). Four clones (PB 28/59, Tjir 1, GT 1 and PB 86) had the girth ranging from 109.25 - 101.67 cm whereas the clones RRIM 703, RRIM 600, Gl 1 and RRII 300, showed the mean girth values within the range of 87.22 – 95.22 cm. The tree-to-tree variation was low in all the clones. Analysis of variance indicated that the clone PB 235 was statistically superior to eight clones *viz.* Tjir 1, GT 1, PB 86, RRII 300, Gl 1, RRIM 600, RRIM 703 and RRII 105 (Table 2). Clones PB 28/59, Tjir 1 and GT 1 were statistically superior to RRII 105.

4.4 Total bark thickness (TBT)

The total bark thickness (TBT) represents the sum of soft bark (SB) and hard bark thickness. Similarly the hard bark thickness was the sum of the thickness of inner hard bark (IHB) and outer hard bark (OHB).

The TBT varied considerably in all the ten clones, and the value was highest in RRIM 703 (13.28 mm) and lowest in RRII 300 (9.06 mm). The TBT was towards the higher side in

Tjir 1 (11.89 mm), GT 1 (11.83 mm) and PB 28/59 (11.56 mm), PB 235 (10.89 mm), PB 86 (10.33 mm) and Gl 1 (10.11 mm) and towards the lower side in RRII 105 (9.78 mm) and RRIM 600 (9.67 mm). All the clones showed low tree to tree variation for this character. ANOVA indicated that RRIM 703 was statistically superior to PB 235, PB 86, Gl 1, RRII 105, RRIM 600 and RRII 300 (Table 2). Similarly the clones Tjir 1 and GT 1 were superior to RRII 105, RRIM 600 and RRII 300 for total bark thickness.

4.4.1 . Soft bark thickness (SBT)

In general, the thickness of the SB was relatively lower than that of the thickness of IHB and OHB zones. The thickness of the SB was maximum (2.24 mm) in PB 28/59 (Fig. 3 a) followed by PB 235 (2.08); RRII 105 (1.90 mm); Tjir 1 (1.77 mm), RRIM 600 (1.57 mm); Gl 1(1.55 mm); RRII 300(1.46 mm); GT 1(1.26 mm); RRIM 703(1.23 mm) and minimum (1.10 mm) in PB 86 (Fig. 3 b). High tree-to-tree variation was observed in RRII 105 whereas low in Tjir 1, Gl 1 and RRIM 600. Rest of the clones had medium tree-to-tree variation. The variation in the thickness of SB region among the clones was not significant (Table 2).

4.4.2 Inner hard bark thickness (IHBT)

The thickness of inner hark bark was the highest in PB 235 (3.82 mm) and lowest in RRII 300 (1.54 mm) (Table 2). The clone PB 86 (3.75 mm) ranked next to PB 235 for this trait. Tree to tree variation was higher in RRII 105 and PB 28/59 and lower in PB 86, RRIM 600 and RRIM 703. Rest of the clones had medium tree to tree variation. The clones PB 235 and PB 86 were statistically superior to RRII 105, RRIM 600, Gl 1, PB 28/59, Tjir 1 and RRII 300 (Table 2). Clones GT 1 and RRIM 703 also showed significant superiority over Tjir 1 and RRII 300.

4.4.3 Outer hard bark thickness (OHBT)

The OHBT (Table 2) was maximum in RRIM 703 (9.15 mm) followed by Tjir 1 (8.29 mm), GT 1 (7.55 mm), PB 28/59 (7.27 mm), Gl 1 (6.38 mm), RRII 300 (6.06 mm), RRIM 600 (5.62 mm), PB 86 (5.48 mm), RRII 105 (5.39 mm) and minimum in PB 235 (4.98 mm). PB 86 and RRII 105 showed medium tree-to-tree variation whereas all the other clones showed low level of variation. ANOVA (Table 2) depicted significant clonal variability where RRIM 703 and Tjir 1 were superior to Gl 1, RRII 300, RRIM 600, PB 86, RRII 105 and PB 235. GT 1and PB 28/59 were superior to PB 235.

4.5 Number of laticifer rows in SB and IHB

The number of latex vessel rows in SB region (Table 3) was the highest (20.06) in PB 28/59 (Fig. 3 c) and the lowest (6.89) in PB 86 (Fig. 3 d) . Within clones, the tree-to-tree variation was high in GT 1 and RRII 105 and medium in rest of the clones. ANOVA indicated that PB 28/59 was statistically superior to RRIM 600, RRIM 703, RRII 300, GT 1 and PB 86; RRII 105 was superior to RRIM 703, RRII 300, GT 1 and PB 86; and PB 235 was statistically superior to GT 1 and PB 86 (Table 3).

The number of latex vessel rows in the IHB (Table 3) was maximum (25.78) in PB 86 (Fig. 3e) and minimum (9.67) in RRII 300 (Fig. 3 f). The number of LV rows varied between trees in all the clones and variation was considerably high in RRII 300 and PB 28/59. The variation was medium in PB 235, GT1, RRII 105, Gl 1 and RRIM 600. Analysis of variation (Table 3) indicated that the clones PB 86, PB235 and RRIM 703 were significantly superior to RRIM 600 and RRII 300. GT 1, RRII 105 and Gl 1 were to RRII 300.

4.6 Distance between laticifer rows in SB and IHB

The average inter row distance between laticifers in both SB and IHB regions is shown in Table 3. In the SB region, the distance ranged from a lowest of 0.07 mm in PB 28/59 (Fig. 4a) and highest of 0.13 mm in PB 86 (Fig. 4 b). Within clones, tree to tree variation was high in RRIM 703 and RRII 300 and medium in PB 86, GT 1, RRIM 600 and PB 235. The remaining four clones *viz.* Gl 1, PB 28/59, RRII 105 and Tjir 1 showed very low tree-to-tree variation. Clonal variation was not significant.

The distance between adjacent laticifer rows in IHB was maximum in RRIM 600 and Tjir 1 (0.13 mm) and minimum in RRII 105 (0.07 mm). Within clones the trees exhibited medium variation in two clones *viz.* RRIM 600 and Gl 1 and the rest of them displayed low tree-to-tree variation. ANOVA (Table 3) revealed that RRIM 600, Tjir 1 and RRII 300 are superior to PB 28/59, GT 1, Gl 1, RRIM 703 and RRII 105. Similarly PB 235 and PB 86 also showed superiority over RRIM 703 and RRII 105.

4.7 Distance between cambium and first row of laticifers

The mean distance between cambium and the first row of laticifers is presented in Table 3. The distance was the highest in PB 235 (0.42 mm) and the lowest in Tjir1 (0.11 mm). Other clones had the mean distance ranged from 0.15 mm – 0.23 mm. Five clones *viz.* PB 235, RRIM 703, RRII 300, RRII 105 and Gl 1 depicted high CV values indicating high tree-to-tree variation. But rest of the clones had medium tree to tree variation. However the variation between clones was not significant (Table 3).

4.7.1. Density of latex vessels contiguous to rays

The density of latex vessels contiguous to rays (Table 4) was maximum (25.44) in GT 1 (Fig. 4 c) and minimum (22.73) in PB 235 (Fig. 4 d). In all the clones the tree-to-tree variation was very low. The clonal variation was statistically significant where the clones GT 1, Gl 1 and RRII 300 were superior to PB 86 and PB 235 (Table 4). Similarly the clones, Tjir 1, RRII 105, RRIM 600, PB 28/59 and RRIM 703 were also superior to PB 235.

4.7.2 Density of latex vessels non-contiguous to rays

The density of latex vessels non-contiguous to rays was considerably reduced in comparison to those latex vessels contiguous to rays in all the clones (Table 4). GT 1 (Fig. 4 e) and PB 86 recorded high density (4.29 and 4.28) and the lowest density (2.27) was noticed in RRIM 703 (Fig. 4 f). The tree-to-tree variation for this character was low in all the clones except in RRIM 703, which showed medium variation. However the clonal variability was statistically significant (Table 4). The clones GT 1, PB 86, RRIM 600 and PB 235 were statistically superior to Tjir 1, RRII 300, Gl 1, PB 28/59, RRII 105 and RRIM 703.

4.7.3 Total density of latex vessels

The total density of latex vessels per row per mm distance (Table 4) was the sum of the density of laticifers contiguous to rays and those non-contiguous to rays. It was maximum (29.73) in GT 1 and minimum (26.40) in RRIM 703. Within clones the tree-to-tree variation was not significant as revealed by low CV values. ANOVA for this character revealed significant clonal variability where GT 1, RRIM 600, Gl 1, PB 86, RRII 300 and Tjir 1 were statistically superior to RRIM 703 (Table 4).

4.8 Frequency of interconnections

The frequency of interconnections (Table 4) between adjacent latex vessels within a row was maximum (22.11) in RRII 105 (Fig. 5 a) and minimum (15.73) in PB 28/59 (Fig. 5 b). RRII 300 (20.94) occupied second position followed by GT 1 (19.59), RRIM 600 (19.58) and Tjir 1 (19.16). Tree to tree variation for the character was very low in all the clones. RRII 105 was statistically superior to eight clones *viz.* GT 1, RRIM 600, Tjir 1, PB 86, Gl 1, RRIM 703, PB 235 and PB 28/59 for thickness for this character (Table 4). Similarly RRII 300 exhibited superiority over PB 86, Gl 1, RRIM 703, PB 235 and PB 28/59; GT 1 was superior to PB 235, PB 28/59; and RRIM 600, Tjir 1, PB 86, Gl 1, RRIM 703 were superior over PB 28/59.

4.9 Diameter of latex vessels

The diameter latex vessels (Table 4) was maximum (25.92 μm) in PB 28/59 (Fig. 5 c) and minimum (21.63 μm) in RRIM 703 (Fig. 5 d). The low CV values indicated low level tree to tree variation for this trait. However, the analysis of variation revealed significant clonal variability. Five clones *viz.* PB 28/59, RRIM 600, Gl 1, RRII 300, PB 86 were superior over GT 1 and RRIM 703 (Table 5). Clone PB 235 and Tjir 1 statistically superior to RRIM 703.

4.10 Total cross sectional area of laticifers (Laticifer area index)

The total cross sectional area of latex vessels was highest in PB 235 (76.17) followed by PB 28/59 (58.50) and lowest in RRII 300 (27.62). Tree-to-tree variation was high in PB 235, whereas RRIM 600 and RRIM 703 exhibited low level of variation. However the remaining seven clones exhibited medium tree-to-tree variation. ANOVA for this character showed significant clonal variability where PB 235 and PB 28/59 were statistically superior to GT 1, Tjir 1, RRII 105, RRIM 600, RRIM 703 and RRII 300 (Table 4).

4.11.1 Angle of inclination of laticifers in SB

The laticifers showed varying degrees of inclination (Fig. 6a) in the SB region within the range 3.36 - 8.42° towards the right in six clones *viz.* RRIM 703, GT 1, RRII 300, Tjir 1, PB 235 and Gl 1 (Table 6). The angle of inclination was maximum (8.42°) in RRIM 703 (Fig. 7 a) and minimum (3.36°) in Gl 1 (Fig. 7 b). The rightward inclination of laticifers observed in other clones were 5.75° in GT 1(Fig. 7 c), 5.13° in RRII 300 (Fig. 7 d), 4.27° in Tjir 1 (Fig. 7 e) and 3.58° in PB 235 (Fig. 7 f).

The inclination of laticifers in four clones *viz.* PB 86, RRII 105, PB 28/59 and RRIM 600 were either towards right, left or even in both directions. Moreover, within these clones the individual trees showed varying degrees of laticifer inclination on either directions. For example seven trees of PB 86 showed leftward inclination with a mean angle of 4.27° (Fig. 7 g); one tree with rightward inclination (4.33°) and another tree with both leftward (1.15°) and rightward (1.08°) inclination (Fig. 7h). The clone RRII 105 depicted both left (2.10°) and rightward (3.24°) inclinations in eight trees (Fig. 8 a) and only rightward inclination in one tree (8.06°). In PB 28/ 59, six trees were noted with both left (1.61°) and rightward (4.01°) laticifer inclination (Fig. 8 b) and three trees were observed with only rightward inclination (4.21°) (Fig. 8 c). Five trees of the clone RRIM 600 had laticifers inclined towards both left and right (Fig. 8 d); three trees were noted with leftward (Fig. 8e) inclination (2.51°) and one tree exhibited rightward (Fig. 8 f) inclination (2.60°).

4.11.2 Angle of inclination of laticifers in the IHB

With respect to the inclination of laticifers in the IHB region (Table 6), six clones were found to have laticifers inclined exclusively towards the right (Fig. 6b) with a maximum degree of 8.73° in RRIM 703 (Fig. 9 a) and minimum of 3.58° in PB 235 (Fig. 9 b). In GT 1 the inclination

was 7.01° followed by RRII 300 (5.50°), Gl 1 (4.63°) and Tjir 1 (4.51°). Tree to tree variation for this trait was low in RRIM 703 and GT 1, medium in Tjir 1 and high in RRII 300 and Gl 1.

Four clones *viz.* PB 86, RRII 105, PB 28/59 and RRIM 600) exhibited left, right or towards both directions. In PB 86 (Fig. 9 c) seven trees showed laticifers inclined exclusively towards the left with a mean value of 4.42°. However one tree of this clone showed rightward inclination (3.20°) and another had laticifers inclined to left (0.08°) and right (1.30°). RRII 105 was noted with both left and rightward laticifer inclination in seven trees with a mean value of 2.42° to the left and 2.68° to the right (Fig. 9 d). Two trees were having rightward inclination with the mean value 7.15°. In PB 28/59, four trees showed rightward inclination with the mean value 6.24° and five trees with inclination towards both left and right (Fig. 9e). The clone RRIM 600 exhibited inclination of laticifers towards both directions. Three trees showed inclination of laticifers towards left and three towards right (Fig. 9f) and three trees were noted the inclination towards both directions.

4.11.3 Angle of inclination of phloic rays in SB

The phloic rays of six clones (RRIM 703, GT 1, RRII 300, Tjir 1, PB 235 and Gl 1) showed inclination exclusively towards the right (Table 7). Among these RRIM 703 (Fig. 10 a) recorded the maximum rightward inclination (7.13°) followed by GT 1 (6.88°), RRII 300 (5.27°), PB 235 (3.59°), Tjir 1(3.50°) and minimum in Gl 1 (3.09°) (Fig. 10 b). The magnitude of tree to tree variation for this trait within clones was high in RRII 300, PB 235 and Gl 1.

The phloic rays were inclined towards left, right or towards both directions in four clones *viz.* PB 86, RRII 105, PB 28/59 and RRIM 600. PB 86 showed leftward inclination of rays

(Fig. 10c) in seven trees with a mean value of 5.21°. One tree with right (7.30°) and another with both left (2.21°) and rightward inclination (1.18°) were also noticed. In RRII 105 seven trees had rays inclined towards both directions (Fig.10 d). Two trees showed rightward inclination of rays at 5.45°. In clone PB 28/59, four trees showed left and rightward inclination with the mean value of 1.73° and 1.28°, respectively. The other five trees recorded rightward inclination of phloic rays. In three trees of RRIM 600, the rays were inclined towards the left (Fig. 10e) with a mean inclination of 1.61°, five trees with left and rightward inclination and one tree with rightward inclination were also noted.

4.11.4 Angle of inclination of phloic rays in the inner hard bark

Table 8 depicts the angle of inclination of phloic rays in IHB zone. Among the clones studied, six of them showed inclination of rays towards the right *viz.* RRIM 703, GT 1, RRII 300, PB 235, Tjir 1 and Gl 1 and four clones *viz.* PB 86, RRII 105, PB 28/59 and RRIM 600 had rays towards left, right or towards both directions.

The angle of inclination of rays towards the right was the highest (Fig. 10f) in RRIM 703 (8.95°) and the lowest in Gl 1 (3.40°). Other clones like GT 1, RRII 300, PB 235 and Tjir 1 recorded rightward inclination with the mean values 6.64°, 5.78°, 3.89° and 3.57°, respectively. Tree to tree variation for this character was higher in RRII 300 and Gl 1; medium in Tjir 1 and PB 235 and lower in RRIM 703 and GT 1.

Seven trees of PB 86 exhibited only leftward inclination (Fig. 10 g) of phloic rays with an average angle of 4.24°. One tree showed rightward inclination (3.25°) and another one showed both right (1.08°) and leftward (2.00°) inclination of rays. Whereas in RRII 105, six trees exhibited both left (3.20°) and rightward (3.59°) inclination (Fig. 10h) and three of them showed only

rightward (4.13°) inclination. In PB 28/59, four trees had both left and rightward inclination with the mean values of 1.55° and 3.18°, respectively. Other five trees with rightward inclination had an average angle of 6.38°. The clone RRIM 600 showed a mixed pattern of ray inclination of both left (2.00°) and right (0.85°) in three trees. Three trees of this clone showed only rightward (3.20°) inclination and another three trees had leftward (3.00°) inclination.

4.11.5 Inclination of laticifers and phloic rays at the juvenile stage

Inclination of laticifers was observed towards the right at 3.84° in the seedling progenies of the cross combination RRII 105 x MT 1005. Whereas in the progenies of the cross RRIM 600 x AC 495, the angle of inclination was 2.55° towards the right. Similar type of rightward inclination at 5.01° was also observed in the young budded plants of RRII 105. In the case of young buddings of RRIM 600, mixed pattern of inclination was noticed, where three plants depicted rightward inclination (3.30°) and one plant with 2.14° leftward inclination (Table 6).

In both the seedling progenies, the phloic rays showed rightward inclination within a range 2.69°- 3.15°. Similar result was also recorded in the young buddings of RRII 105, but the angle of inclination was slightly higher (5.62°) than that of the seedling progenies. Whereas in RRIM 600, the phloic rays of three plants had 3.60° rightward inclination and one plant with leftward inclination of 1.75° (Table 7).

4.12.1 Frequency of uni-; bi-; and multi-seriate phloic rays contiguous to laticifers in SB and IHB

In the SB region the frequency of uniseriate rays was maximum in RRII 105 (1.88) and minimum in RRIM 600 (0.69) (Fig. 11). As the uniseriate rays were very few in trees of all clones the tree to tree variation was not worked out. Similarly, ANOVA also showed non-significant clonal variability (Table 9).

Frequency of biseriate rays is given in Table 9. The mean value was maximum in GT 1 (1.17) and in three clones *viz.* PB 86, Tjir1 and RRII 300 had a uniform minimum frequency (0.06) of biseriate rays. Biseriate rays were absent in three clones *viz.* RRIM 600, RRII 105 and PB 235. Due to the absence of biseriate rays in many trees, CV values were not worked out. ANOVA for this character indicated significant clonal variability where GT 1 was statistically superior to all clones except PB 28/59. The clone PB 28/59 showed superiority over PB 86, Tjir 1, RRII 300, RRIM 600, RRII 105 and PB 235 (Table 9).

Maximum frequency of multiseriate rays was noticed in PB 235 (7.00) and minimum in RRII 105 (5.98). Frequency of multiseriate rays did not show significant clonal variation as well as tree to tree variation (Table 9).

Occurrence of uniseriate rays contiguous to laticifers per unit distance in was considerably reduced in IHB. In RRII 105, RRII 300 and GT 1 were noted with uniseriate rays (Table 9). ANOVA showed that the clonal variability was not statistically significant (Table 9).

The frequency of biseriate rays in this region was maximum in GT 1 (0.17) and minimum in RRII 105 (0.03). The clonal variations for this character was not significant (Table 9).

The frequency of multiseriate rays was maximum in GT 1 (6.56), followed by PB 28/59 (6.23), Tjir 1 (6.22) and minimum (5.22) in Gl 1 (Table 9). Tree to tree variation within clones was not significant as obvious from the low CV values. None of the clones showed significant clonal variation (Table 9).

4.12.2 Total frequency of phloic rays contiguous to latex vessels in SB and IHB

Total frequency of phloic rays was the sum of uni, bi and multiseriate rays. Rays contiguous to LV in SB zone (Table 9) recorded the maximum number (8.58) in GT 1 and minimum

(7.39) in RRIM 703. Tree to tree variation within clones was low as revealed by the low CV values. ANOVA indicated significant clonal variation. GT 1 and RRII 300 were statistically superior to PB 86, RRIM 600 and RRIM 703. Clone PB 28/59 also had superiority over RRIM 600 and RRIM 703 (Table 10).

The ray frequency was considerably reduced in IHB region compared to SB region (Table 9). The frequency of phloic rays contiguous to LV in the inner hard bark zone was maximum in GT 1 (6.83) and minimum in Gl 1 (5.22). Tree to tree variation was very low. Analysis of variance indicated that the clonal variation was not statistically significant (Table 9).

4.13.1 Frequency of uni-; bi-; and multiseriate rays in latex vessel free zone in SB and IHB

The occurrence of uniseriate rays in SB was observed in all the clones except RRIM 703 and its frequency was higher in RRII 300 (1.50) and lower in PB 86 (0.22) (Fig. 11). However, the clonal variation was not significant as revealed by the analysis of variance (Table 10).

Biseriate rays were maximum in RRIM 703 (0.78) and minimum in PB 86 (0.06) (Table 10). Such rays were absent in many of the trees and hence the tree-to-tree variation was not worked out. The clones RRIM 703, PB 28/59 and GT 1 were statistically superior to the rest of the clones for this trait (Table 10).

The frequency of multiseriate rays was the highest in Tjir 1 (7.18) and the lowest in Gl 1(5.58). Tree to tree variation was not significant in all the clones. Tjir 1 was observed with significant clonal superiority for this character over PB 86, RRIM 703, RRII 105, PB 28/59, RRII 300 and Gl 1 (Table 10). The clones PB 235, RRIM 600 and GT 1 were statistically superior to RRII 300 and Gl 1; and PB 86 and RRIM 703 were superior to Gl 1.

The frequency of uniseriate rays was drastically reduced in IHB. Only two clones, GT 1 (0.22) and PB 86 (0.11) were accounted for the presence of uniseriate phloic rays in low frequency in the LV free zone (Table 10). The tree-to-tree variation and clonal variation were insignificant (Table 10). None of the clones showed the presence of biseriate rays.

The frequency of multiseriate rays is presented in Table 10. Clones PB 86 (6.28), GT 1 (6.11) and PB 28/59 (6.08) were ranked top for this character. Gl 1 recorded the minimum value (5.00). Tree to tree variation was very low. ANOVA indicated that the clonal variation was highly significant (Table 10). PB 86 exhibited marked superiority over RRII 300, Tjir 1, RRIM 703, RRIM 600, RRII 105 and Gl 1. Likewise GT 1 showed superiority over RRIM 703, RRIM 600, RRII 105 and Gl 1; and PB 28/59 over RRIM 600, RRII 105, Gl 1. The clones PB 235 and RRII 300 were also showed superiority over Gl 1.

4.13.2 Total frequency of phloic rays in LV free zone in SB and IHB

The total frequency of rays in laticifer free zone showed considerable variation among clones (Table 10). A highest frequency of was observed in Tjir 1 (8.06), and lowest in Gl 1 (6.17). The tree-to-tree variation within clones was negligible. Clones Tjir 1 and GT 1 had superiority for this character over RRIM 703, PB 86 and Gl 1 (Table 10). PB 235 showed superiority for this trait over PB 86 and Gl 1. Similarly five clones *viz.* RRII 105, RRII 300, PB 28/59, RRIM 600 and RRIM 703 also showed superiority for this trait over Gl 1.

The total frequency of phloic rays in laticifer free zone in IHB is given in Table 10. The frequency was highest in PB 86 (6.39) and lowest in Gl 1 (5.00). None of the clones showed significant tree-to-tree variation. ANOVA (Table 10) indicated significant clonal superiority by PB 86 and GT 1 over seven clones *viz.* PB 235, RRII 300, Tjir 1, RRIM 703,

RRIM 600, RRII 105 and Gl 1. Like wise PB 28/59 was statistically superior to RRIM 600, RRII 105 and Gl 1; and PB 235 and RRII 300 were superior to Gl 1.

4.14.1 Height and width of phloic rays contiguous to laticifers in SB and IHB

The height of rays contiguous to laticifers in SB varied considerably in all the clones (Fig. 12). The maximum (Fig. 13 a) ray height was observed in PB 235 (400.64 µm) and minimum (Fig. 13b) in RRIM 703 (292.55 µm). Tree to tree variation within clones was considerably reduced. Analysis of variance indicated that PB 235 was significantly superior to seven clones *viz.* PB 28/59, RRII 300, Tjir 1, Gl 1, RRII 105, RRIM 600 and RRIM 703 (Table 11). Similarly GT 1 was statistically superior to RRII 105, RRIM 600 and RRIM 703; and PB 86 was superior to RRIM 703.

The width of phloic rays in the SB region was maximum in RRII 105 (46.71 µm) and minimum in PB 235 (36.10 µm) (Table 11). The tree to tree variation within clones for this character was very low. ANOVA indicated that the clonal variation was insignificant.

The height of the rays was considerably reduced in IHB. Within the clones the ray height was maximum in PB 86 (382.94 µm) and minimum in RRIM 703 (267.03 µm) (Table 11). Tree to tree variation for ray height within clones was very low. ANOVA revealed that PB 86 was statistically superior to RRII 300, Tjir 1, RRII 105, RRIM 600 and RRIM 703 (Table 11) and GT 1 was superior to RRII 105, RRIM 600 and RRIM 703. The clones Gl 1, PB 28/59 and PB 235 showed superiority over RRIM 703.

The width of phloic rays in IHB was maximum (72.85 µm) in Gl 1 (Fig. 13 c) and minimum (54.25 µm) in GT 1 (Fig. 13 d). The CV values did not depict any marked tree-to-tree variation. Similarly the variation between clones was also not significant (Table 11).

4.14.2 Height and width of phloic rays in laticifer free zone in SB and IHB

Height of rays in laticifer free zone of the SB region showed considerable variation among the ten clones (Table 12). Ray height was higher (Fig. 13 e) in PB 235 (387.87 µm) and lower (Fig. 13 f) in RRIM 703 (307.30 µm). Tree to tree variation within clones was low in all the clones. ANOVA revealed significant clonal superiority by PB 235, Gl 1 and PB 86 over RRIM 600, RRII 300, PB 29/59, Tjir 1, RRII 105 and RRIM 703 (Table 12). Superiority of GT 1 over RRIM 703 was also noticed for ray height.

The width of phloic rays in SB region was higher in Gl (49.39 µm) and lowest in GT 1 (34.05 µm). Within clones the tree-to-tree variation was low. ANOVA indicated that the clonal variability was not significant (Table 12). An increase in ray height associated with a corresponding increase in ray width was noticed in PB 235 and PB 86, whereas the trend was just the reverse in RRII 105.

The height of rays in IHB region is depicted in Table 12. Comparatively the height of rays was the highest in Gl 1 (380.51 µm) and the lowest in RRIM 703 (277.03 µm). The tree-to-tree variation within clone was relatively low. Analysis of variance revealed that the difference in ray height between clones were statistically significant where Gl 1 was superior over GT 1, PB 28/59, Tjir 1, PB 235, RRIM

600, RRII 300, RRII 105 and RRIM 703 (Table 12). Likewise the clone PB 86 was also statistically superior to RRII 300, RRII 105 and RRIM 703 for this trait.

The width of the rays was increased in this zone with the maximum value of 78.04 µm in Gl 1 and minimum of 52.88 µm in GT 1 (Table 12). Within clones the variations in ray width were negligible. Clonal variation for this character was statistically significant (Table 12). Clone GT 1 was statistically superior to PB 28/ 59, RRII 300, PB 235, Tjir 1, RRIM 703, PB 86 and GT 1. RRII 105 showed superiority over Tjir1, RRIM 703, PB 86 and GT 1. Like wise RRIM 600, PB 28/ 59 and RRII 300 showed superiority over PB 86 and GT 1; and PB 235 and Tjir 1 were superior to GT 1.

4.15.1 Height/width ratio of phloic rays contiguous to laticifers in SB and IHB

The height/width ratio of rays contiguous to laticifers was higher in SB region and lower in the IHB regions of the bark tissue (Fig. 12). In SB region the ratio was maximum in PB 235 (11.09) followed by GT 1 (10.50) and minimum in RRII 105 (6.44) (Table 11). None of the clones showed significant tree-to-tree variation for this trait. ANOVA revealed significant clonal variability, where PB 235 was statistically superior to PB 28/59, Gl 1, RRIM 600, RRIM 703 and RRII 105 (Table 11). Clone GT 1 recorded superiority over Gl 1, RRIM 600, RRIM 703 and RRII 105. The clone PB 86 was superior to RRII 105.

The height/width ratio of phloic rays contiguous to LVs was reduced in IHB region. The ratio was maximum (6.59) in GT 1 and minimum (4.18) in RRIM 600 (Table 11). The CV values

showed low tree-to-tree variation. The ANOVA revealed significant clonal variation for this character (Table 11). GT 1 was statistically superior to eight clones *viz.* PB 86, PB 28/59, RRII 105, RRII 300, Tjir 1, Gl 1, RRIM 703 and RRIM 600. Similarly clone PB 235 was superior to five clones *viz.* RRII 300, Tjir 1, Gl 1, RRIM 703 and RRIM 600. Two PB clones *viz.* PB 86 and PB 28/59 were superior to RRIM 600.

4.15.2 Height/Width ratio of phloic rays in laticifer free zone in SB and IHB

The height/width ratio of phloic rays in laticifer free zone in SB is given in Table 12. GT 1 exhibited maximum height/width ratio (10.33) and minimum in RRII 105 (7.04). The tree-to-tree variation was insignificant in all the clones studied. The clonal variation was not significant as revealed by the analysis of variance (Table 12).

The H/W ratio of phloic rays in laticifer free zone in IHB was higher in GT 1 (6.17) and lower in RRII 105 (3.86) (Table 12). The tree-to-tree variation within clone was not significant. The ANOVA (Table 12) indicated significant clonal superiority by GT 1 and PB 86 over rest of the clones. Tjir 1 and Gl 1 also had statistical superiority over RRII 105.

4.16 Length and diameter of sieve tubes

Considerable variation in the length of sieve tubes was noticed (Fig. 14a). The length of sieve tube was maximum (875.02 µm) in PB 235 (Fig. 15 a) and minimum (329.02 µm) in RRIM 703 (Fig. 15 b). In all the clones, the CV values were very low reflecting low tree-to-tree variation within clones. Analysis of variance (Table 13) 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.

The diameter of sieve tubes was maximum (45.17 µm) in PB 86 (Fig. 11 f) and minimum (27.08 µm) in RRIM 703 (Fig. 11g). The low CV values explains 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 13). RRIM 600 was 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.

Sieve tubes are oriented one above the other with well separated end walls made up of long oblique sieve plates (Fig. 15 c) in *Hevea.* Companion cells were relatively small with well defined nucleus. In majority of cases two companion cells were attached to the sieve tubes (Fig. 15 d). However rare occurrence of tubes with one companion cell, very close to the sieve plate and two sieve tubes sharing a common companion cell was also noticed (Fig. 15 e).

4.18 Number of stone cell rows in the inner hard bark

Number of stone cell rows in IHB zone was maximum in Tjir 1 (8.11), followed by PB 235 (7.67) and PB 86 (7.67) and minimum in Gl 1 (3.39). This character showed considerable tree-to-tree variation. The variation was high in RRII 105 and low in PB 86, GT 1 and RRIM 600. Rest of the clones were depicted medium tree-to-tree variation. The clonal variation for this trait was not statistically significant (Table 13).

4.19 Area occupied by stone cells per unit CS area ($255x10^{-3}mm^2$) in IHB and OHB

The area occupied by stone cells ($1x10^{-3}mm^2$) in IHB and OHB is given in Table 13. In IHB region, an increase of area occupied by stone cells was noticed in clone PB 86 (42.48 mm^2). The area occupied by stone cells was also towards the higher side in clones RRIM 600 (37.27 mm^2) and PB 235 (27.85 mm^2) compared to other clones like RRII 300 (22.29 mm^2); Tjir 1 (21.58 mm^2); GT 1 (21.39 mm^2) and Gl 1 (18.46 mm^2), and it was lower in PB 28/59 (2.45 mm^2). Tree to tree variation was high in RRIM 600, PB 235, PB 86, Tjir 1 and Gl 1. Medium variations was observed in RRII 300, GT 1 and RRIM 703. The ANOVA (Table 13) indicated significant clonal variability. The clones PB 86 and RRIM 600 were statistically superior to RRIM 703, RRII 105 and PB 28/59. Likewise PB 235 also showed superiority over PB 28/59.

The area occupied by stone cells ($1x10^{-3}mm^2$) in OHB (Table 13) was higher in seven clones (PB 86, RRII 300, RRIM 600, Tjir 1, PB 235, Gl 1 and GT 1 (126.35 - 101.61 cm^2) and it was considerably reduced in three clones RRII 105 (8.20 cm^2), PB 28/59 (6.74 cm^2) and RRIM 703 (6.68 cm^2). The tree-to-tree variation was negligible in all the clones as indicated by low CV values. Significant clonal variability was observed, where PB 86 was statistically superior to GT 1, RRII 105, PB 28/59 and RRIM 703 (Table 13). Similarly the clones RRII 300, RRIM 600, Tjir 1, PB 235, Gl 1, GT 1 showed significant superiority over RRII 105, PB 28/59 and RRIM 703.

4.20 Correlation among bark characters

Simple correlation among various bark characters by considering all trees together, irrespective of latex vessels (LVs) inclination, exemplified the positive and negative interrelations. For convenience, different parameters were grouped together for analysis *viz.* i). Phloic ray characters in the soft bark (SB) ii). Phloic ray characters in the inner hard bark (IHB) and iii) all

other parameters. Correlation at 1% significant level has only been described in the text and other significant correlations are given in the table.

4.20.1 Correlation among phloic ray characters in SB

In the SB region correlation between rays contiguous to LVs and rays present in LV free zone were made (Table 14).

Most of the characters of the phloic rays contiguous to LVs had significant positive or negative associations. Width of rays showed significant negative correlation with height/width ratio (-0.740) and total ray frequency (-0.452). The H/W ratio had significant positive association with ray height (0.726). The total ray frequency was positively associated with multiseriate (0.338) and uniseriate ray frequencies (0.363). Similarly, multiseriate ray frequency had significant positive association with the frequency of biseriate rays (0.302) but significant negative correlation with uniseriate ray frequency (-0.522).

Most of the ray characters contiguous to LVs exhibited significant correlations with that of rays in LVs free zone in SB. Width of rays contiguous to LVs showed highly significant positive correlation with the ray width in LV free zone (0.406); negative association with H/W ratio (-0.457) and frequency of rays in LV free zone in SB (-0.355). Height of the rays contiguous to LVs correlated with height (0.457) and H/W ratio (0.535)of rays in LVs free zone. The H/W ratio of rays contiguous to LVs had significant negative association with ray width (-0.407) and significant positive association with H/W ratio (0.650) and frequency of total rays (0.348) in LVs free zone in SB. Total frequency (0.398) and biseriate ray frequency (0.694) of phloic rays in both zones were exhibited significant positive correlation.

Significant correlations between phloic ray characters in LV free zone include, negative correlation between ray width with H/W ratio (-0.684), whereas significant positive asso-

ciation with H/W ratio (0.519). The H/W ratio and total ray frequency were positively correlated (0.305). Total ray frequency had significant association with frequencies of multiseriate (0.468) and uniseriate rays (0.494) in LVs free zone but multiseriate and uniseriate ray frequencies showed significant negative association (-0.358).

4.20.2 Correlation among phloic ray characters in IHB

In the IHB also the correlation coefficients were worked out among rays contiguous to LVs and rays in LVs free zone (Table 15).

Different parameters of the rays contiguous to LVs recorded a wide range of correlation coefficient among themselves. Width of the rays had significant negative correlation with H/W ratio (-0.712), total ray frequency (-0.547) and frequency of multiseriate rays (-0.432). The height of the ray was positively associated with H/W ratio (0.567). The H/W ratio had a significant positive relation with total ray frequency (0.494), multiseriate (0.317) and biseriate ray frequencies (0.484). The total ray frequency had a very high positive and significant association with multiseriate ray frequency (0.930).

Width of the rays contiguous to LVs showed significant positive correlation with width of the rays (0.557) in LVs free zone and negative but significant relation with H/W ratio (-0.336), total ray (-0.381) and multiseriate ray frequencies (-0.307). Height of the rays contiguous to LVs had highly significant positive association with ray height (0.680) and H/W ratio (0.567 of the rays in LVs free zone. The H/W characteristics of the rays contiguous to LVs had highly significant positive associations with many characters of the rays in LVs free zone *viz.*, ray height (0.339), H/W ratio (0.668), total ray frequency (0.462), multiseriate ray frequency (0.321), uniseriate ray frequency (0.508) and negative correlation with ray width (-0.556). Total ray frequency contiguous to LVs was also significantly correlated (positive) with most of the characters of the rays in LVs free zone, like H/W ratio (0.369), total ray frequency (0.542),

multiseriate ray frequency (0.471), uniseriate frequency and negatively with ray width (-0.407). The multiseriate ray frequency of rays contiguous to LVs was found significantly and positively correlated H/W ratio (0.306), total ray frequency (0.513), multiseriate ray frequency (0.495) and negatively correlated with ray width (-0.349) of rays in LVs free zone in IHB. Likewise biseriate ray frequency showed significant positive associations with uniseriate ray frequency (0.502).

Ray width in the LV free zone had significant negative correlation with H/W ratio (-0.764), total ray frequency (-0.658) and positive association with multiseriate ray frequency (0.589), while the ray height was significantly and positively correlated with H/W ratio (0.588). The H/W ratio possessed significant positive associations with total ray frequency (0.635), multi (0.565) and uniseriate ray frequencies (0.275). The total ray frequency had the highest positive and significant correlation coefficient (0.964) with multiseriate rays.

4.20.3 Correlation among all other characters

The simple correlation coefficients among those characters other than phloic ray characters are presented in Table 16.

Sieve tube length was positively correlated with, sieve tube diameter (0.504) and stone cell area in HB (0.373) and significant negative association with LVs density contiguous to rays (-0.340) and outer hard bark thickness (-0.426). But sieve tube diameter showed only positive significant association with LVs density non contiguous to rays (0.404) and IHB thickness (0.328).

LV diameter had significant positive influence on the thickness of SB (0.326) and total cross sectional area of latex vessels (0.449). Frequency of interconnections exhibited significant positive correlations with total LVs density (0.311) and LV density contiguous to rays (0.313). Also it had negative correlations with total bark thickness (-0.330), girth (-0.407) and

total cross sectional area of LVs (-0.459). Total LVs density possessed very high significant positive correlations with LVs density contiguous to rays (0.784), LVs density non contiguous to rays (0.566) and area occupied by stone cells in outer HB (0.474). But the density of laticifers non contiguous to rays was positively correlated with stone cell area in IHB (0.447) and stone cell area in outer HB (664).

Thickness of the SB had significant positive correlations with number of LV rows in SB (0.848) and total cross sectional area of LVs (0.425). Number of LV rows in SB showed significant negative association with many characters *viz.* distance between LVs rows in SB (-0.476), IHB thickness (-0.332), stone cell area in HB (-0.338) and positively with total cross sectional area of LVs (0.381). The thickness of IHB recorded highly significant positive correlation with many characters, *viz.* number of LVs rows in IHB (0.879), number of stone cell rows in IHB (0.633), total bark thickness (0.433), girth (0.491) and total cross sectional area of LVs (0.554). Similarly, such type of significant positive correlations were noted between the number of LV rows in IHB with the distance between LV rows in IHB (0.523), number of stone cell rows in IHB (0.550), total bark thickness (0.518), tree girth (0.447) and total cross sectional area of LVs (0.584). The distance between LV rows in IHB had significant positive correlation with stone cell area in IHB (0.501) and HB (0.509) and also had significant negative association with total bark thickness (0.363) and total cross sectional area of LVs (-0.321).

The number of stone cell rows in IHB recorded very high positive and significant associations with total bark thickness (0.308), girth of the tree (0.358) and total cross sectional area of LVs (0.359). Outer hard bark thickness showed very high positive association with total bark thickness (0.859) and negative association with stone cell area in IHB (-0.366). Total bark thickness also made similar significant negative correlation with stone cell area in IHB (-0.308) and very high positive correlation with tree girth (0.560) and total cross sectional area of LVs (0.507). Other important significant positive correlations were stone cell area in IHB with that in HB (0.611); and girth with total cross sectional area of LVs (0.759).

4.20.4 Correlation between phloic ray characters in SB and phloic ray characters in IHB

The correlation coefficients of phloic ray characters in SB with that in IHB are presented in Table 17.

Characters of rays contiguous to LVs in SB were correlated significantly with characters of rays contiguous to LV in IHB. Width of the rays contiguous to LV in SB was positively correlated with ray width (0.318) and negatively correlated with H/W ratio (-0.326). Height of the rays contiguous to LV in SB showed significant positive correlation with ray height (0.413) and H/W ratio (0.481). H/W ratio of rays contiguous to LV in SB made significant positive correlation with ray height (0.318), H/W ratio (0.611) and negatively with width of rays contiguous to LVs in IHB (-0.442). The total ray frequency contiguous to LV in SB made significant positive correlations with total ray (0.347) and multiseriate ray frequencies (0.320); and negative association with ray width (-0.330) contiguous to LV in IHB. Biseriate rays contiguous to LV present in SB exhibited significant positive correlation with H/W ratio (0.353), total ray (0.352) *and multiseriate ray frequencies (0.305).*

The height of the rays contiguous to LV in SB was correlated positively with H/W ratio (0.379) and negatively correlated with width of the rays (-0.326) in LVs free zone in IHB. H/W ratio of the rays contiguous to LV in SB was correlated with characters of rays in LVs free zone in IHB, positively with H/W ratio (0.408) and uniseriate ray frequency (0.341) and negatively with ray width (-0.362). The total frequency of the rays contiguous to LV in SB was correlated with total ray frequency (0.343) and multiseriate ray frequency (0.335) in LV free zone in IHB. Similar correlation was also shown by multiseriate rays contiguous to LV in SB with frequency of multiseriate rays (0.305) in LVs free zone in IHB. The biseriate ray frequency of the rays contiguous to LV in SB showed negative correlation with ray width (-0.306) in LVs free zone in IHB.

Characters of rays in LVs free zone in SB associated significantly with many of the characters of rays contiguous to LVs in IHB. Height of rays in LV free zone in SB was having very high positive association with height of the rays (0.445) contiguous to LV in IHB. The character H/W ratio of rays in LV free zone in SB made significant positive correlation with ray height (0.326) and H/W ratio (0.387) of rays contiguous to LVs in IHB. The total frequency of rays in LV free zone in SB indicated significant positive association with total ray frequency (0.486) and multiseriate ray frequency (0.518) of rays contiguous to LVs in IHB. Also it possessed significant negative association with ray width (-0.383). Another significant correlation is the frequency of multiseriate rays in LVs free zone in SB with total ray frequency (0.306) and multiseriate ray frequency (0.306) contiguous to LVs in IHB.

Characters of phloic rays in LV free zone in SB made very few significant correlations with characters of phloic rays in LV free zone in IHB. The height of rays in LV free zone in SB was found associated with ray height (0.398) in LV free zone in IHB. The H/W ratio of rays in LV free zone in SB were correlated positively with H/W in LV free zone in IHB.

4.20.5 Correlation between all other characters and phloic ray characters in SB

Characters grouped under all other characters showed significant correlations with many of the ray characters in SB. The results are presented in Table 18. Sieve tube length exhibited significant positive correlations H/W ratio (0.347) and negative association with biseriate ray frequency (-0.324). Another highly significant association was LV density non contiguous to rays positively with ray height (0.364) and H/W ratio (0.453) and negatively with ray width (-0.361). Number of LVs rows in SB had significant negative association with frequency of multiseriate rays (-0.342). Distance between rows in SB was correlated positively with multiseriate ray frequency (0.364) and negatively with uniseriate ray frequency (-0.309). Thickness of IHB zone had significant positive association with ray height (0.320). The area of stone cells in the HB

region showed significant positive associations with H/W ratio (0.366). Similarly, positive correlation of girth with ray height (0.327) was also noticed. The total cross sectional area of LVs exhibited positive significant association with ray height (0.345).

Certain characters of rays in LV free zones in SB also has significant correlations with all other parameters. Sieve tube length and biseriate ray frequency were negatively associated each other (-0.362). LVs density non contiguous to rays possessed positive correlation with H/W ratio (0.371). Number of stone cell rows in HB had significant positive associations with multiseriate ray frequency (0.312). Stone cell area in IHB (-0.245) and outer HB (-0.303) possessed negative association with biseriate ray frequency.

4.20.6 Correlation between all other characters and phloic ray characters in IHB

The correlation results describing the association of the other characters with phloic ray characters in IHB are presented in Table 19. LV density non contiguous to rays showed significant positive association with ray height (0.355). Thickness of IHB (0.377) and number of LVs rows in IHB (0.381) had significant positive association with H/W ratio. Total cross section area of LV also made very high positive correlations with ray height (0.353) and ray width (0.329).

4.21 Correlation of characters with latex vessel inclination

Simple correlation was worked out between bark structural characters and laticifer inclination.

4.21.1 Rightward inclination of laticifers

The laticifer inclination towards the right was positively correlated with the phloic rays inclined to the right in LV free zone in both SB and IHB (Table 20). Characters which showed negative correlations were diameter of laticifers, distance between latex vessel rows in SB and area occupied by stone cells in IHB.

4.21.2 Leftward inclination of laticifers

Leftward inclination of latex vessels in SB had highly significant correlation with inclination of phloic rays in the SB zone, whereas the thickness of IHB and stone cell area in IHB were negatively correlated (Table 21). Inclination of latex vessels in IHB were associated positively with leftward inclined LV in the IHB but the frequency of biseriate rays contiguous to LV in SB and LV free zone in SB and number of stone cell rows in IHB regions were showed negative correlations.

4.21.3 Inclination of laticifers to both right and left direction

Certain trees exhibited both left and rightward inclination of latex vessels within the bark of same tree. Different factors were also found associated with each other on latex vessels inclination at various regions (SB and IHB) of the bark to left and right (Table 22).

Number of characters influencing left and rightward inclination of LVs also showed considerable differences. The leftward inclination of LVs in the SB and IHB showed significant positive correlation with only one character, the leftward inclined phloic rays in LV free zone in the IHB and negatively correlated with the number of LV rows in SB.

The rightward inclination of LVs in SB was having very high significant positive correlation with many characters such as the rightward inclination of phloic rays in the LVs free zone in SB and IHB, the rightward inclined LVs in IHB and tree girth. Few other characters exhibited significant negative correlations includes total density of LV, density LV non contiguous to rays and area occupied by stone cells in HB.

The rightward inclination of LVs in the IHB was also depicted highly significant positive correlation with rightward inclination of phloic rays in LV free zone in SB and IHB, frequency of biseriate rays contiguous to LVs in IHB and tree girth. Two other characters which showed correlations were total density of laticifers and density of laticifers non contiguous to rays.

4.22 Regression Analysis

Regression analysis was done separately for trees with rightward, leftward and and right to leftward inclination of latex vessels to identify the most important character responsible for the laticifer inclination in SB and IHB. The results indicated that effect of various independent variables were positively and negatively associated with the dependent variable (laticifer inclination).

4.22.1 Trees having only rightward inclination of laticifers

Different characters associated with rightward inclination of rays were presented in Table 23. The inclination of phloic rays in SB had highly significant positive effect on inclination of LV in SB, whereas the sieve tube diameter showed negative role on LV inclination. Likewise, in the IHB region also, the most significant positive character identified was phloic ray inclination in IHB. However, the sieve tube length played a significant negative role on the inclination of LV.

Regression analysis could not done due to inadequate number of variables in those trees which depicted leftward inclination of laticifers.

4.22.2 Trees having left and rightward inclination of laticifers

Table 24 represents the regression analysis in trees with left-rightward inclined LVs and phloic rays. The rightward inclination of LVs was positively influenced by the rightward inclination of phloic rays in SB, along with negative influence of LV density non contiguous to rays. The leftward inclination of LVs in SB was also influenced positively by the leftward inclination of phloic rays in SB and diameter of the sieve tubes. The rightward inclination of phloic rays also influenced negatively on LV inclination to left in SB.

In the IHB region, the rightward inclined LVs were also positively influenced by the rightward inclination of phloic rays in IHB. The number of stone cell rows in IHB depicted a negative influence on the number of LVs inclined to left in the IHB region.

4.23 Histochemical localization

4.23.1 Starch

Starch grains stained bluish-black with Iodine - Potassium Iodide (I_2KI) and were mainly localized in axial parenchyma and rarely in ray cells. The frequency of starch bearing cells, as well as, the number of grains per cell varied considerably in different zones of bark. Soft bark region region contiguous to cambium, had low level of starch reserves (Fig. 16 a, arrow head) whereas the outer IHB region (Fig.16 a, arrow) ,as well as, the entire HB region (Fig. 16 b) showed high storage of starch reserves. The storage of starch was more in axial parenchyma cells than in rays (Fig. 16c). In axial parenchyma, the starch grains were mostly accumulated as groups (Fig. 16 d) and in certain cases the grains were randomly distributed within the cells. The starch grains appeared as circular/oval in shape (Fig. 16 e). In the OHB region, starch grains were distributed in almost all the cells except in stone cells (Fig. 16 f).

The total area occupied by starch grains per unit CS area of $430x10^{-3}$ mm^2 and the average area of starch grains ($1x10^{-4}$ mm^2) in IHB and outer HB are presented in Figure 14b The area occupied by them was maximum in PB 28/59 (66.01 mm^2), followed by RRII 105 (48.67 mm^2) and the minimum was recorded in GT 1 (3.97.49 mm^2). The variation in starch grain size was also noticed in different clones (Fig 16g)

4.23.2 Total polysaccharides

Total polysaccharides were stained reddish in Periodic acid and Schiff's reagent. Cell walls of all tissues showed such stainability (Fig. 17 a to c). In the SB region, total polysaccharides were localized in the cytoplasm of the ray cells (Fig. 17 a, broad arrows). The staining

intensity was considerably reduced towards the IHB region (Fig. 17 a narrow arrow). Axial parenchyma adjacent to ray (Fig. 17 b, broad arrow), sieve plates (Fig. 17 arrow head) and longitudinal walls of latex vessels showed deep stainability indicating content of total polysaccharides (Fig. 17 b, thin arrow). Deposition of polysaccharides on sieve plate were also noticed (Fig. 17c)

4.23.3 Lipids

Lipids stained bluish black with Sudan Black B and its localization was observed as granules in both ray and axial parenchyma cells, in the SB and IHB. Most of the ray cells (Fig. 17 d, arrow head) and axial parenchyma cells (Fig. 17 d, broad arrow) possessed lipid globules in the inner most SB zone contiguous to cambium. The quantity of lipid increased in IHB region, both in ray (Fig.17 e, arrow head) and axial parenchyma cells (Fig. 17 e, arrow). The tissue systems of the OHB zone (Fig. 17 f, broad arrow) were also showed the presence of lipid globules, whereas the stone cell did not show any lipid localization (Fig. 17 f, thin arrow).

4.23.4. Proteins

Total proteins stained blue with Mercuric bromophenol blue. Among the different group of cells in the bark tissue, ray parenchyma cells took uniform stainability. Ray cells contiguous to cambial zone showed intense stainability and the stainability further extended towards the outer bark zone indicating high content of total protein (Fig. 18 a, b and c, arrow head). In the SB region, sieve plates (Fig. 18 a and d) of the sieve tubes showed protein localization. Many of the axial parenchyma cells in the SB region showed localization of protein throughout the cytoplasm (Fig. 18 e, arrow head). Localization of proteins was also noticed in the latex vessels (Fig. 18 f, arrow head). In the OHB region, most of the parenchymatous tissues showed protein localization, whereas the stone cells did not show any protein localisation (Fig. 18a, broad arrows).

4.23.5 Phenols

Phenolic substances stained dark-blue in tannic acid - ferric chloride reagent. Cells having phenolic content are less frequent in the SB region (Fig. 18g, arrow) whereas in the IHB (Fig. 18g and h arrow head) intensity of phenolic localization was very high. The parenchymatous tissue contiguous to laticifers had high phenolic storage. However, the latex vessels were devoid of phenolics accumulation (Fig 18 i, arrow head). In general, axial parenchyma had high phenolic content in comparison to ray parenchyma cells. Stone cells were generally devoid of phenolic substances (Fig. 18 h, broad arrow). Sieve tubes in the inner zone of the SB were also lacking phenolics (Fig. 18 j, arrow head).

4.23.6 Tannin

Tannin stained bluish-black in ferric sulphate and were localized in the axial and ray parenchyma cells. Tanniferous cells were usually absent in the SB region contiguous to cambium (Fig. 19 a) whereas the frequency of such cells were high towards the IHB (Fig. 19 b), OHB (Fig. 19 c) and outer most hard bark zone (Fig. 19 d).

Tanniferous cells were absent in the LV free zone of SB. Latex vessels are free from tannin deposition as revealed by its non-stainability (Fig. 19 e, arrow head). Many of the axial parenchyma cells contiguous to rays (Fig. 19 f) in the outer SB and IHB region were stained deeply due to the high content of tannin. The rays in the the OHB region showed intense tannin localization as patches (Fig. 19 g).

4.23.7 Lignin

Lignin stained purplish red with Phloroglucinol-HCl which indicated the presence of lignin biopolymer. Lignified cells were absent in the SB region (Fig. 19 h, arrow). Lignification was relatively more in axial parenchyma than the ray cells in the IHB region (Fig. 19 h, arrow head).

Such cells are seen as groups and are randomly distributed in the secondary phloem tissue (Fig. 19 i, arrow head). The phloem fibres also showed intense lignification leading to sclerefication and many of the sclerified cells are grouped together, to form stone cells (Fig. 19 j). Nevertheless, the cell wall of laticifers did not show lignification (Fig. 19 h).

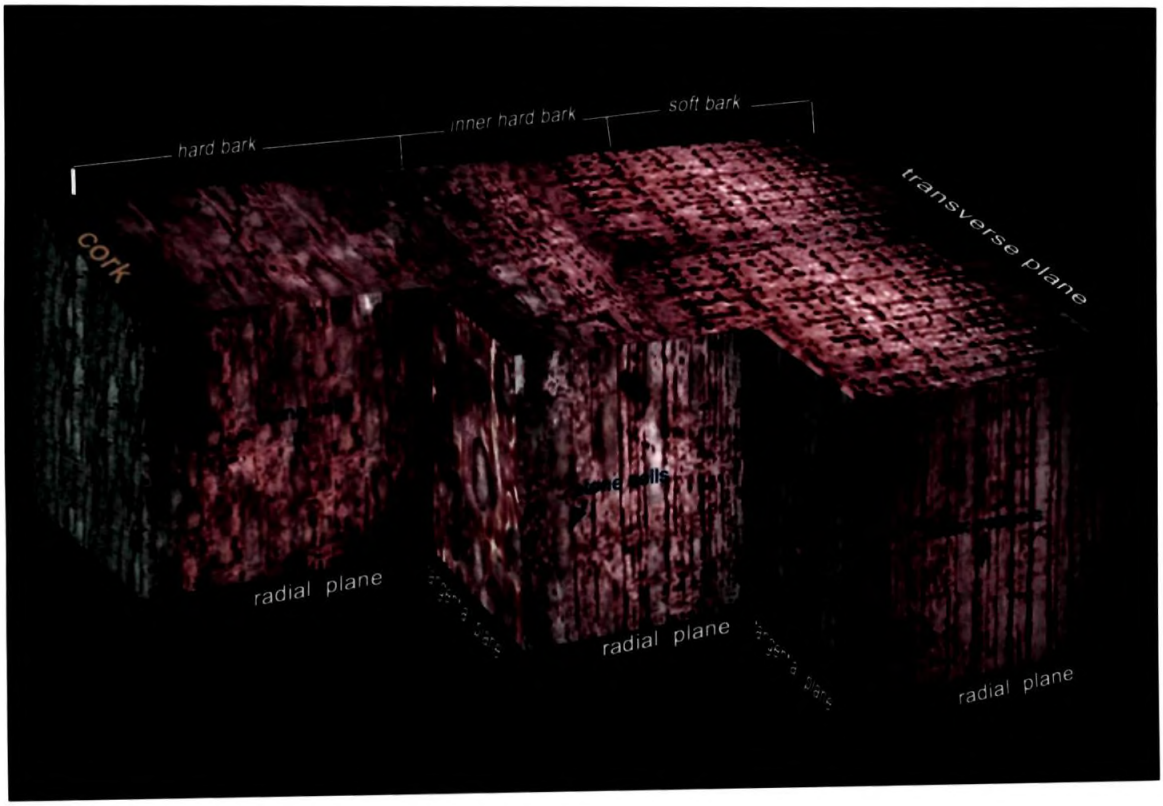


Figure 2. Three dimensional picture of bark of *H. brasiliensis*

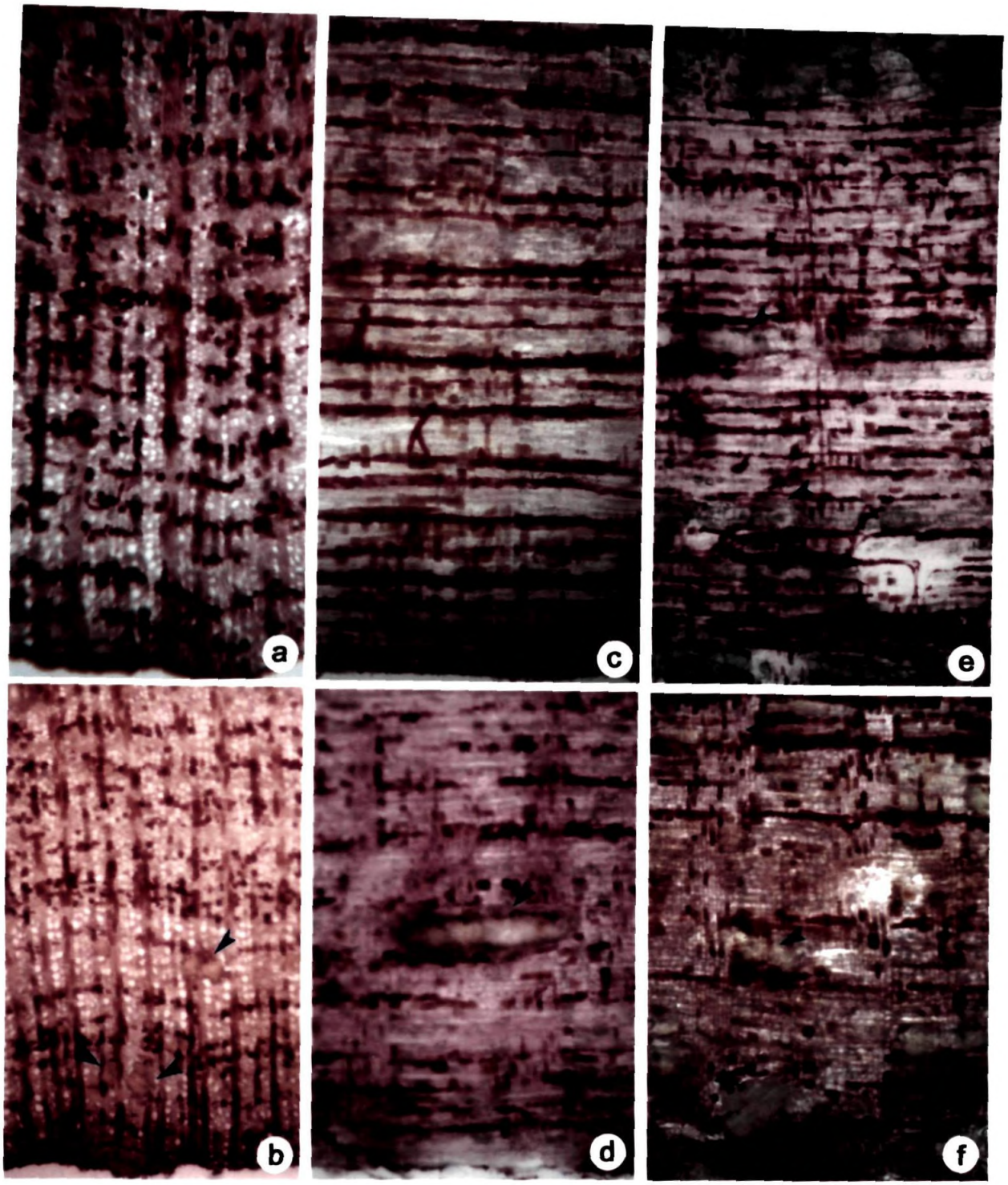


Figure 3. a-f: Bark sections stained with Oil Red O. a- PB 28/59 showing maximum soft bark thickness. b- PB 86 showing minimum soft bark thickness (stone cell at arrows). c- PB 28/59 with maximum number of laticifer rows in soft bark. d- PB 86 with minimum laticifer rows in soft bark. e- PB 86 with maximum laticifer rows in inner hard bark. f- RRII 300 with minimum laticifer rows in inner hard bark (stone cell at arrows)
a&b -cross section (CS) X30; c-f- Radial longitudinal section (RLS) X75

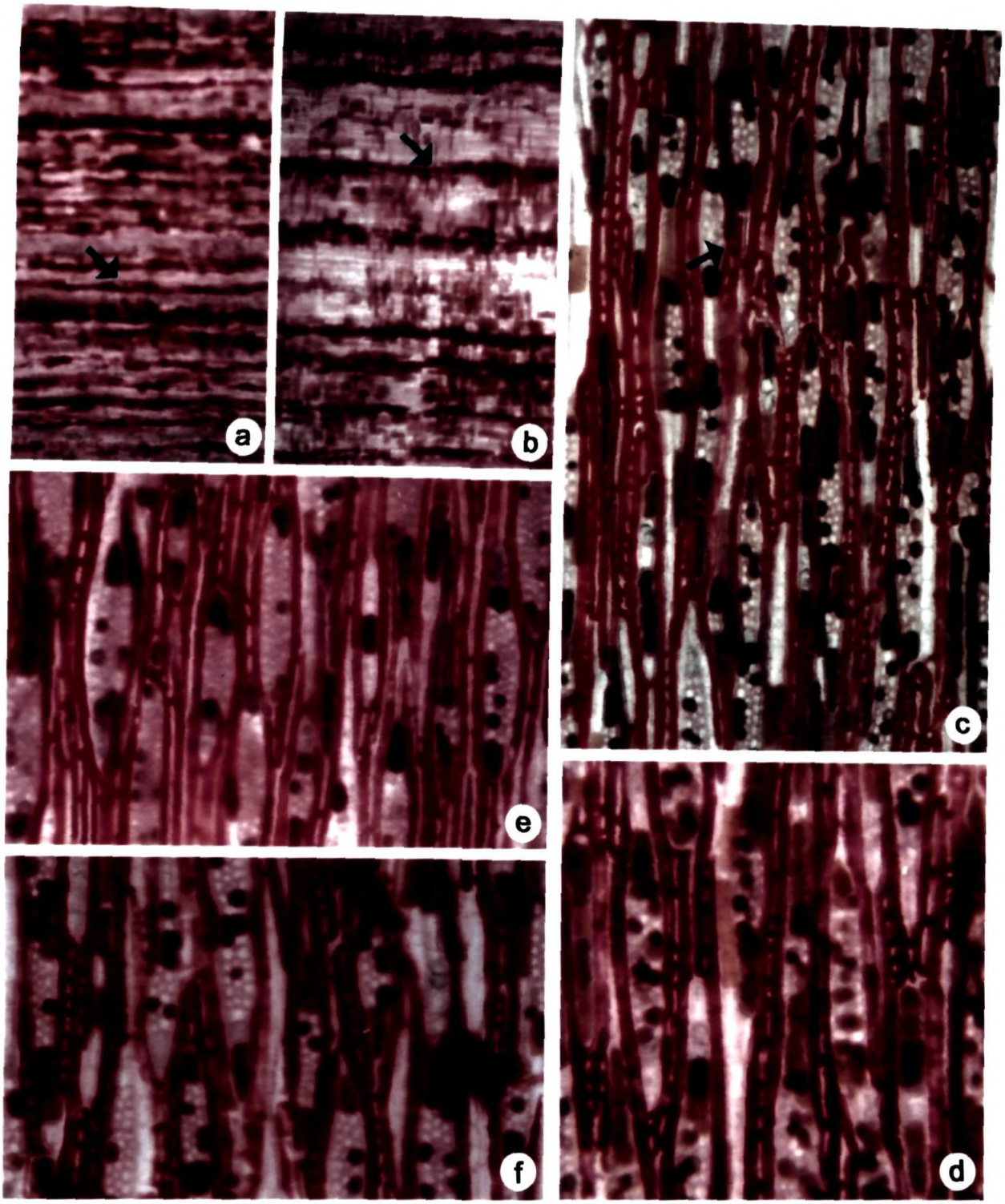


Figure 4. Bark sections showing distribution of latex vessels (at arrows) a- PB 86 minimum laticifer inter-row distance. b- PB 28/59 –maximum laticifer inter-row distance. c- GT 1- maximum density of latex vessels contiguous to rays. d- PB 235 minimum density of latex vessels contiguous to rays. e-GT 1 maximum density of latex vessels non-contiguous to rays. f- RRIM 703 minimum density of latex vessels non-contiguous to rays..

a&b- radial longitudinal sections (RLS) X75; c to f- tangential longitudinal sections (TLS) X200

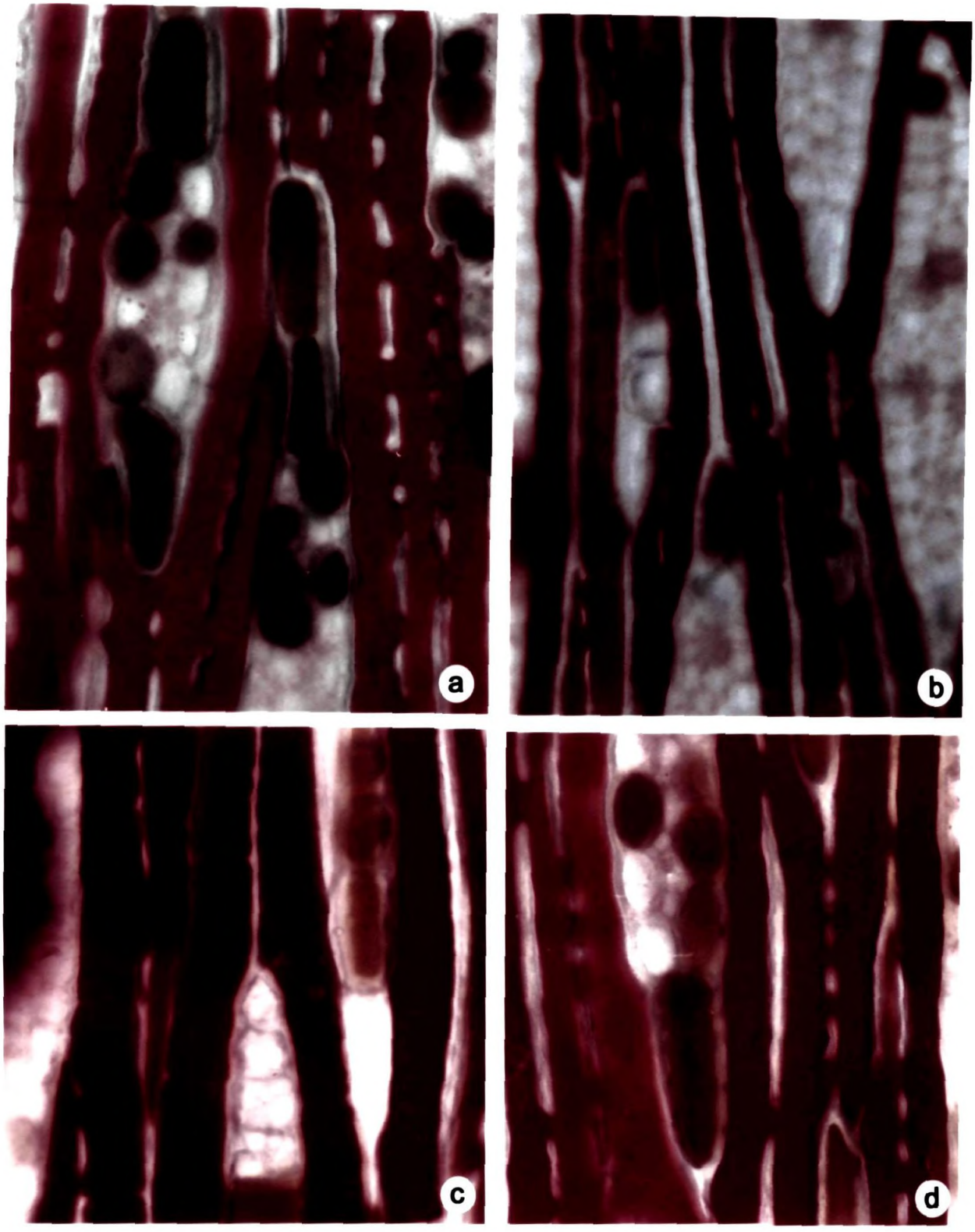


Figure 5. TLS of bark showing frequency of interconnections between latex vessels and diameter. a- RRII 105 frequency of interconnections (maximum). b- PB 28/59 frequency of interconnections (minimum) . c- PB 28/59 latex vessel diameter (maximum). d- RRIM 703 latex vessel diameter (minimum).
a&b – X200; c&d – X300

Figure 6. Inclination of laticifers in soft bark and inner hard bark regions of *Hevea* clones

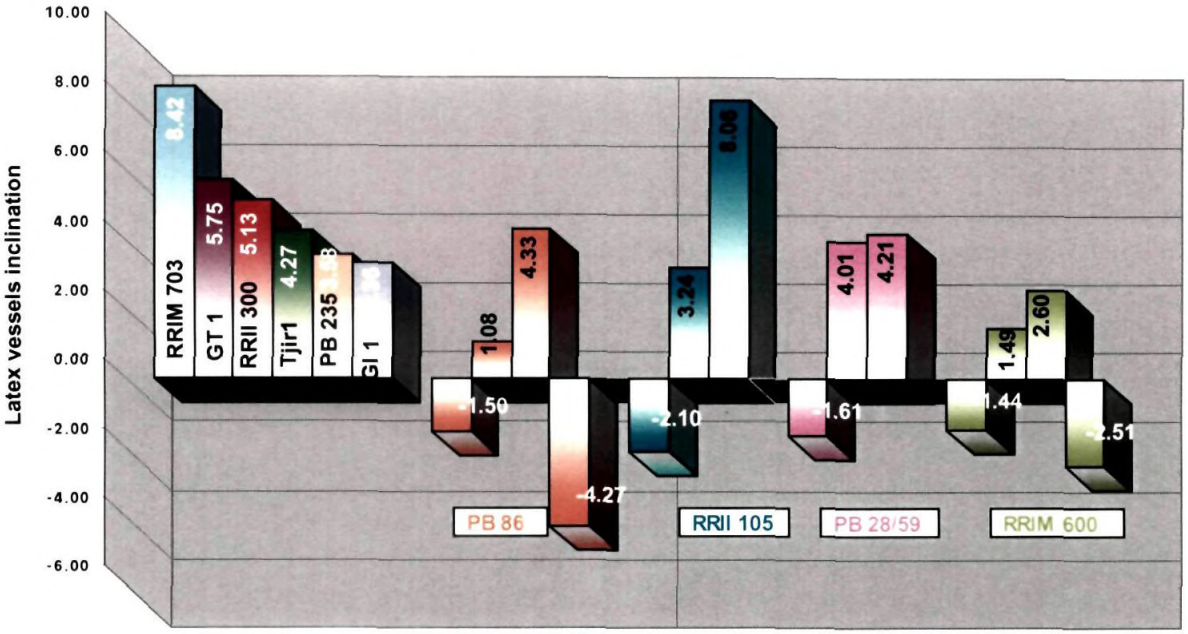


Figure 6a. Inclination of laticifers in the soft bark region (degrees). Six clones with rightward inclination in RRIM 703, GT 1, RRII 300, Tjir 1, PB 235 and Gl 1. But PB 86 with left-right (1), right (1) and left (7); RRII 105 with left-right (7) and right (2); PB 28/59 with left-right (5) and right (4); RRIM 600 with left-right (3), right (3) and left (3). Numbers in parenthesis indicate the number of trees with respective inclination.

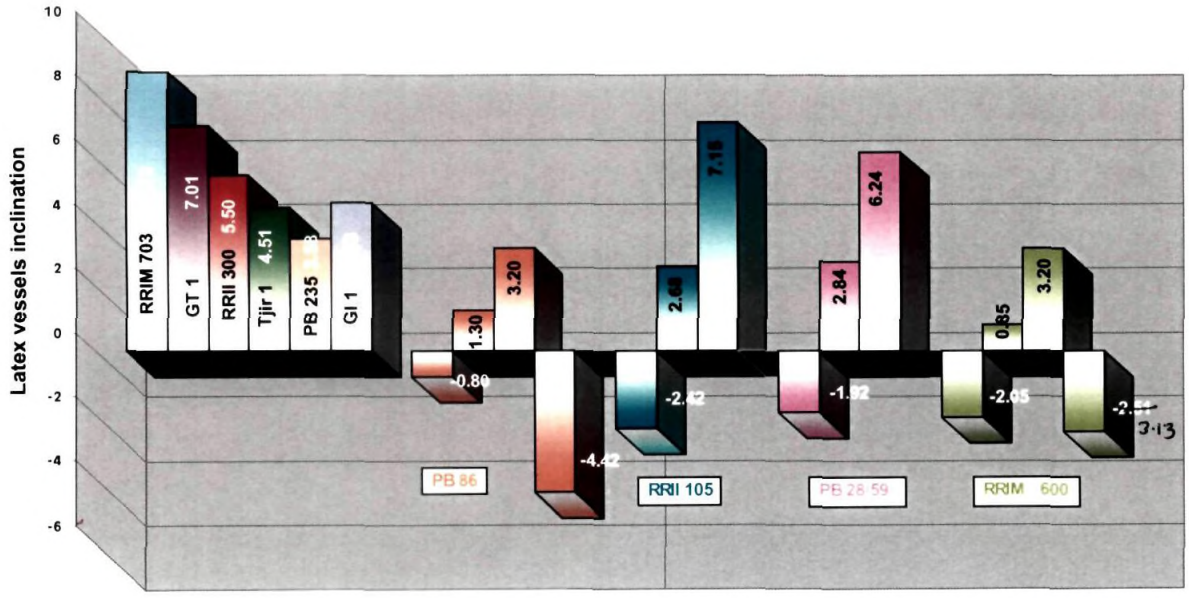


Figure 6b. Inclination of laticifers in the inner hard bark region (degrees). Six clones RRIM 703, GT 1, RRII 300, Tjir 1, PB 235 and Gl 1 were having only rightward inclined LVs. But PB 86 with left-right (1), right (1) and left (7); RRII 105 with left-right (7) and right (2); PB 28/59 with left-right (5) and right (4); RRIM 600 with left-right (3), right (3) and left (3). Numbers in parenthesis indicate the number of trees with respective inclination.

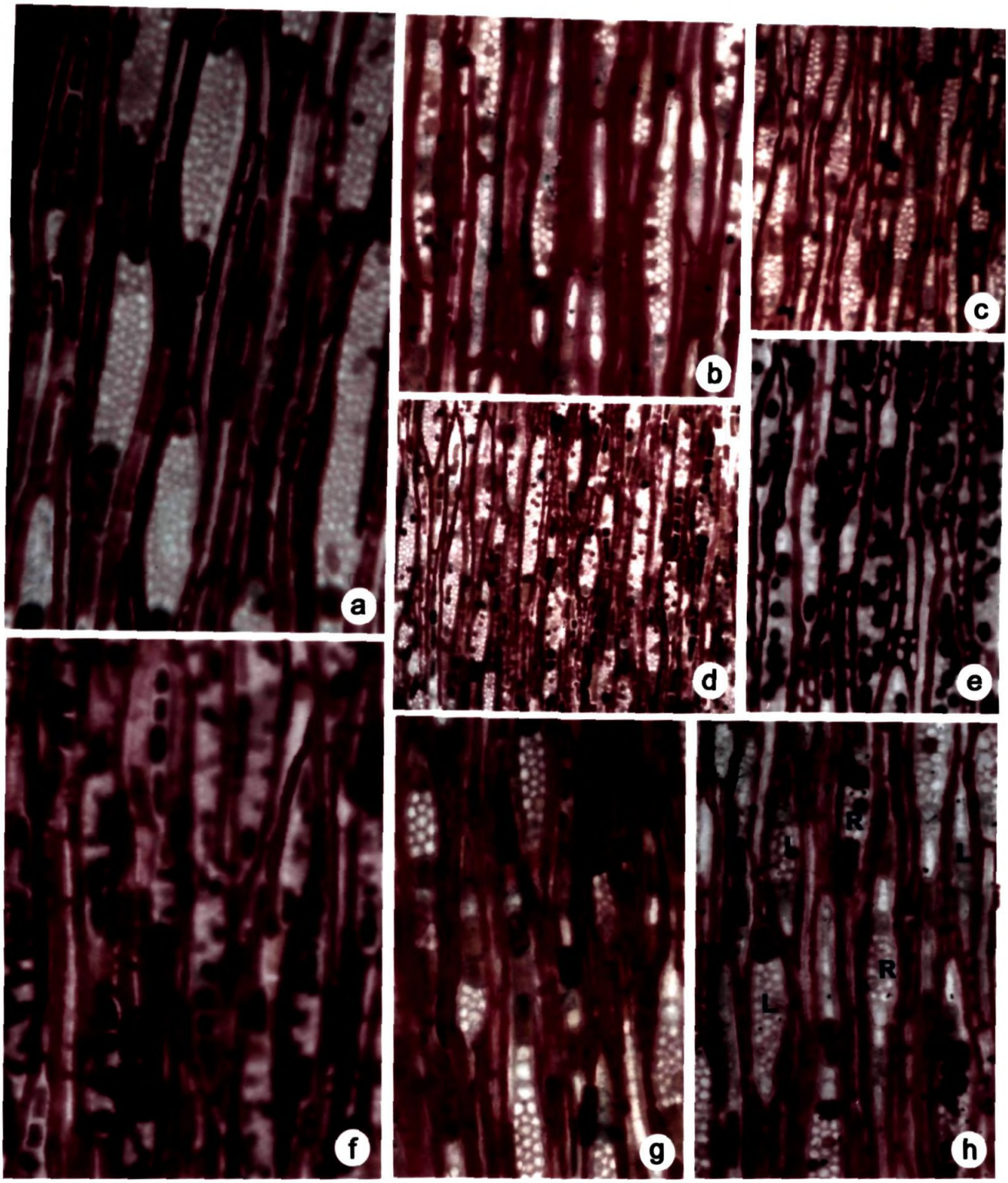


Figure 7. TLS of bark showing inclination of laticifers in soft bark. a- RRIM 703 rightward inclination (maximum). b- Gl 1 rightward inclination (minimum). c- GT 1 rightward inclination. d- RRII 300 rightward inclination. e- Tjir 1 rightward inclination. f- PB 235 rightward inclination. g- PB 86 leftward inclination. h- PB 86 both rightward & leftward inclination.

a- X125; b, e,f,g and h- X75; c- X50; d- X30

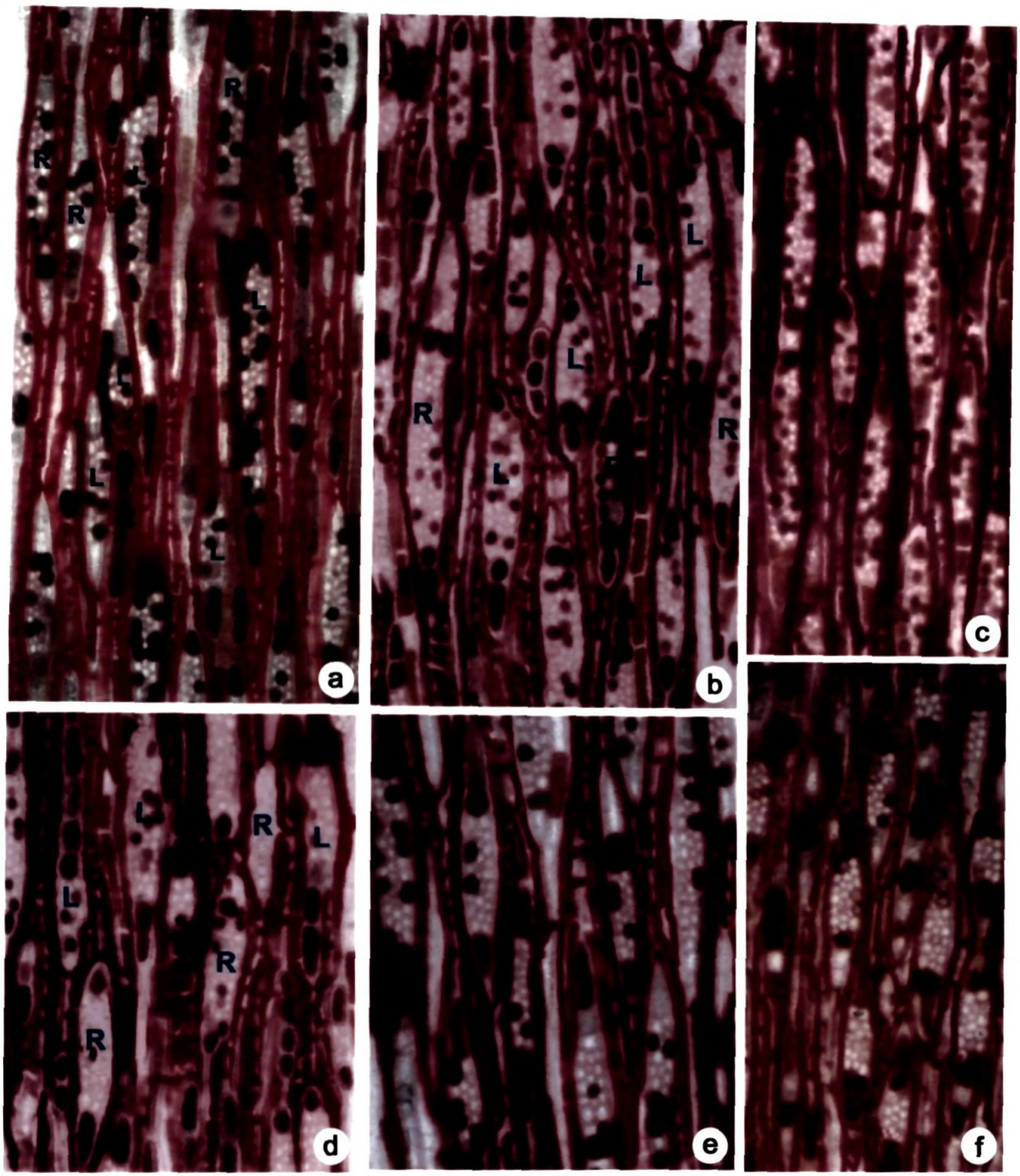


Figure 8. TLS of bark showing inclination of latex vessels in soft bark. a- RRII 105 leftward and rightward inclination. b- PB 28/59 both leftward and rightward inclination. c- PB 28/59 only rightward inclination. d- RRIM 600 both leftward and rightward inclination. e- RRIM 600 only leftward inclination. f- RRIM 600 only rightward inclination.

a, b, d, e & f- X75; c- X125

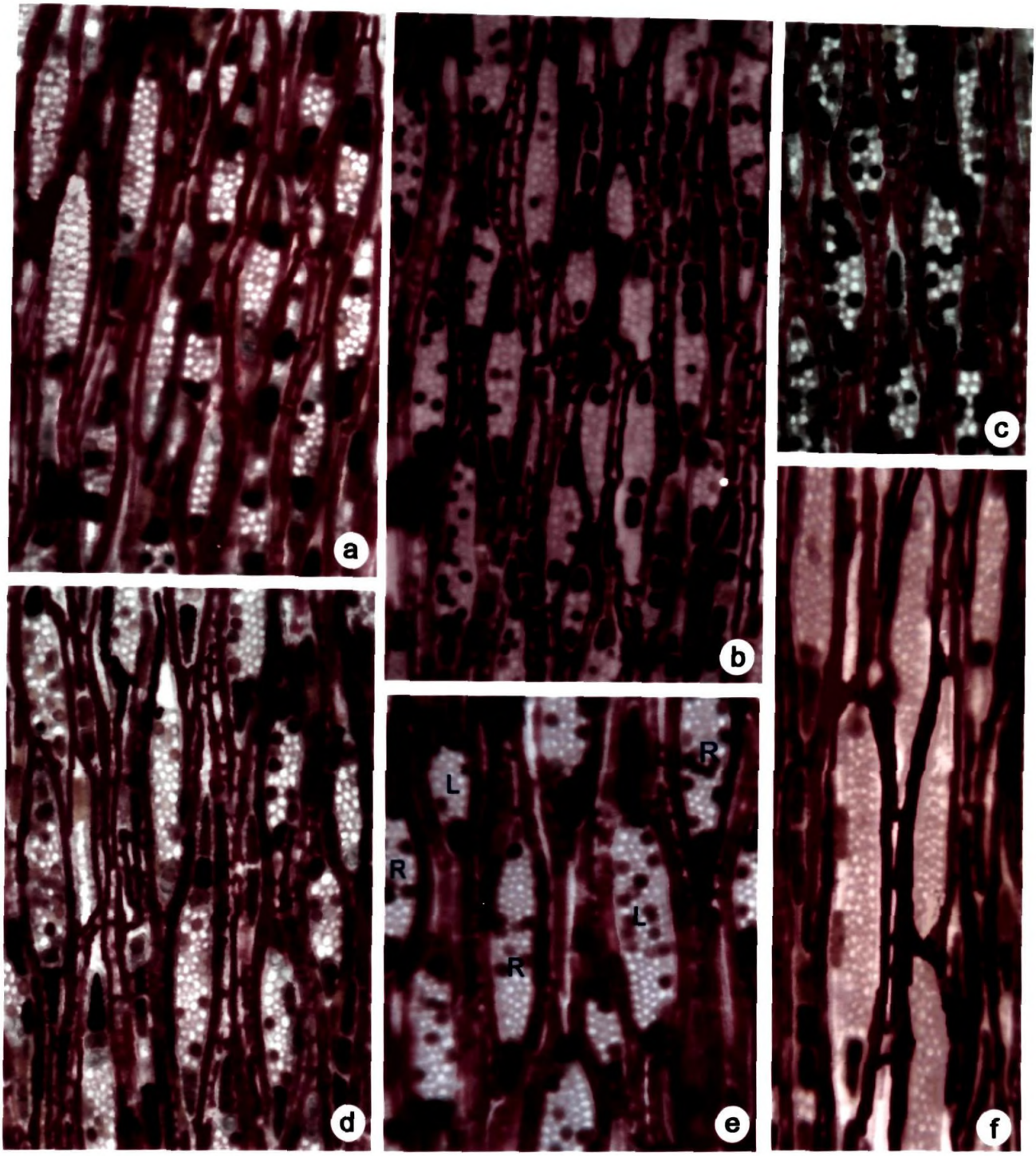


Figure 9. TLS showing inclination of laticifers inner hard bark. a- RRIM 703 rightward inclination (maximum). b- PB 235 rightward inclination (minimum). c- PB 86 leftward inclination. d- RRII 105 both left & rightward inclination. e- PB 28/59 both left & rightward inclination. f- RRIM 600 rightward inclination
a to e- X75; f- X125

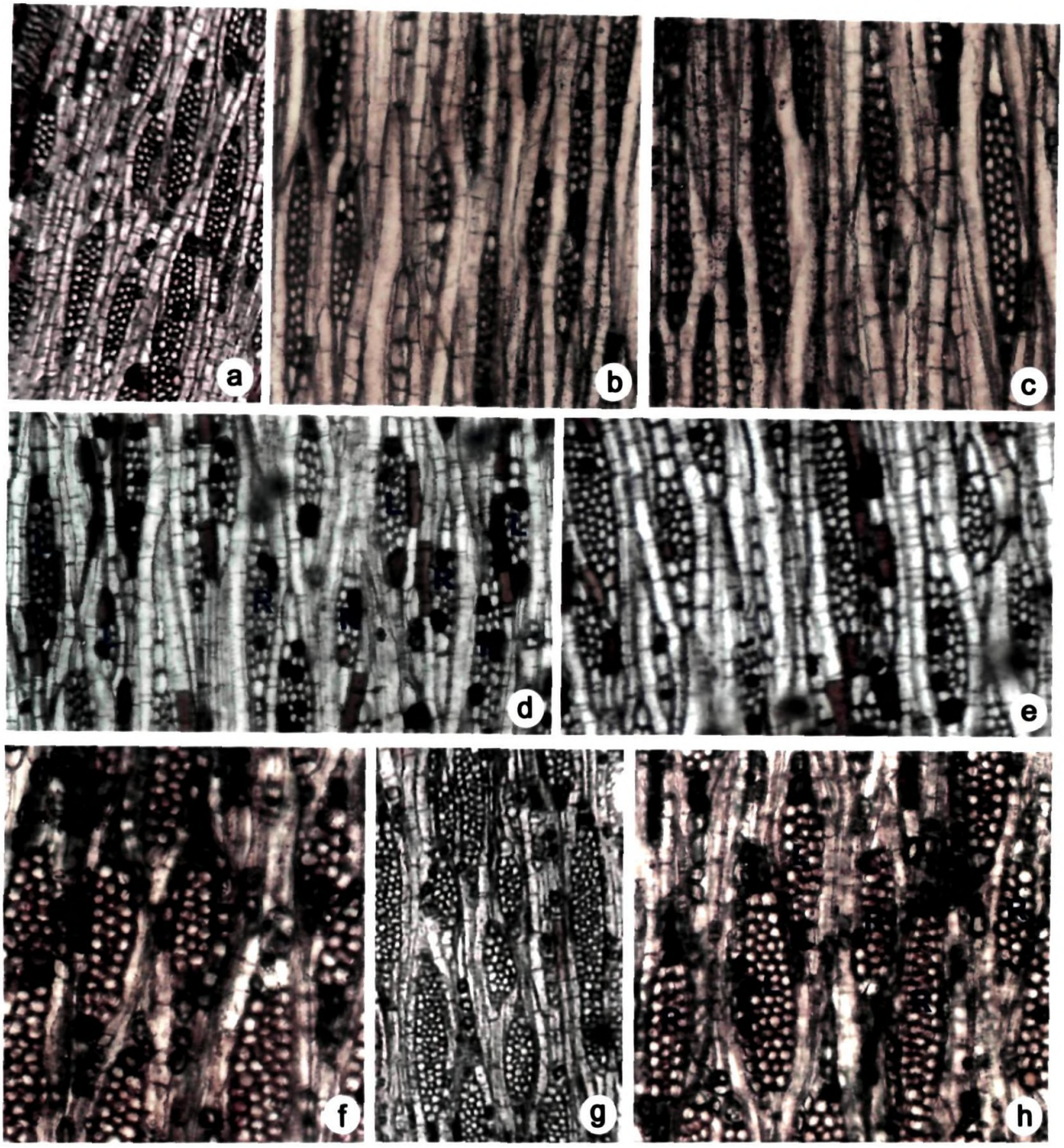


Figure 10. TLS of bark showing inclination of phloic rays in latex vessel free zone in soft bark and inner hard bark. a- RRIM 703 rightward inclination (maximum). b- Gl 1 rightward inclination (minimum). c- PB 86 leftward inclination (soft bark). d- RRII 105 left and rightward inclination e- RRIM 600 leftward inclination in IHB. f, g & h- rays inclination in laticifer free zone in inner hard bark, f- RRIM 703 rightward . g-PB 86 leftward & h- both left and rightward in RRII 105.

a-h – X75

Figure 11. Frequency of different phloic rays in soft bark inner hard regions of *Hevea* clones

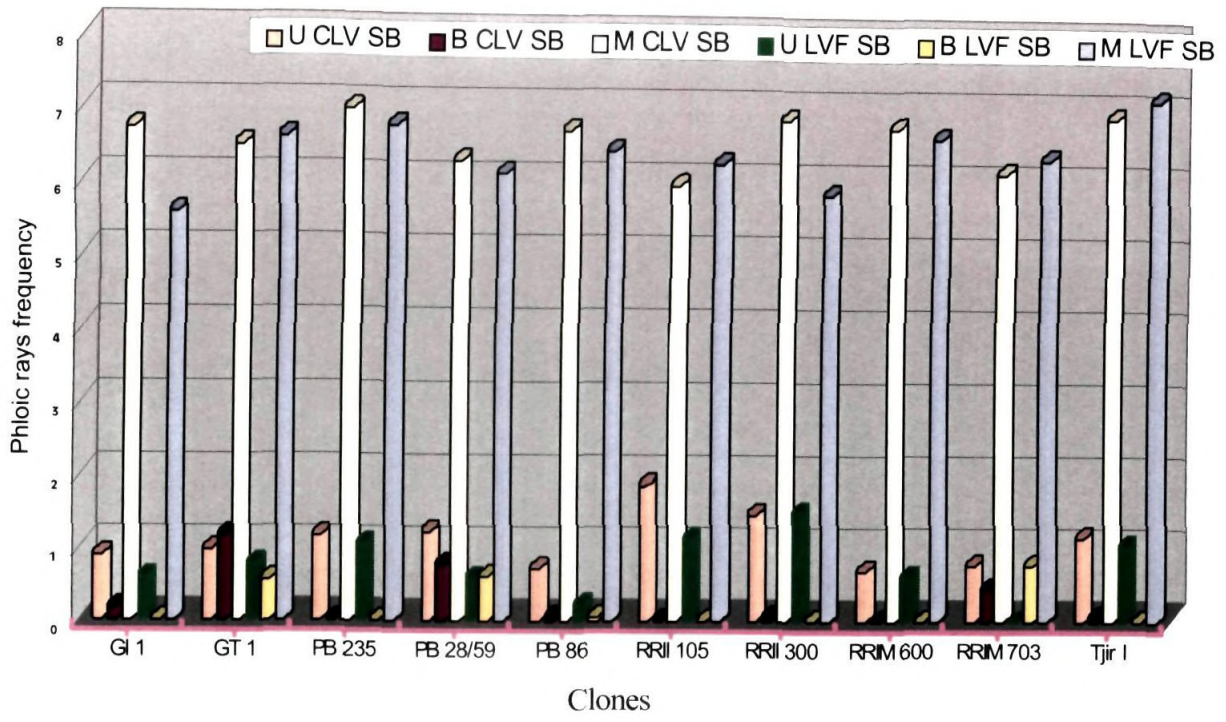


Figure 11a . Frequency of phloic rays contiguous to laticifers and laticifer free zone in soft bark

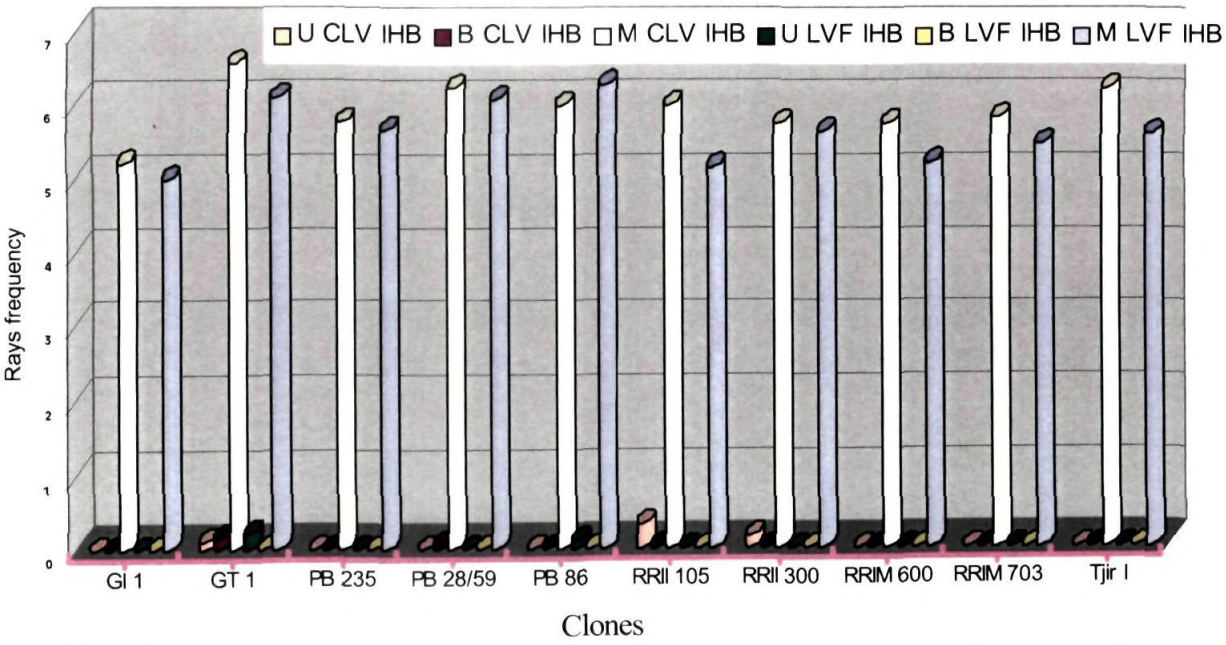


Figure 11 b . Frequency of phloic rays contiguous to laticifers & laticifer free zone in inner hard bark

U-uniseriate rays, B- biseriate rays, M-multiseriate rays, CLV- contiguous to latex vessels, LVF- latex vessel free zone, SB-soft bark, IHB-inner hard bark

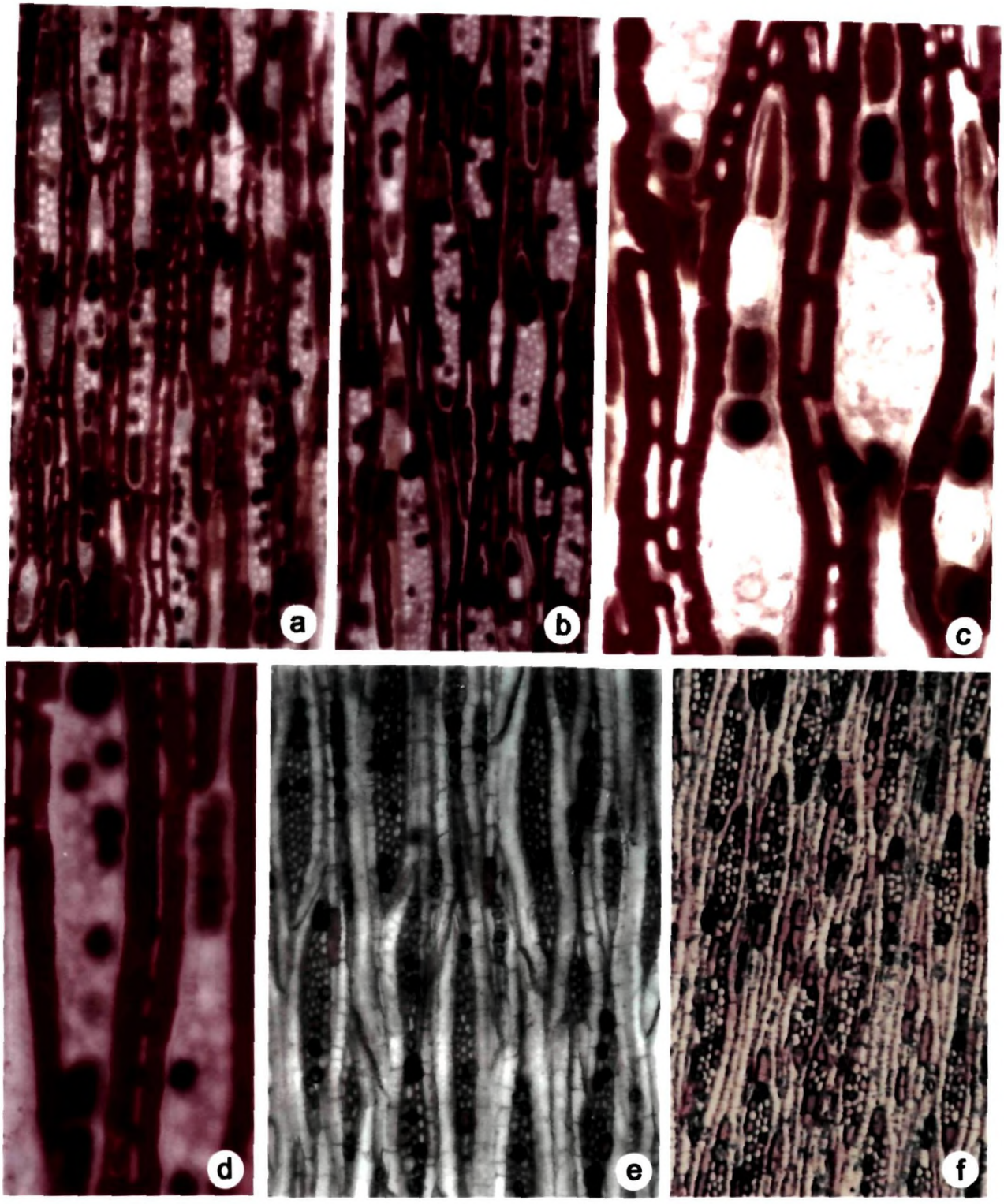
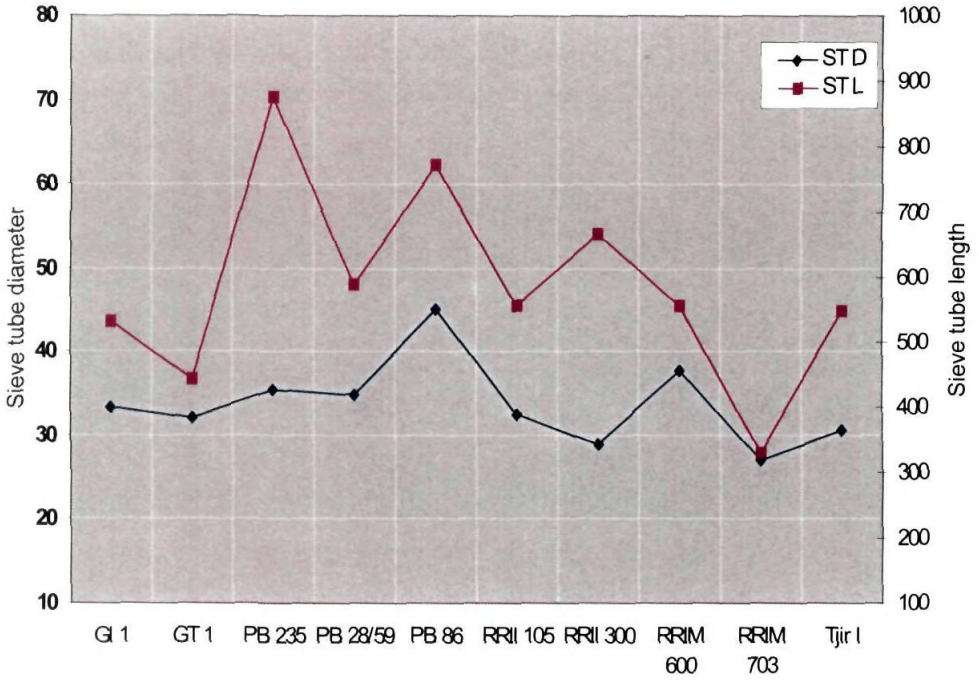


Figure 13. TLS of bark showed height and width of phloic rays in soft bark and inner hard bark. a- PB 235 maximum ray height in soft bark. b- RRIM 600 ray height (minimum) in soft bark. c- Gl 1 ray width (maximum). d- GT 1 ray width minimum. e- PB 235 ray height in latex vessel free zone (maximum). f- RRIM 703 ray height (minimum).

a & b- X50; c & d- X100; e & f- X75

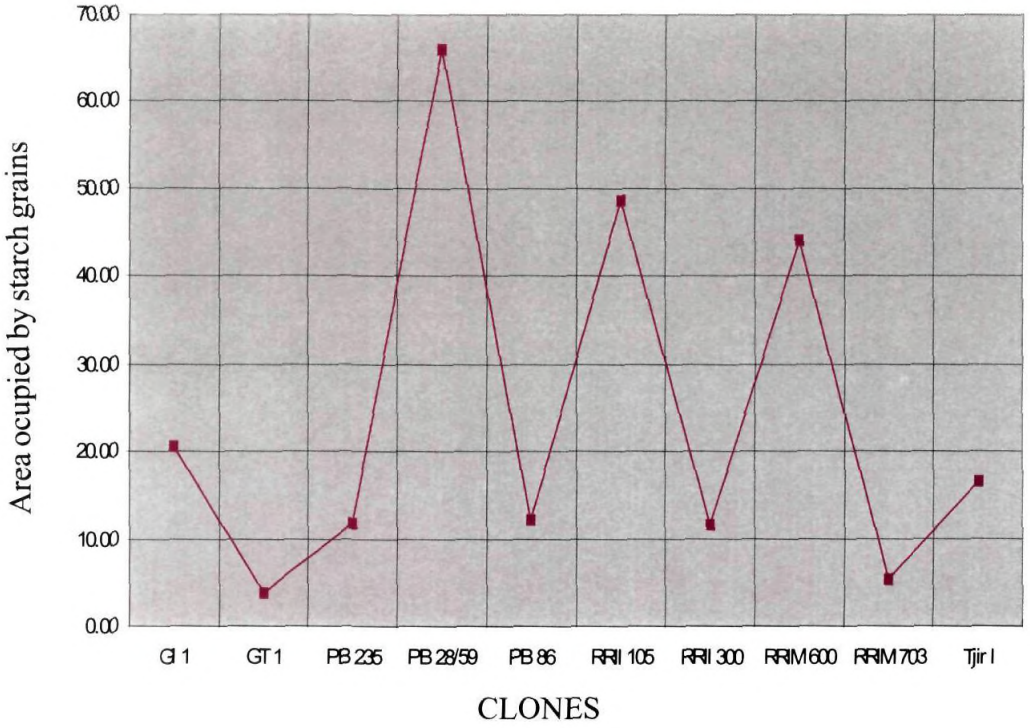
Figure 14. Sieve tube dimensions and area occupied by starch grains in different clones of *Hevea*

Figure 14a. Sieve tube diameter and length (μm)



ST D-sieve tube diameter, ST L- sieve tube length

Figure 14b. Area ocupied by starch grains ($1x10^{-4}mm^2$)



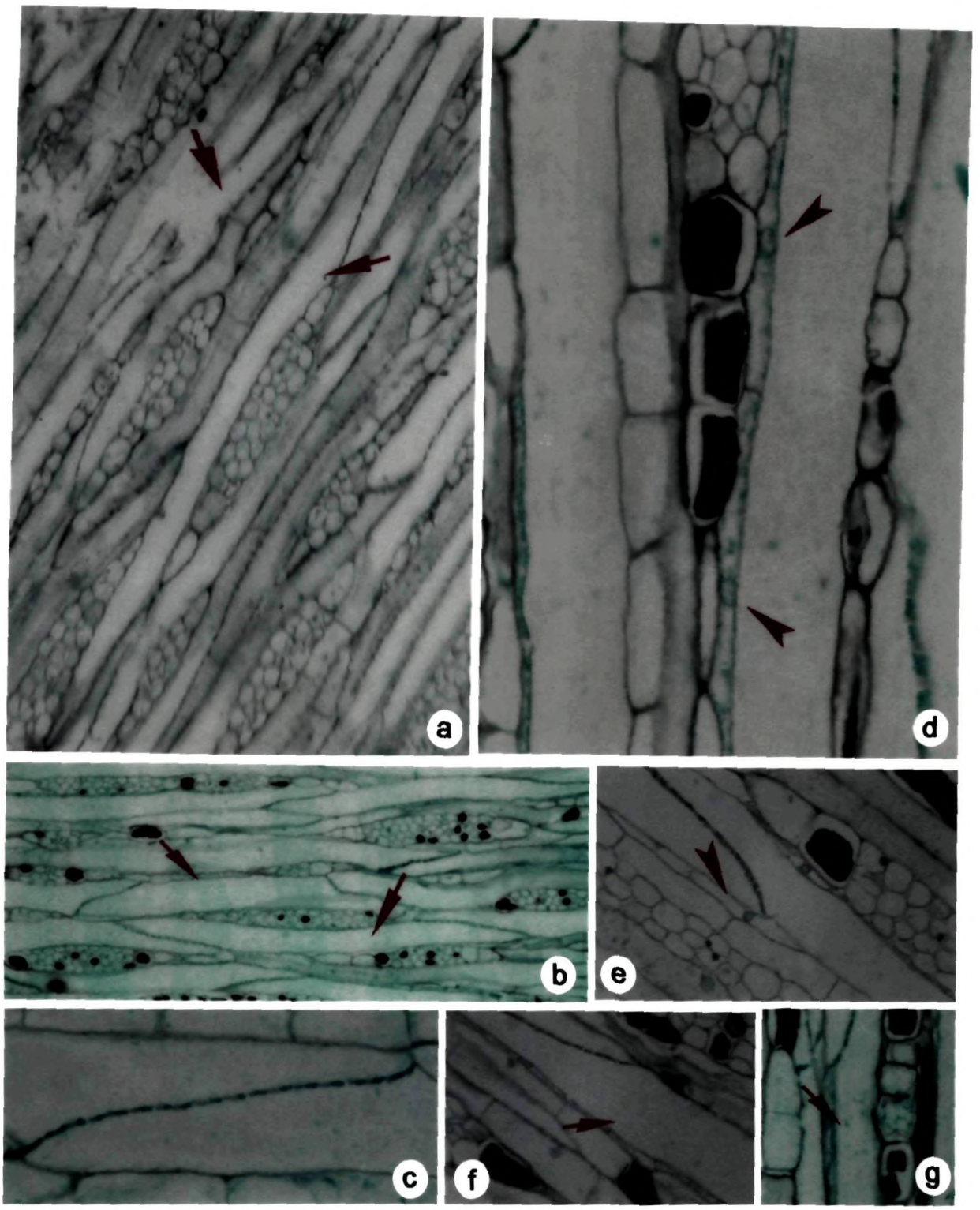


Figure 15. a to f- TLS of bark stained with safranin- fast green showing morphology of sieve tubes . a- PB 235 very long sieve tubes (arrows). b- RRIM 703 short sieve tubes (arrow). c- long and oblique sieve plates. d- sieve tube with two companion cells (arrow). e- companion cells very close to sieve plate (arrow). f- PB 86 sieve tube diameter (maximum). g- RRIM 703 sieve tube diameter (minimum).

a&b- X75; c&d- X300; e, f & g- X200

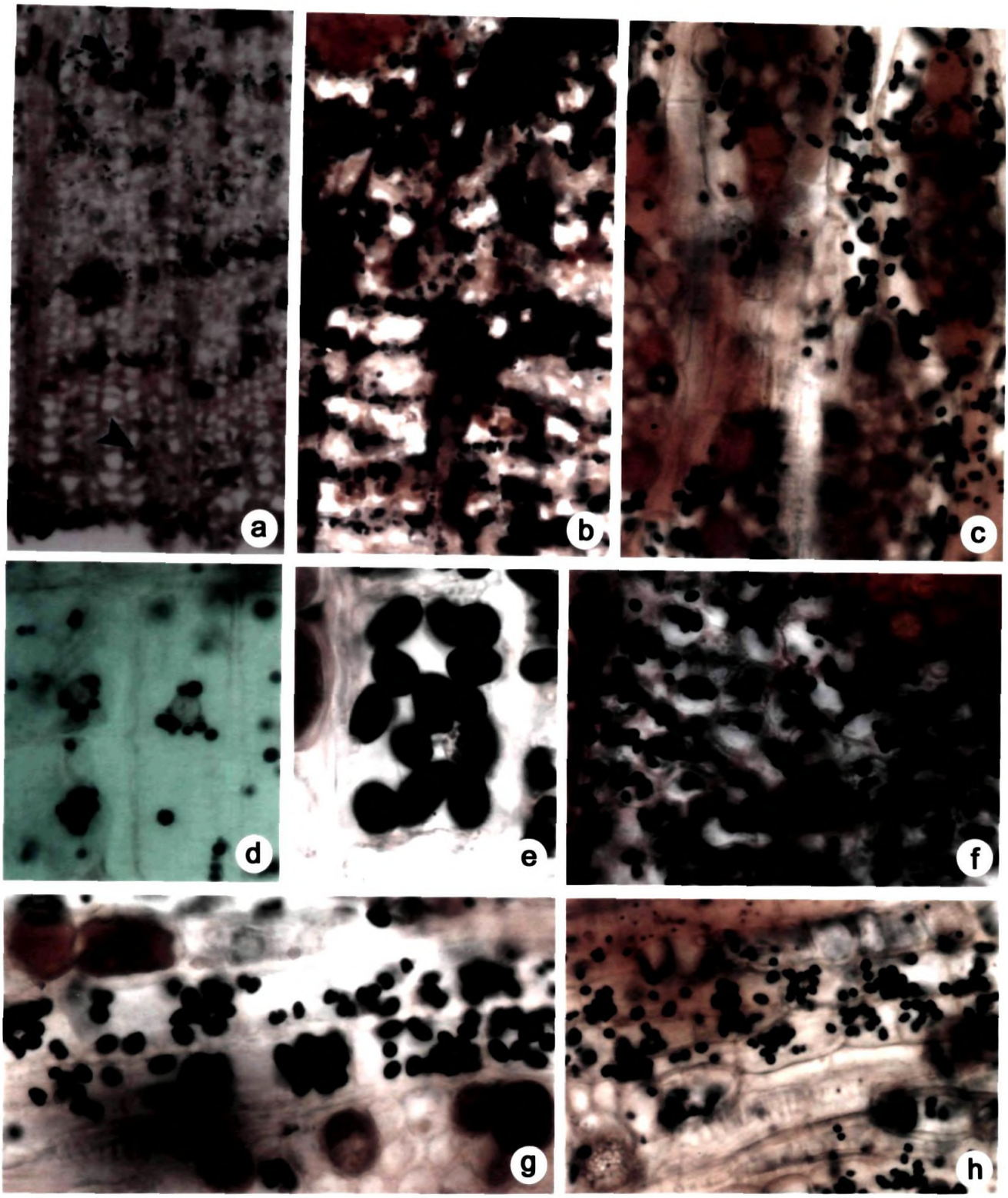


Figure 16. a to h - Histochemical localization of starch in bark tissue. a- less starch grains in soft bark (arrow head) and more starch grains in inner hard bark. b&c- outer hard bark showing high storage of starch. d- starch grains grouped. e- oval shape starch grains. f- starch surrounding stone cells in outer hard bark. g- PB 28/59 grain size (maximum). h- GT 1 grain size (minimum).
a, b & f- CS; c,d,e,f,g and h- RLS
a&b- X10; c,d,f,g & h- X200; e- X300

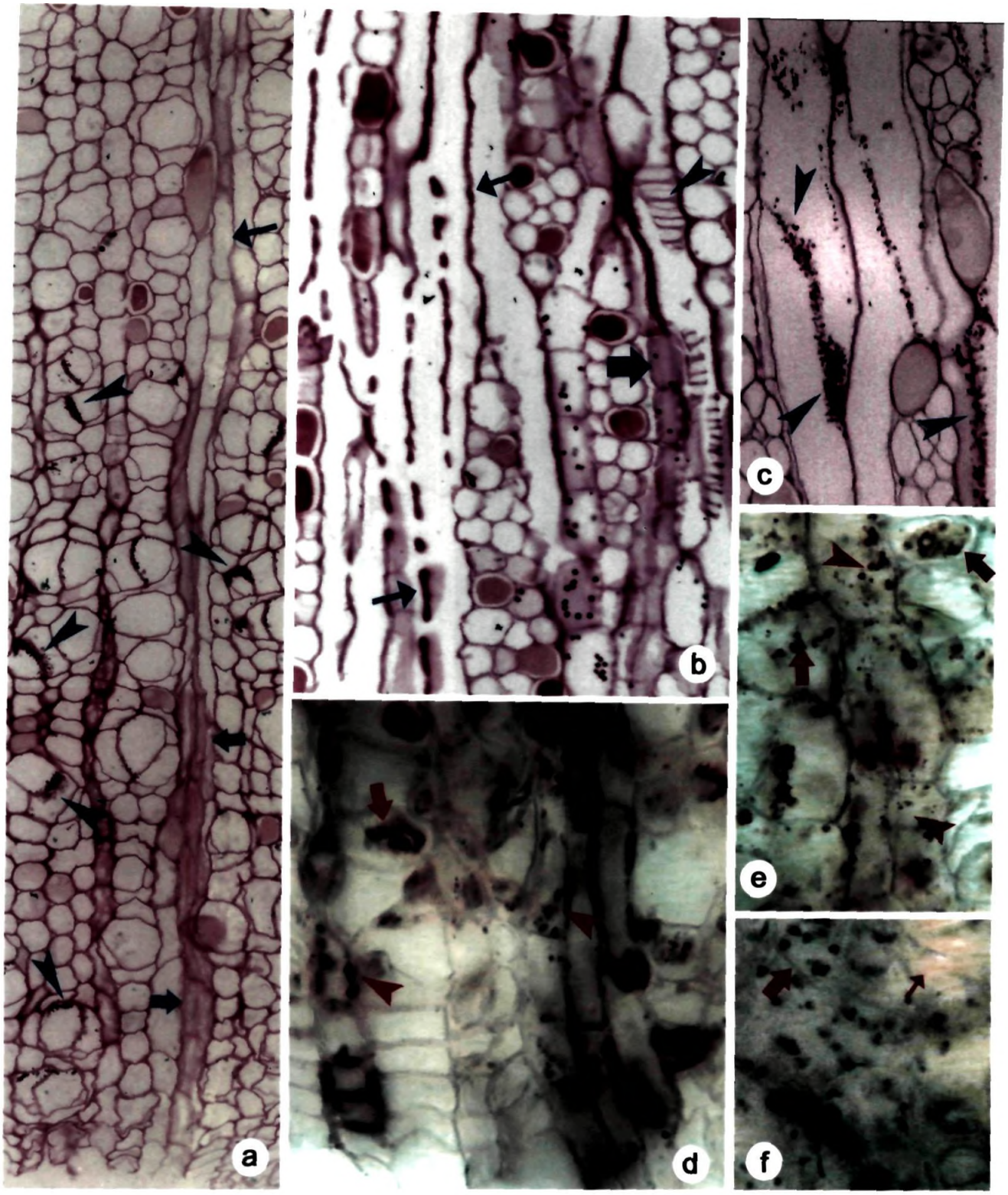


Figure 17. a to c - Histochemical localization of total polysaccharides in bark tissue. a- localization of polysaccharides in the cell walls and cytoplasm of rays (brad arrow) and sieve plates (arrow head) in soft bark. b- intense localization of polysaccharides in latex vessel walls. LV walls took thick and hard staining (thin arrows), and axial parenchyma (broad arrow) and sieve plates (arrow head). c- deposition of polysaccharides on sieve plates (arrow head)

d-f: Localization of lipids: d- lipid globules in ray cells (arrow head) and axial parenchyma (arrow). e- towards IHB more lipid localization in ray cells (arrow head) and axial parenchyma cells (arrow) in inner hard bark. f- lipid localization in hard bark in all unsclerified cells (broad arrow) & stone cells devoid of lipids (thin arrow).

a,d,e & f- CS, b &c – TLS. a- X75, b&c- X200, d,e&f- X100

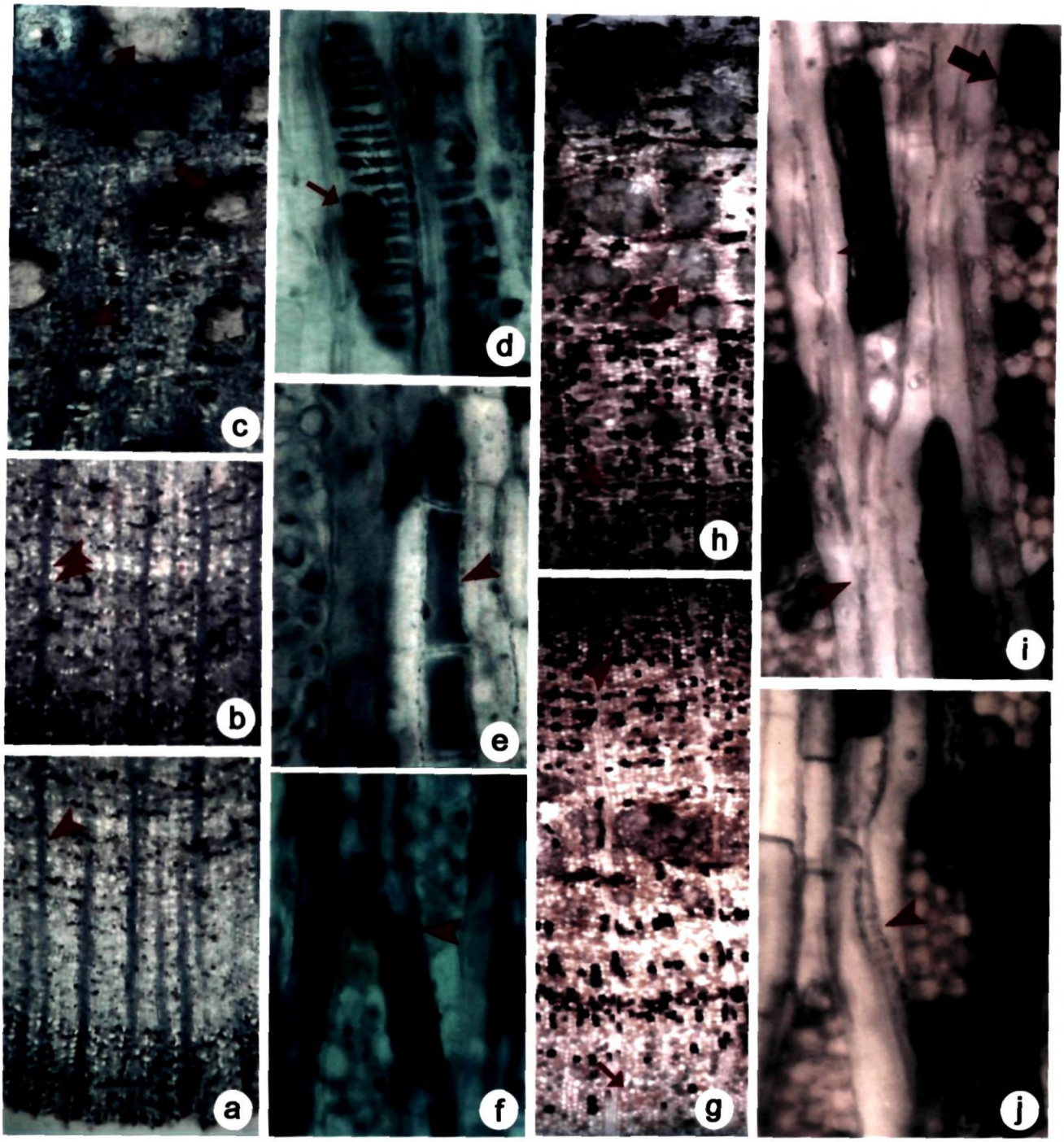


Figure 18. a-f- Histochemical localization of total proteins in soft bark, inner hard bark and outer hard bark. a- phloic rays in soft bark (arrow head), b- phloic rays in inner hard bark (arrow head), c- phloic rays in outer hard bark (arrow head) and surrounding stone cells (arrows)d- sieve plate (arrow) e- axial parenchyma. f- latex vessels.
g-h: Histochemical localization of Phenols in soft bark, inner hard bark and outer hard bark. g- less phenol accumulation in soft bark (arrow) and more phenolics towards the inner hard bark (arrow head). i- phloic ray showing intense localization (arrow) and latex vessels devoid of phenol (arrow head). j- sieve tubes lacking phenol (arrow head).
a, b, c, g & h-CS X30; d, e, f, i&j- TLS X300

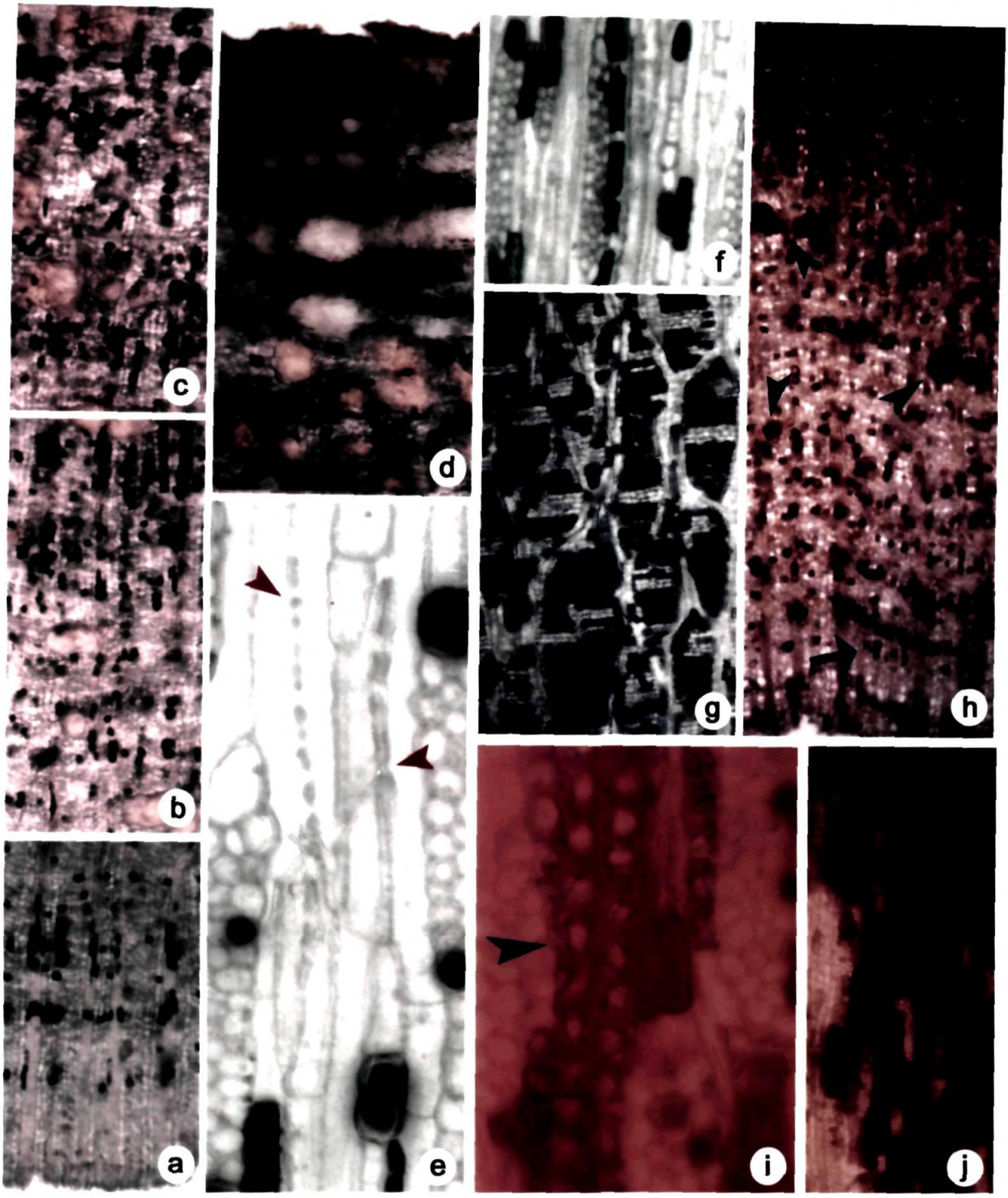


Figure 19. a-g: Histochemical localization of tannin content in soft bark, inner hard bark and outer hard bark. a- soft bark region. b- inner hard bark. c- hard bark. d- outermost hard bark zone. e- latex vessels lacking tannin content (arrow head); f- densely stained tanniferous axial parenchyma contiguous to rays. g- densely stained tanniferous rays in the outer hard bark.
h- j: Histochemical localization lignin. h- absence of lignification in soft bark zone (arrow). and lignified sclereids (arrow head) in the inner hard bark. i- lignification of axial parenchyma leading to stone cell formation (arrow head) j- later stage of lignification forming thick mass of stone cells.
a,b,c,d &h- CS. X30; e,f,g, i & j TLS. X200

Table 2. Angle of tree leaning, girth and bark thickness

Clones	Tree Leaning		Girth (cm)		Total bark thickness (mm)		Soft bark thickness (mm)			Inner hard Bark thickness (mm)			Outer hard bark thickness (mm)		
	Mean	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	%	**Mean**	CV (%)	%	**Mean**	CV (%)	%
Gl 1	0.12	71	90.83	19	10.11	25	1.55	28	15.33	2.17	44	21.46	6.38	29	63.11
GT 1	0.08	85	102.00	10	11.83	11	1.26	46	10.65	3.02	31	25.53	7.55	22	63.82
PB 235	0.08	73	128.22	29	10.89	28	2.08	33	19.10	3.82	46	35.08	4.98	28	45.73
PB 28/59	0.12	41	109.25	23	11.56	20	2.24	36	19.38	2.04	51	17.65	7.27	29	62.89
PB 86	0.12	61	101.67	12	10.33	18	1.10	41	10.65	3.75	19	36.30	5.48	34	53.05
RRII 105	0.13	58	79.11	18	9.78	19	1.90	58	19.43	2.49	55	25.46	5.39	31	55.11
RRII 300	0.08	72	95.22	25	9.06	21	1.46	42	16.11	1.54	42	17.00	6.06	27	66.89
RRIM 600	0.17	32	89.39	12	9.67	21	1.57	29	16.24	2.48	20	25.65	5.62	28	58.12
RRIM 703	0.22	36	87.22	12	13.28	8	1.23	43	9.26	2.90	25	21.84	9.15	9	68.90
Tjir I	0.11	90	103.78	10	11.89	18	1.77	13	14.89	1.82	35	15.31	8.29	24	69.72
V R (F)	*2.12*^{NS}		***3.24*****		***3.49*****		***1.89***^{NS}			***4.49*****			***4.77*****		
C D' (5%)			***22.73***		***2.05***					***1.08***			***1.89***		

**Significant for $p < 0.01$ ^{NS} Not significant

Table 3. Number and distance between laticifer rows in soft bark and inner hard bark and distance from cambium to 1st row of laticifers

Clones	Number of laticifer rows in soft bark			Number of laticifer rows in inner hard bark			Inter laticifer row distance in SB (mm)		Inter laticifer row distance in IHB (mm)		Distance from cambium to 1st LVR (mm)	
	Mean	CV (%)	%	**Mean**	CV (%)	%	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)
Gl 1	11.72	40	37.28	19.72	43	62.72	0.11	29	0.09	38	0.15	55
GT 1	8.83	62	27.26	23.56	43	72.74	0.12	42	0.09	15	0.19	48
PB 235	15.00	33	36.88	25.67	49	63.12	0.08	40	0.11	14	0.42	65
PB 28/59	20.06	44	51.94	18.56	57	48.06	0.07	11	0.09	27	0.21	39
PB 86	6.89	48	21.09	25.78	28	78.91	0.13	42	0.11	25	0.23	35
RRII 105	16.33	55	42.42	22.17	47	57.58	0.08	20	0.07	28	0.19	57
RRII 300	9.56	42	49.71	9.67	61	50.29	0.11	51	0.12	24	0.20	57
RRIM 600	11.28	31	42.12	15.50	33	57.88	0.10	34	0.13	32	0.18	45
RRIM 703	9.78	49	27.86	25.33	29	72.14	0.09	54	0.08	20	0.22	66
Tjir I	12.67	31	42.55	17.11	27	57.45	0.11	23	0.13	29	0.11	31
V R (F)	***3.61***			***2.69***			***2.04*** NS		***4.94***		***1.36*** NS	
C D (5%)	***6.12***			***9.51***					***0.03***			

*Significant for $p < 0.05$ **Significant for $p < 0.01$ NS Not significant

Table 4. Density, diameter, laticifer area index and frequency of interconnections between latex vessels

Clones	LV density contiguous to rays/row/1mm			LV density non contiguous to rays/row/1mm			Total LV density /row/1mm		Frequency of interconnections/ Unit area		Diameter of LV (µm)		Laticifer area index	
	Mean	CV (%	%	**Mean**	CV (%	%	**Mean**	CV (%	**Mean**	**CV (%)**	**Mean**	CV (%)	**Mean**	CV (%
Gl 1	25.27	4	88.11	2.85	23	10.55	28.12	4	18.54	7	25.52	12	43.15	45
GT 1	25.44	2	85.57	4.29	9	14.43	29.73	3	19.59	8	22.51	6	39.31	35
PB 235	22.73	3	86.10	3.96	9	14.14	26.69	3	17.64	11	24.72	15	76.17	58
PB 28/59	24.30	5	89.93	2.48	28	9.26	26.79	5	15.73	9	25.92	8	58.50	31
PB 86	23.72	4	88.87	4.28	6	14.92	28.00	4	18.71	13	24.99	7	46.18	33
RRII 105	24.65	4	88.32	2.37	27	8.88	27.02	4	22.11	6	23.64	7	37.91	45
RRII 300	24.95	4	88.73	2.96	29	10.65	27.91	6	20.94	15	25.49	13	27.62	50
RRIM 600	24.62	4	88.59	4.07	6	14.47	28.68	4	19.58	3	25.56	9	35.07	25
RRIM 703	24.13	8	90.07	2.27	35	8.60	26.40	7	18.20	20	21.63	7	29.80	24
Tjir I	24.78	2	88.50	3.01	14	10.78	27.79	2	19.16	4	24.06	15	39.09	44
V R (F)	***4.42***			***9.01***			***5.39***		***7.15***		***3.19***		***5.05***	
C D (5%)	***1.12***			***0.80***			***1.31***		***1.95***		***2.38***		***19.14***	

**Significant for $p < 0.01$

Table 5. Angle of inclination of latex vessels in soft bark

Clones	No. of trees	Left/Right	Mean (degrees)	*CV (%)
RRIM 703	9	RIGHT	**8.42**	33
GT 1	9	RIGHT	**5.75**	35
RRII 300	9	RIGHT	**5.13**	57
Tjir I	9	RIGHT	**4.27**	37
PB 235	9	RIGHT	**3.58**	80
GI 1	9	RIGHT	**3.36**	43
PB 86	1	LEFT and L	**1.15**	
		RIGHT R	**1.08**	
	1	RIGHT	**4.33**	
	7	LEFT	**4.27**	
RRII 105	8	LEFT and L	**2.10**	
		RIGHT R	**3.24**	
	1	RIGHT	**8.06**	
	Nil	LEFT		
PB 28/59	6	LEFT and L	**1.61**	
		RIGHT R	**4.01**	
	3	RIGHT	**4.21**	
	Nil	LEFT		
RRIM 600	5	LEFT and L	**1.44**	
		RIGHT R	**1.49**	
	1	RIGHT	**2.60**	
	3	LEFT	**2.51**	
Juvenile Seedling (RRII 105xMT 1005)	4	RIGHT	**3.84**	
Juvenile Seedling (RRIM 600xAC 495)	4	RIGHT	**2.55**	
Juvenile Budded plants(RRII 105)	4	RIGHT	**5.01**	
Juvenile Budded plants(RRIM 600)	3	RIGHT	**3.30**	
	1	LEFT	**2.14**	

* CV has been worked out only for rightward inclination of laticifers

Table 6. Angle of inclination of latex vessels in the inner hard bark

Clones	No. of trees	Left/Right	Mean (degrees)	*CV (%)
RRIM 703	9	RIGHT	**8.73**	29
GT 1	9	RIGHT	**7.01**	19
RRII 300	9	RIGHT	**5.50**	26
Tjir I	9	RIGHT	**4.51**	35
PB 235	9	RIGHT	**3.52**	42
GI 1	9	RIGHT	**4.63**	55
PB 86	1	LEFT and L	**0.80**	
		RIGHT R	**1.30**	
	1	RIGHT	**3.20**	
	7	LEFT	**4.42**	
RRII 105	7	LEFT and L	**2.42**	
		RIGHT R	**2.68**	
	2	RIGHT	**7.15**	
	Nil	LEFT		
PB 28/59	5	LEFT and L	**1.92**	
		RIGHT R	**2.84**	
	4	RIGHT	**6.24**	
	Nil	LEFT		
RRIM 600	3	LEFT and L	**2.05**	
		RIGHT R	**0.85**	
	3	RIGHT	**3.20**	
	3	LEFT	**3.13**	

* CV has been worked out only for rightward inclination of laticifers

Table 7. Angle of inclination of phloic rays in soft bark

Clones	No. of trees	Left / Right	Mean (degrees)	*CV (%)
RRIM 703	9	RIGHT	**7.13**	30
GT 1	9	RIGHT	**6.88**	25
RRII 300	9	RIGHT	**5.27**	58
Tjir I	9	RIGHT	**3.50**	42
PB 235	9	RIGHT	**3.59**	70
Gl 1	9	RIGHT	**3.09**	56
PB 86	1	LEFT and L	**2.21**	
		RIGHT R	**1.18**	
	1	RIGHT	**7.30**	
	7	LEFT	**5.21**	
RRII 105	7	LEFT and L	**2.02**	
		RIGHT R	**2.68**	
	2	RIGHT	**5.45**	
	Nil	LEFT		
PB 28/59	4	LEFT and L	**1.73**	
		RIGHT R	**1.28**	
	5	RIGHT	**5.81**	
	Nil	LEFT		
RRIM 600	5	LEFT and L	**1.33**	
		RIGHT R	**1.31**	
	1	RIGHT	**2.42**	
	3	LEFT	**1.61**	
Juvenile Seedling (RRII 105xMT 1005)	4	RIGHT	**3.15**	
Juvenile Seedling (RRIM 600xAC 495)	4	RIGHT	**2.69**	
Juvenile Budded plants(RRII 105)	4	RIGHT	**5.62**	
Juvenile Budded plants(RRIM 600)	3	RIGHT	**3.60**	
	1	LEFT	**1.75**	

* CV has been worked out for only rightward inclination of laticifers

Table 8. Angle of Inclination rays in the inner hard bark

Clones	No. of trees	Left / Right	Mean (degrees)	*CV (%)
RRIM 703	9	RIGHT	**8.95**	29
GT 1	9	RIGHT	**6.64**	27
RRII 300	9	RIGHT	**5.78**	57
Tjir I	9	RIGHT	**3.57**	33
PB 235	9	RIGHT	**3.89**	39
GI 1	9	RIGHT	**3.40**	58
PB 86	1	LEFT and L	**2.00**	
		RIGHT R	**1.08**	
	1	RIGHT	**3.25**	
	7	LEFT	**4.24**	
RRII 105	6	LEFT and L	**3.20**	
		RIGHT R	**3.59**	
	3	RIGHT	**4.13**	
	Nil	LEFT		
PB 28/59	4	LEFT and L	**1.55**	
		RIGHT R	**3.18**	
	5	RIGHT	**6.38**	
	Nil	LEFT		
RRIM 600	3	LEFT and L	**2.00**	
		RIGHT R	**0.85**	
	3	RIGHT	**3.20**	
	3	LEFT	**3.00**	

* CV has been worked out only for rightward inclination of laticifers

Table 9. Frequency of uni-; bi-; and multiseriate and total rays contiguous to laticifers per 765 µm distance in soft bark and inner hard bark

Clones	In SB								In IHB							
	Uniseriate		Biseriate		Multiseriate		Total ray Frequency		Uniseriate		Biseriate		Multiseriate		Total ray frequency	
	Mean	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)
Gl 1	0.89		0.17		6.72	22	7.78	14	0.00		0.00		5.22	11	5.22	11
GT 1	0.97		1.17		6.50	20	8.58	7	0.11		0.17		6.56	10	6.83	8
PB 235	1.17		0.00		7.00	9	8.17	9	0.00		0.00		5.78	9	5.78	9
PB 28/59	1.22		0.77		6.31	9	8.31	7	0.00		0.06		6.23	12	6.29	11
PB 86	0.72		0.06		6.72	13	7.50	6	0.00		0.00		6.00	16	6.00	16
RRII 105	1.88		0.00		5.98	11	7.86	13	0.33		0.03		6.00	11	6.20	12
RRII 300	1.47		0.06		6.89	12	8.47	5	0.17		0.00		5.75	14	5.92	10
RRIM 600	0.69		0.00		6.78	11	7.47	6	0.00		0.00		5.75	11	5.75	11
RRIM 703	0.78		0.44		6.17	12	7.39	11	0.00		0.00		5.83	15	5.83	15
Tjir I	1.17		0.06		6.94	15	8.17	3	0.00		0.00		6.22	7	6.22	7
V R (F)	***0.93*** *NS*		***3.10****		***0.46*** *NS*		***2.41*** *NS*		***2.43*** *NS*		***0.89*** *NS*		***1.59*** *NS*		***2.05*** *NS*	
C D (5%)			***0.69***													

NS Not significant

Table 10. Frequency of uni-; bi-; multiseriate rays in latex vessel free zone per 765 µm distance in SB and IHB

Clones	Soft bark								Hard bark							
	Uniseriate		Biseriate		Multiseriate		Total ray frequency		Uniseriate		Biseriate		Multiseriate		Total ray frequency	
	Mean	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)
Gl 1	0.58		0.00		5.58	10	6.17	11	0.00		0.00		5.00	11	5.00	11
GT 1	0.78		0.56		6.61	13	7.94	7	0.22		0.00		6.11	10	6.33	8
PB 235	1.06		0.00		6.78	12	7.72	8	0.00		0.00		5.67	16	5.67	16
PB 28/59	0.59		0.61		6.14	10	7.34	12	0.00		0.00		6.08	7	6.08	7
PB 86	0.22		0.06		6.44	8	6.72	11	0.11		0.00		6.28	17	6.39	15
RRII 105	1.15		0.00		6.28	13	7.42	13	0.00		0.00		5.16	9	5.16	9
RRII 300	1.50		0.00		5.86	16	7.42	10	0.00		0.00		5.64	11	5.64	11
RRIM 600	0.61		0.00		6.64	10	7.25	5	0.00		0.00		5.22	9	5.22	9
RRIM 703	0.00		0.78		6.36	8	7.06	11	0.00		0.00		5.47	10	5.47	10
Tjir I	1.06		0.00		7.18	8	8.06	7	0.00		0.00		5.61	11	5.61	11
V R (F)	*2.34 NS*		*6.23***		*3.55***		*3.76***		*0.87 NS*				*3.93***		*5.25***	
C D (5%)			*0.38*		*0.73*		*0.86*						*0.64*		*0.62*	

**Significant for $p < 0.01$ NS Not significant

Table 11. Height, width and height/width ratio of phloic rays contiguous to latex vessels per 765 µm distance in soft bark and inner hard bark

Clones	Soft bark						Hard bark					
	Height (µm)		Width (µm)		H/W Ratio		Height (µm)		Width (µm)		H/W Ratio	
	Mean	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)
GI 1	314.70	22	40.99	9	7.78	22	345.31	12	72.85	8	4.75	14
GT 1	376.04	8	36.11	19	10.50	14	354.02	11	54.25	19	6.59	23
PB 235	400.64	18	36.10	12	11.09	17	334.03	7	55.89	17	6.03	15
PB 28/59	326.26	11	39.51	18	8.42	19	335.34	16	62.64	14	5.38	17
PB 86	367.09	9	41.63	14	9.04	18	382.94	14	70.28	20	5.48	22
RRII 105	300.56	9	46.71	14	6.44	18	296.95	11	57.62	12	5.16	17
RRII 300	319.52	8	36.34	17	8.83	16	299.62	8	60.19	15	5.01	14
RRIM 600	297.08	13	40.90	11	7.26	7	293.45	9	70.27	5	4.18	9
RRIM 703	292.55	5	40.73	8	7.21	8	267.03	7	59.43	19	4.55	18
Tjir I	318.57	17	36.55	17	8.90	21	298.26	14	61.52	9	4.85	14
V R (F)	***2.45****		***2.33*** ^{NS}		***2.88****		***3.43****		***2.33*** ^{NS}		***4.29*** **	
C D (5%)	***70.20***				***2.56***		***56.37***				***1.02***	

*Significant for $p < 0.05$ **Significant for $p < 0.01$ ^{NS} Not significant

Table 12. Height, width and height/width ratio of phloic rays in latex vessel free zone in soft bark and inner hard bark

Clones	Soft bark						Hard bark					
	Height (µm)		Width (µm)		H/W ratio		Height (µm)		Width (µm)		H/W ratio	
	Mean	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)
Gl 1	381.65	8	49.39	16	7.85	18	380.51	11	78.04	9	4.88	16
GT 1	351.57	15	34.05	14	10.33	11	326.37	15	52.88	15	6.17	19
PB 235	387.87	16	43.96	17	9.48	21	315.66	11	66.63	9	4.73	9
PB 28/59	320.95	9	38.80	16	8.34	19	324.80	9	67.78	12	4.81	17
PB 86	380.36	11	40.88	20	9.53	22	344.74	14	57.13	21	6.10	23
RRII 105	315.79	11	45.82	14	7.04	13	287.06	12	74.54	11	3.86	16
RRII 300	321.68	22	35.47	16	9.10	24	287.25	7	67.76	21	4.30	24
RRIM 600	326.74	10	43.44	12	7.57	12	293.45	4	70.27	12	4.18	11
RRIM 703	307.30	4	40.73	23	7.61	18	277.03	8	62.77	17	4.44	19
Tjir I	318.84	14	36.00	15	8.86	18	315.82	12	64.36	8	4.93	12
V R (F)	***4.42****		***1.30**NS*		***1.59**NS*		***3.19****		***5.03****		***5.26****	
C D (5%)	***44.08***						***52.22***		***9.94***		***0.99***	

* Significant for $p < 0.05$ ** Significant for $p < 0.01$ *NS* Not significant

Table 13. Length and diameter of sieve tubes; number and area occupied by stone cells in inner hard bark and outer hard bark .

Clones	Sieve tube length (μm)		Sieve tube diameter (μm)		No. of stone cells rows in inner hard bark			Stone cell area in inner hard bark ($1x10^{-3}$ mm^2)		Stone cell area in outer hard bark ($1x10^{-3}$ mm^2)	
	Mean	CV (%)	**Mean**	CV (%)	**Mean**	CV (%)	In %	**Mean**	CV (%)	**Mean**	CV (%)
Gl 1	531.22	14	33.36	14	3.39	28	10.78	18.46	90	103.51	23
GT 1	441.90	14	31.97	12	7.22	22	22.29	21.39	46	101.61	29
PB 235	875.02	15	35.48	12	7.67	32	18.86	27.85	53	118.95	13
PB 28/59	588.23	11	34.70	7	5.67	39	14.68	2.45	24	6.74	25
PB 86	773.40	13	45.17	17	7.67	24	23.48	42.48	94	126.35	11
RRII 105	555.88	16	32.54	20	5.44	70	14.13	3.37	13	8.20	14
RRII 300	666.75	9	28.91	12	4.39	47	22.83	22.29	43	124.84	17
RRIM 600	555.92	7	37.70	13	5.72	28	21.36	37.27	73	121.42	8
RRIM 703	329.02	18	27.08	7	6.22	49	17.72	3.33	34	6.68	29
Tjir I	549.95	11	30.50	7	8.11	36	27.23	21.58	52	120.64	14
V R (F)	***16.5****		***10.92****		***1.84*** ^{NS}			***2.73****		***42****	
C D (5%)	***113.83***		***4.58***					***25.00***		***23.43***	

* Significant for $p < 0.05$ ** Significant for $p < 0.01$ ^{NS} Not significant

Table 14. Correlation among phloic ray characters in soft bark

				Phloic ray characters													
				Rays contiguous to latex vessels							Rays in LV free zone						
				R. W	R. H	H/W	TRF	M	B	U	R. W	R. H	H/W	TRF	M	B	U
				1	2	3	4	5	6	7	8	9	10	11	12	13	14
Phloic ray characters	Rays contiguous to latex vessels	R. W	1	1.000	-0.106	**-0.740****	**-0.452****	0.074	**-0.293***	**-0.284***	**0.406****	0.010	**-0.457****	**-0.355****	-0.136	-0.123	-0.180
		R. H	2		1.000	**0.726****	0.021	0.150	0.045	-0.169	-0.201	**0.457****	**0.535****	0.171	0.079	0.039	0.081
		H/W	3			1.000	**0.348****	0.032	**0.261***	0.101	**-0.407****	**0.271***	**0.650****	**0.348****	0.153	0.109	0.168
		T.R.F	4				1.000	**0.338****	**0.293***	**0.363****	-0.181	-0.102	0.120	**0.398****	0.236*	0.090	0.150
		M	5					1.000	**0.302****	**-0.522****	0.091	0.090	-0.043	0.071	**0.267***	**-0.289***	-0.025
		B	6						1.000	-0.135	-0.211	-0.072	0.193	**0.265***	-0.041	**0.694****	-0.095
		U	7							1.000	-0.112	-0.124	0.026	0.120	-0.025	-0.123	**0.249***
	Rays in latex vessel free zone	R. W	8								1.000	0.187	**-0.684****	**-0.293***	-0.030	-0.190	-0.176
		R. H	9									1.000	**0.519****	-0.061	0.157	-0.087	-0.161
		H/W	10										1.000	**0.305****	0.107	0.153	0.137
		T.R.F	11											1.000	**0.468****	0.182	**0.494****
		M	12												1.000	-0.131	**-0.358****
		B	13													1.000	**-0.247***
		U	14														1.000

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

R.W-Ray width; R.H- Ray height; H/W- height/width ratio; TRF- total ray frequency; M-frequency of multiseriate rays; B- frequency of biseriate rays; U- frequency of uniseriate rays

Table 15. Correlation among phloic ray characters in inner hard bark

				Phloic ray characters													
				Rays contiguous to latex vessels							Rays in LV free zone						
				R. W	R. H	H/W	TRF	M	B	U	R. W	R. H	H/W	TRF	M	B	U
				15	16	17	18	19	20	21	22	23	24	25	26	27	28
Phloic ray characters	Rays contiguous to latex vessels	R. W	15	1.000	0.120	**-0.712****	**-0.547****	**-0.432****	**-0.241***	-0.183	**0.557****	0.151	**-0.336****	**-0.381****	**-0.307****	a	**-0.274***
		R. H	16		1.000	**0.567****	0.052	-0.017	0.180	0.086	-0.182	**0.680****	**0.567****	0.210	0.131	a	**0.279***
		H/W	17			1.000	**0.494****	**0.317****	**0.484****	0.191	**-0.556****	**0.339****	**0.668****	**0.462****	**0.321****	a	**0.508****
		T.R.F	18				1.000	**0.930****	0.200	0.099	**-0.407****	0.038	**0.369****	**0.542****	**0.471****	a	**0.321****
		M	19					1.000	-0.027	-0.157	**-0.349****	0.034	**0.306****	**0.513****	**0.495****	a	0.133
		B	20						1.000	-0.044	-0.170	0.177	**0.297***	0.176	0.036	a	**0.502****
		U	21							1.000	-0.040	-0.120	-0.039	-0.019	-0.078	a	0.200
	Rays in latex vessel free zone	R. W	22								1.000	0.022	**-0.764****	**-0.658****	**0.589****	a	**-0.287***
		R. H	23									1.000	**0.588****	0.111	0.098	a	0.046
		H/W	24										1.000	**0.635****	**0.565****	a	**0.275***
		T.R.F	25											1.000	**0.964****	a	0.195
		M	26												1.000	a	-0.062
		B	27													a	a
		U	28														1.000

* Significant for $p < 0.05$ ** Significant for $p < 0.01$ [a] variable is absent

R.W-Ray width; R.H- Ray height; H/W- height/width ratio; TRF- total ray frequency; M-frequency of multiseriate rays; B- frequency of biseriate rays; U-frequency of uniseriate rays

Table 16. Correlation among all other characters

		STL	STD	LV dia.	FIC	TLV Den	LVD CR	LVD N CR	DC 1 LVR	SBT	NLVR SB	DR SB	IHBT	NLVR IHB	DR IHB	NSR IHB	OHBT	TBT	SCA IHB	SCA HB	Girth	Slope	LAI
		29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
STL	29	1.000	**0.504****	0.209	0.074	-0.131	**-0.340****	**0.245***	0.198	0.063	0.023	-0.083	0.190	0.005	0.207	**0.260***	**-0.426****	-0.263	**0.274***	**0.373****	0.212	-0.195	**0.274***
STD	30		1.000	0.207	0.014	0.125	-0.152	**0.404****	0.033	-0.068	-0.114	0.074	**0.328****	0.146	0.107	**0.247***	-0.211	-0.643	0.091	**0.247***	0.106	0.121	0 207
LV dia.	31			1.000	-0.200	-0.036	-0.098	0.073	0.050	**0.326****	0.172	0.053	-0.039	-0.094	0.040	-0.107	0.011	0.086	0.058	0.167	0.157	0.056	**0.449****
FIC	32				1.000	**0.311****	0.313**	0.081	-0.073	**-0.233***	-0.213	0.075	-0.180	**-0.239***	0.191	-0.142	-0.194	**-0.330****	0.132	**0.246***	**-0.407****	0.012	**-0.459****
TLV Den	33					1.000	**0.784****	**0.556****	**-0.302***	-0.168	-0.164	0.089	-0.068	-0.128	0.163	0.062	-0.098	-0.170	**0.236***	**0.474****	-0.140	-0.143	-0.030
LVD CR	34						1.000	-0.080	**-0.292***	-0.098	-0.027	-0.026	**-0.265***	-0.173	-0.009	-0.107	0.053	-0.114	-0.051	0.073	**-0.272***	-0.113	**-0.238***
LVD NCR	35							1.000	-0.094	-0.137	-0.227	0.177	**0.246***	0.026	**0.273***	**0.242***	-0.229	-0.120	**0.447****	**0.664****	0.140	-0.079	0.110
DC 1 LVR	36								1.000	0.162	0.031	-0.068	0.176	0.063	0.050	-0.106	-0.226	-0.066	0.112	-0.045	0.166	0.057	0.088
SBT	37									1.000	**0.848****	-0.128	**-0.244***	-0.218	-0.094	-0.140	-0.0.98	0.085	0.007	-0.211	0.175	0.029	**0.425****
NLVR SB	38										1.000	**-0.476****	**-0.332****	**-0.232***	-0.174	-0.218	-0.023	0.063	-0.120	**-0.338****	0.103	-0.017	**0.381****
DR SB	39											1.000	0.063	-0.102	**0.292***	0.022	0.082	0.067	0.231	**0.287***	0.034	-0.016	-0.214
IHBT	40												1.000	**0.879****	-0.174	**0.633****	0.006	**0.433****	0.027	-0.014	**0.491****	0.079	**0.554****
NLVR IHB	41													1.000	**0.523****	**0.550****	0.162	**0.518****	-0.166	-0.228	**0.447****	0.039	**0.584****
DR IHB	42														1.000	-0.009	**-0.281***	**-0.363****	**0.501****	**0.589****	-0.100	-0.007	**-0.321****
NSR IHB	43															1.000	0.038	**0.308****	0.090	0.080	**0.358****	-0.008	**0.359****
OHBT	44																1.000	**0.859****	**-0.366****	**-0.241***	**0.298***	0.108	0.120
TBT	45																	1.000	**-0.308****	**-0.281***	**0.580****	0.143	**0.507****
SCA IHB	46																		1.000	**0.611****	0.019	-0.171	-0.044
SCA HB	47																			1.000	0.072	-0.148	-0.074
Girth	48																				1.000	-0.128	**0.759****
Slope	49																					1.000	-0.086
LAI	50																						1.000

* Significant for $p < 0.05$ ** Significant for $p < 0.01$ [a] variable is absent

29. STL -Sieve tube length; 30.STD-Sieve tube diameter; 31.LV Dia- Latex vessel diameter; 32. FIC- Frequency of interconnections /unit area; 33. TLV Den -Total vessel density; 34. LVD CR - Latex vessel density contiguous to rays; 35. LVD NCR- Latex density non contiguous to rays; 36. DC 1LVR -Distance from cambium to 1st latex vessel row, 37. SBT- Soft bark thickness; 38. NLVR SB- Number of latex vessel rows in soft bark; 39. DR SB- Distance between adjacent rows in SB; 40. IHB- Thickness of inner hard bark; 41. NLVR IHB- Number of latex vessel rows in inner had bark; 42. DR IHB-Distance between adjacent rows in inner hard bark; 43. NSR IHB- Number of stone cell rows in inner hard bark; 44. OHBT-Thickness of Outer hard bark; 45. TBT-Total bark thickness; 46. SCA IHB- Stone cell area in inner hard bark; 47. SCA HB- Stone cell area in Hard bark; 48. Girth- Girth of the tree; 49. Slope- Leaning angle of Trees; 50. LAI- Total cross sectional area of latex vessels (Laticifer Area Index)

Table 17. Correlation between phloic ray characters in soft bark and inner hard bark

				Phloic ray characters in inner hard bark													
				Rays contiguous latex vessels							Rays in latex vessels free zone						
				R. W	R. H	H/W	TRF	M	B	U	R. W	R. H	H/W	TRF	M	B	U
				15	16	17	18	19	20	21	22	23	24	25	26	27	28
Phloic ray characters in soft bark	Rays contiguous to latex vessels	R. W	1	**0.318****	-0.038	**-0.326****	**-0.253***	-0.216	-0.176	0.149	0.171	-0.070	-0.162	-0.106	-0.040	a	**0.249***
		R. H	2	**-0.260***	**0.413****	**0.481****	0.119	0.097	0.033	-0.024	**-0.326****	0.225	**0.379****	**0.258***	0.204	a	0.180
		H/W	3	**-0.442****	**0.318****	**0.611****	**0.288***	0.217	**0.233***	-0.05	**-0.362****	0.224	**0.408****	**0.280***	0.185	a	**0.341****
		T.R.F	4	**-0.330****	-0.023	**0.259***	**0.347****	**0.320****	0.143	-0.107	-0.189	-0.038	0.146	**0.343****	**0.335****	a	0.046
		M	5	-0.009	-0.047	-0.036	0.188	**0.234***	-0.078	-0.151	-0.143	-0.037	0.082	**0.287***	**0.305****	a	-0.014
		B	6	**-0.300***	0.097	**0.353****	**0.352****	**0.305****	0.215	-0.025	**-0.306****	0.023	**0.281***	**0.292***	0.229	a	0.225
		U	7	-0.078	-0.028	0.033	-0.126	-0.166	0.057	0.079	0.193	0.008	-0.134	-0.197	-0.176	a	-0.110
	Rays in LV free zone	R. W	8	0.211	-0.011	-0.178	**-0.260***	**-0.249***	-0.075	0.068	**0.242***	-0.002	-0.183	-0.216	-0.181	a	-0.135
		R. H	9	0.171	**0.445****	0.165	-0.130	-0.174	-0.038	0.061	-0.040	**0.398****	**0.262***	-0.010	-0.041	a	0.068
		H/W	10	-0.203	**0.326****	**0.387****	0.225	0.191	0.064	-0.041	**-0.297***	**0.259***	**0.369****	**0.239***	0.178	a	0.196
		T.R.F	11	**-0.383****	-0.017	**0.296***	**0.486****	**0.518****	0.061	-0.227	-0.13	0.009	0.136	0.169	0.151	a	0.079
		M	12	-0.202	-0.028	0.158	**0.306****	**0.306****	-0.008	-0.094	-0.198	-0.001	0.191	0.196	0.164	a	0.107
		B	13	-0.104	-0.017	0.061	0.179	0.227	-0.071	-0.108	-0.127	-0.121	0.018	0.126	0.130	a	-0.025
		U	14	-0.161	0.008	0.125	0.082	0.092	0.106	-0.091	0.118	0.088	-0.043	-0.071	-0.060	a	-0.014

* Significant for $p < 0.05$ ** Significant for $p < 0.01$ [a] variable is absent

R.W-Ray width; R.H- Ray height; H/W- height/width ratio; TRF- total ray frequency; M-frequency of multiseriate rays; B- frequency of biseriate rays; U- frequency of uniseriate rays

Table 18. Correlation between phloic ray characters in soft bark and other parameters

			Phloic ray characters in soft bark													
			Rays contiguous to latex vessels							Rays in latex vessel free zone						
			R. W	R. H	H/W	TRF	M	B	U	R. W	R. H	H/W	TRF	M	B	U
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
All other parameters	STL	29	-0.207	**0.287***	**0.347****	0.177	0.147	**-0.324****	0.240	0.074	0.219	0.145	0.094	0.129	**-0.362****	0.204
	STD	30	-0.127	**0.275***	**0.243***	-0.021	0.122	-0.100	-0.072	-0.24	0.196	0.162	-0.065	0.125	-0.151	-0.085
	LV dia.	31	-0.070	0.144	0.113	-0.052	0.071	-0.103	-0.050	0.092	0.012	-0.007	-0.034	-0.227	-0.115	0.175
	FIC	32	0.021	-0.014	0.008	0.072	0.088	-0.111	0.049	0.043	-0.028	-0.036	0.048	0.124	**-0.246***	0.088
	TLV Den	33	-0.221	0.123	0.218	0.021	-0.011	0.156	-0.075	-0.119	-0.001	0.080	0.040	0.014	-0.082	0.065
	LVD CR	34	0.005	-0.124	-0.077	0.043	-0.010	0.107	-0.025	-0.008	-0.213	-0.182	-0.110	-0.109	-0.063	0.007
	LVD NCR	35	**-0.361****	**0.364****	**0.453****	-0.025	-0.004	0.106	-0.086	-0.180	**0.283***	**0.371****	0.212	0.168	-0.048	0.095
	DC 1 LVR	36	0.034	0.221	0.147	0.049	0.155	-0.053	-0.094	-0.051	-0.018	0.036	-0.219	0.012	-0.068	-0.191
	SBT	37	0.098	-0.020	-0.082	-0.129	-0.191	-0.018	0.102	-0.027	-0.052	0.046	-0.027	-0.101	0.006	0.099
	NLVR SB	38	0.156	-0.092	-0.154	-0.162	**-0.342****	0.021	0.208	0.119	-0.065	-0.084	-0.032	-0.079	0.027	0.056
	DR SB	39	0.000	0.188	0.097	0.014	**0.364****	-0.093	**-0.309****	-0.188	0.151	**0.256***	-0.030	-0.055	-0.051	0.014
	IHBT	40	-0.053	**0.320****	**0.257***	-0.092	0.087	0.035	-0.187	0.000	0.203	0.156	0.049	0.222	-0.002	-0.163
	NLVR IHB	41	-0.042	**0.258***	0.220	0.005	0.022	0.091	-0.064	-0.026	0.133	0.122	0.081	0.157	0.069	-0.097
	DR IHB	42	-0.075	-0.089	-0.041	-0.127	0.185	-0.152	-0.223	0.078	0.054	-0.043	-0.104	0.091	**-0.244***	-0.070
	NSR IHB	43	-0.198	0.195	**0.254***	0.070	0.009	0.050	0.036	-0.056	0.121	0.188	**0.291***	**0.312****	0.050	-0.036
	OHBT	44	0.125	0.026	-0.066	0.064	0.197	0.050	-0.172	-0.044	0.030	0.120	0.019	0.176	0.121	0.040
	TBT	45	0.113	0.177	0.046	-0.028	0.161	0.057	-0.215	-0.047	0.049	0.118	0.123	0.098	0.156	-0.017
	SCA IHB	46	-0.084	0.037	0.079	-0.041	0.091	-0.154	-0.020	0.020	0.177	0.091	-0.056	0.211	**-0.245***	-0.094
	SCA OHB	47	**-0.296***	**0.291***	**0.366****	0.014	0.145	-0.124	-0.051	-0.112	**0.284***	**0.295***	0.046	0.052	**-0.303****	0.178
	Girth	48	-0.056	**0.327****	**0.245***	0.025	0.176	0.009	-0.154	-0.058	0.227	**0.249***	0.055	0.014	-0.069	0.097
	Slope	49	0.090	-0.113	-0.167	**-0.265***	-0.006	-0.083	-0.172	-0.157	-0.225	-0.045	-0.040	-0.015	0.043	-0.045
	LAI	50	-0.062	**0.345****	**0.267***	-0.074	-0.042	0.004	-0.008	0.083	0.223	0.161	0.029	-0.015	-0.064	0.107

* Significant for $p < 0.05$ ** Significant for $p < 0.01$ [a] variable is absent

R.W-Ray width; R.H- Ray height; H/W- height/width ratio; TRF- total ray frequency; M-frequency of multiseriate rays; B- frequency of biseriate rays; U- frequency of uniseriate rays

29. STL -Sieve tube length; 30.STD-Sieve tube diameter; 31.LV Dia- Latex vessel diameter; 32. FIC- Frequency of interconnections /unit area; 33. TLV Den -Total vessel density; 34. LVD CR -Latex vessel density contiguous to rays; 35. LVD NCR- Latex density non contiguous to rays; 36. DC 1LVR -Distance from cambium to 1st latex vessel row, 37. SBT- Soft bark thickness; 38. NLVR SB- Number of latex vessel rows in soft bark; 39. DR SB- Distance between adjacent rows in SB; 40. IHB- Thickness of inner hard bark; 41. NLVR IHB- Number of latex vessel rows in inner hard bark; 42. DR IHB-Distance between adjacent rows in inner hard bark; 43. NSR IHB- Number of stone cell rows in inner hard bark; 44. OHBT-Thickness of Outer hard bark; 45. TBT- Total bark thickness; 46. SCA IHB- Stone cell area in inner hard bark; 47. SCA OHB- Stone cell area in outer hard bark; 48. Girth- Girth of the tree; 49. Slope- Leaning angle of Trees; 50. LAI- Total cross sectional area of latex vessels (Laticifer Area Index)

Table 19. Correlation between phloic ray characters in inner hard bark and all other parameters

			Phloic ray characters in inner hard bark													
			Rays contiguous to latex vessels							Rays in LV free zone						
			R. W	R. H	H/W	TRF	M	B	U	R. W	R. H	H/W	TRF	M	B	U
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
All other parameters	STL	29	-0.096	0.213	0.205	-0.039	-0.034	-0.076	0.031	0.049	0.223	0.091	0.104	0.128	a	-0.083
	STD	30	-0.008	0.189	0.125	0.061	0.138	-0.096	-0.180	-0.167	0.196	**0.236***	0.217	0.220	a	-0.001
	LV dia.	31	0.109	0.191	0.003	-0.086	-0.039	-0.153	-0.077	0.126	0.148	-0.017	0.025	0.070	a	-0.165
	FIC	32	-0.225	-0.148	0.075	0.095	0.030	-0.062	0.025	0.031	-0.116	-0.088	-0.136	-0.165	a	0.099
	TLV Den	33	-0.076	0.199	0.214	-0.012	-0.027	0.033	0.002	**-0.240***	0.199	**0.322****	0.006	-0.043	a	0.211
	LVD CR	34	-0.153	-0.026	0.097	-0.011	-0.032	0.068	0.031	-0.158	0.071	0.170	-0.088	-0.112	a	0.106
	LVD NCR	35	0.082	**0.355****	0.213	-0.004	-0.001	-0.038	-0.039	-0.174	0.224	**0.290***	0.128	0.082	a	0.197
	DC 1 LVR	36	-0.122	-0.033	0.107	0.080	0.077	0.031	0.019	-0.152	-0.182	-0.018	0.237	0.243	a	-0.025
	SBT	37	-0.010	0.061	0.010	-0.153	-0.096	-0.125	-0.058	0.072	-0.024	-0.136	-0.162	-0.122	a	-0.205
	NLVR SB	38	0.011	-0.005	-0.045	-0.149	-0.112	-0.026	-0.022	0.132	0.021	-0.126	-0.183	-0.133	a	-0.225
	DR SB	39	0.023	0.130	0.047	0.062	0.092	-0.086	-0.074	-0.203	-0.005	0.110	0.128	0.079	a	0.157
	IHBT	40	-0.186	**0.255***	**0.377****	0.117	0.067	0.164	-0.061	-0.192	0.127	**0.270***	**0.273***	0.211	a	**0.241***
	NLVR IHB	41	-0.228	0.225	**0.381****	0.163	0.101	0.231	-0.063	-0.162	0.186	**0.277***	**0.236***	0.171	a	**0.240***
	DR IHB	42	0.215	-0.100	-0.213	-0.104	-0.101	-0.096	0.018	0.075	-0.137	-0.147	-0.072	-0.052	a	-0.071
	NSR IHBOHBT	43	-0.129	0.224	**0.272***	0.195	0.206	-0.021	-0.121	-0.124	0.200	**0.248***	0.161	0.131	a	0.112
	OHBT	44	-0.069	-0.100	-0.017	0.153	0.144	0.168	-0.145	-0.179	0.050	0.190	**0.235***	**0.236***	a	0.013
	TBT	45	-0.156	0.056	0.176	0.149	0.133	0.193	-0.176	**-0.233***	0.101	**0.262***	**0.297***	**0.278***	a	0.071
	SCA IHB	46	**0.298***	**0.283***	-0.033	-0.212	-0.182	-0.124	-0.051	-0.011	0.140	0.097	-0.040	-0.050	a	0.012
	SCA HB	47	0.166	0.212	0.022	-0.156	-0.128	-0.120	-0.078	-0.062	**0.241***	0.198	0.003	-0.008	a	0.059
	Girth	48	-0.050	0.214	0.185	0.023	0.009	0.096	-0.121	-0.034	0.152	0.111	**0.251***	0.229	a	0.081
	Slope	49	0.052	**-0.122**	**-0.171**	-0.048	0.039	-0.212	-0.112	0.040	-0.199	-0.137	-0.097	-0.067	a	-0.086
	LAI	50	-0.126	**0.353****	**0.329****	-0.040	-0.044	0.066	-0.104	-0.027	**0.281***	0.185	0.155	0.148	a	0.008

* Significant for $p < 0.05$ * * Significant for $p < 0.01$ [a] variable is absent

R.W-Ray width; R.H- Ray height; H/W- height/width ratio; TRF- total ray frequency; M-frequency of multiseriate rays; B- frequency of biseriate rays; U- frequency of uniseriate rays

29. STL -Sieve tube length; 30.STD-Sieve tube diameter; 31.LV Dia- Latex vessel diameter; 32. FIC- Frequency of interconnections /unit area; 33. TLV Den -Total vessel density; 34. LVD CR -Latex vessel density contiguous to rays; 35. LVD NCR- Latex vessel density non contiguous to rays; 36. DC 1LVR -Distance from cambium to 1st latex vessel row, 37. SBT- Soft bark thickness; 38. NLVR SB- Number of latex vessel rows in soft bark; 39. DR SB- Distance between adjacent rows in SB; 40. IHB- Thickness of inner hard bark; 41. NLVR IHB- Number of latex vessel rows in inner hard bark; 42. DR IHB- Distance between adjacent rows in inner hard bark; 43. NSR IHB- Number of stone cell rows in inner hard bark; 44. OHBT-Thickness of Outer hard bark; 45. TBT- Total bark thickness; 46. SCA IHB- Stone cell area in inner hard bark; 47. SCA OHB- Stone cell area in outer hard bark; 48. Girth- Girth of the tree; 49. Slope- Leaning angle of Trees; 50. LAI- Total cross sectional area of latex vessels (Laticifer Area Index)

Table 20. Correlation of laticifer inclination with all other characters in soft bark and inner hard ba (trees having only rightward inclination)

			Inclination of laticifers to right in soft bark	*Inclination of laticifers to right in inner hard bark*
Soft bark	*Inclination of laticifers to right*		1.000	**0.699****
	Rays contiguous to LV in SB	Ray width	0.205	0.140
		Ray height	-0.088	-0.083
		Height/Width Ratio	-0.199	-0.130
		Total Ray frequency	-0.169	-0.239
		Frequency of Multiseriate Rays	0.066	-0.170
		Frequency of Biseriate Rays	0.048	0.163
		Frequency of Uniseriate Rays	-0.245	-0.125
	Rays in LV free zone in SB	*Inclination of rays to Right*	**0.862****	**0.688****
		Ray width	-0.111	-0.231
		Ray height	-0.139	-0.182
		Height/Width Ratio	-0.049	0.045
		Total Ray frequency	0.001	0.016
		Frequency of Multiseriate Rays	0.157	0.010
		Frequency of Biseriate Rays	0.269	0.215
		Frequency of Uniseriate Rays	-0.267	-0.183
Inner hard bark	*Inclination of LVs to Right*		**0.699****	1.000
	Rays contiguous to LV in IHB	Ray width	-0.039	-0.050
		Ray height	-0.246	-0.117
		Height/Width Ratio	-0.120	0.027
		Total Ray frequency	0.177	0.171
		Frequency of Multiseriate Rays	0.217	0.104
		Frequency of Biseriate Rays	-0.043	0.207
		Frequency of Uniseriate Rays	-0.063	0.021
	Rays in LV free zone in IHB	*Inclination of rays to Right*	**0.778****	**0.850****
		Ray width	-0.057	-0.071
		Ray height	-0.326	-0.185
		Height/Width Ratio	-0.123	0.007
		Total Ray frequency	0.026	0.071
		Frequency of Multiseriate Rays	-0.002	0.000
		Frequency of Biseriate Rays	a	a
		Frequency of Uniseriate Rays	0.092	0.238
All other parameters		STL	-0.293	0.001
		STD	-0.084	-0.187
		LV dia.	**-0.384***	**-0.430***
		FIC	-0.018	0.078
		TLV Den	-0.053	0.143
		LVD CR	0.089	0.230
		LVD NCR	-0.230	-0.084
		DC 1 LVR	-0.142	0.334
		SBT	-0.137	-0.239
		NLVR SB	0.033	-0.101
		DR SB	**-0.320***	-0.099
		IHBT	0.205	0.018
		NLVR IHB	0.297	0.134
		DR IHB	**-0.389***	**-0.339***
		NSR IHBOHBT	-0.040	-0.144
		OHBT	0.298	0.003
		TBT	0.277	0.262
		SCA IHB	**-0.487***	**-0.377***
		SCAO HB	**-0.519***	**-0.365***
		Girth	0.084	-0.227
		Slope	0.239	0.152
		LAI	-0.026	-0.287

* Significant for $p < 0.05$ ** Significant for $p < 0.01$ [a] variable is absent

STL -Sieve tube length; STD-Sieve tube diameter; LV Dia- Latex vessel diameter; FIC- Frequency of interconnections /unit area; TLV Den vessel density; LVD CR -Latex vessel density contiguous to rays; LVD NCR- Latex vessel density non contiguous to rays; DC 1LVR -Di from cambium to 1st latex vessel row, SBT- Soft bark thickness; NLVR SB- Number of latex vessel rows in soft bark; DR SB- Distance be adjacent rows in SB; IHB- Thickness of inner hard bark; NLVR IHB- Number of latex vessel rows in inner hard bark; DR IHB-Distance be adjacent rows in inner hard bark; NSR IHB- Number of stone cell rows in inner hard bark; OHBT-Thickness of Outer hard bark; TBT- Tota thickness; SCA IHB- Stone cell area in inner hard bark; SCA OHB- Stone cell area in outer hard bark; Girth- Girth of the tree; Slope- Le angle of Trees; LAI- Laticifer Area Index

Table 21. Correlation of laticifer inclination with all other characters in soft bark and inner hard bark (in trees having only leftward inclination)

			Inclination of laticifers to left in soft bark	*Inclination of laticifers to left in Inner hard bark*
Soft bark		*Inclination of LV to left*	1.000	0.597
	Rays contiguous to LV in SB	Ray width	-0.075	0.344
		Ray height	-0.505	-0.441
		Height/Width Ratio	-0.380	-0.580
		Total Ray frequency	0.279	0.117
		Frequency of Multiseriate Rays	0.018	0.027
		Frequency of Biseriate Rays	-0.521	-0.125
		Frequency of Uniseriate Rays	0.284	0.242
	Rays in LV free zone in SB	*Inclination of rays to left*	**0.910****	0.562
		Ray width	0.021	0.341
		Ray height	-0.031	0.445
		Height/Width Ratio	-0.028	-0.009
		Total Ray frequency	0.578	-0.048
		Frequency of Multiseriate Rays	0.362	-0.082
		Frequency of Biseriate Rays	-0.521	-0.214
		Frequency of Uniseriate Rays	0.350	0.377
Inner hard bark		*Inclination of LVs to left*	0.597	1.000
	Rays contiguous to LV in IHB	Ray width	-0.165	0.320
		Ray height	-0.231	-0.151
		Height/Width Ratio	-0.569	-0.540
		Total Ray frequency	-0.137	-0.487
		Frequency of Multiseriate Rays	-0.137	-0.487
		Frequency of Biseriate Rays	a	a
		Frequency of Uniseriate Rays	a	a
	Rays in LV free zone in IHB	*Inclination of rays to left*	**0.888****	**0.761***
		Ray width	0.446	-0.145
		Ray height	-0.228	0.033
		Height/Width Ratio	-0.642	0.158
		Total Ray frequency	-0.441	0.133
		Frequency of Multiseriate Rays	-0.205	0.480
		Frequency of Biseriate Rays	a	a
		Frequency of Uniseriate Rays	-0.521	-0.421
All other parameters		STL	-0.293	0.001
		STD	-0.084	-0.187
		LV dia.	0.389	0.300
		FIC	-0.140	-0.667
		TLV Den	-0.237	-0.353
		LVD CR	-0.216	-0.408
		LVD NCR	-0.138	0.370
		DC 1 LVR	-0.142	0.334
		SBT	0.279	-0.196
		NLVR SB	0.535	0.182
		DR SB	-0.578	-0.560
		IHBT	**-0.815***	-0.628
		NLVR IHB	-0.624	-0.347
		DR IHB	-0.111	-0.174
		NSR IHBOHBT	-0.510	**-0.724***
		OHBT	0.298	0.003
		TBT	-0.166	-0.511
		SCA IHB	**-0.713***	-0.107
		SCAO HB	-0.286	0.007
		Girth	-0.188	-0.121
		Slope	0.623	0.119
		LAI	-0.159	-0.086

* Significant for $p < 0.05$ ** Significant for $p < 0.01$ [a] variable is absent

STL -Sieve tube length; STD-Sieve tube diameter; LV Dia- Latex vessel diameter; FIC- Frequency of interconnections /unit area; TLV Den - Total vessel density; LVD CR -Latex vessel density contiguous to rays; LVD NCR- Latex vessel density non contiguous to rays; DC 1LVR - Distance from cambium to 1st latex vessel row, SBT- Soft bark thickness; NLVR SB- Number of latex vessel rows in soft bark; DR SB- Distance between adjacent rows in SB; IHB- Thickness of inner hard bark; NLVR IHB- Number of latex vessel rows in inner hard bark; DR IHB-Distance between adjacent rows in inner hard bark; NSR IHB- Number of stone cell rows in inner hard bark; OHBT-Thickness of Outer hard bark; TBT- Total bark thickness; SCA IHB- Stone cell area in inner hard bark; SCA OHB- Stone cell area in outer hard bark; Girth- Girth of the tree; Slope- Leaning angle of Trees; LAI- Laticifer Area Index

Table 22. Correlation of laticifer inclination with all the other characters in soft bark and inner hard bark (in trees having both left and rightward inclination)

			Soft bark		Inner hard bark	
			Inclination of LV to left	*Inclination of LV to right*	*Inclination of LV to left*	*Inclination of LV to right*
Soft bark		*Inclination of LV to left*	1.000	0.076	0.036	0.360
		Inclination of LV to Right	0.076	1.000	-0.140	**0.867****
	Rays contiguous to LV in SB	Ray width	-0.282	0.217	-0.269	0.249
		Ray height	0.262	-0.178	0.062	-0.214
		Height/Width Ratio	0.324	-0.255	0.164	-0.299
		Total Ray frequency	-0.212	0.284	0.225	0.026
		Freq. of Multiseriate Rays	0.032	0.163	0.309	-0.009
		Frequency of Biseriate Rays	-0.010	0.314	0.135	0.268
		Frequency of Uniseriate Rays	-0.278	-0.022	-0.045	-0.149
	Rays in LV free zone in SB	*Inclination of rays to Left*	**0.521***	0.120	-0.142	0.202
		Inclination of rays to Right	0.226	**0.686****	-0.303	**0.809****
		Ray width	-0.283	0.025	-0.141	0.100
		Ray height	0.260	-0.285	0.360	-0.232
		Height/Width Ratio	0.333	-0.211	0.244	-0.236
		Total Ray frequency	0.137	0.035	-0.215	-0.171
		Freq. of Multiseriate Rays	-0.031	-0.098	-0.179	-0.304
		Frequency of Biseriate Rays	-0.082	0.213	0.206	0.098
		Frequency of Uniseriate Rays	0.307	-0.031	-0.328	0.003
Inner hard bark		*Inclination of LVs to Left*	0.036	-0.140	1.000	-0.163
		Inclination of LVs to Right	0.360	**0.867****	-0.163	1.000
	Rays contiguous to LV in IHB	Ray width	0.336	-0.219	0.118	0.036
		Ray height	0.124	-0.358	0.106	-0.201
		Height/Width Ratio	-0.223	-0.031	-0.092	-0.186
		Total Ray frequency	-0.047	0.314	-0.202	0.144
		Freq. of Multiseriate Rays	0.000	0.182	-0.113	0.028
		Frequency of Biseriate Rays	0.420	0.314	0.105	0.118
		Frequency of Uniseriate Rays	-0.372	-0.053	0.145	-0.024
	Rays in LV free zone in IHB	*Inclination of rays to Left*	**0.590***	0.021	**0.533***	0.087
		Inclination of rays to Right	0.091	**0.855****	-0.081	**0.939****
		Ray width	-0.180	0.083	-0.438	0.101
		Ray height	0.146	-0.397	0.254	-0.207
		Height/Width Ratio	0.189	-0.291	0.404	-0.207
		Total Ray frequency	-0.043	0.092	0.114	0.048
		Freq. of Multiseriate Rays	-0.043	0.092	0.114	0.048
		Frequency of Biseriate Rays	a	a	a	a
		Frequency of Uniseriate Rays	0.191	0.032	0.141	0.184
All other parameters		STL	-0.439	0.266	-0.396	0.139
		STD	-0.129	0.411	-0.141	0.306
		LV dia.	-0.065	-0.082	0.066	-0.071
		FIC	-0.328	-0.213	-0.307	-0.248
		TLV Den	-0.072	**-0.627****	0.288	**-0.600****
		LVD CR	-0.370	-0.163	0.290	-0.294
		LVD NCR	0.298	**-0.710****	0.054	**-0.529***
		DC 1 LVR	0.117	-0.157	0.177	-0.111
		SBT	-0.109	0.329	-0.358	0.252
		NLVR SB	0.110	0.399	**-0.545***	0.449
		DR SB	0.056	-0.177	0.233	-0.257
		IHBT	-0.360	-0.205	0.388	-0.324
		NLVR IHB	-0.409	-0.061	0.462	-0.284
		DR IHB	0.298	-0.217	-0.137	0.043
		NSR IHBOHBT	-0.403	-0.196	0.392	-0.361
		OHBT	0.508*	0.221	0.548*	0.277
		TBT	-0.100	0.410	0.381	0.380
		SCA IHB	0.141	-0.385	-0.015	-0.289
		SCA OHB	0.408	**-0.619****	0.378	-0.387
		Girth	-0.050	**0.467***	0.318	**0.506***
		Slope	0.082	-0.346	0.089	-0.234
		LAI	-0.181	0.179	0.318	0.127

** Significant for $p < 0.05$ * Significant for $p < 0.01$ [a] variable is absent

ɹ -Sieve tube length; STD-Sieve tube diameter; LV Dia- Latex vessel diameter; FIC- Frequency of interconnections /unit area; TLV Den -Total sel density; LVD CR -Latex vessel density contiguous to rays; LVD NCR- Latex density non contiguous to rays; DC 1LVR -Distance from ıbium to 1st latex vessel row, SBT- Soft bark thickness; NLVR SB- Number of latex vessel rows in soft bark; DR SB- Distance between adjacent 's in SB; IHB- Thickness of inner hard bark; NLVR IHB- Number of latex vessel rows in inner hard bark; DR IHB-Distance between adjacent 's in inner hard bark; NSR IHB- Number of stone cell rows in inner hard bark; OHBT-Thickness of Outer hard bark; TBT- Total bark thickness; ٨ IHB- Stone cell area in inner hard bark; SCA OHB- Stone cell area in outer hard bark; Girth- Girth of the tree; Slope- Leaning angle of Trees; I- Laticifer Area Index

Table 23. Regression analysis on laticifer inclination in soft bark and inner hard bark (in trees having only rightward inclination)

Dependent variable	Independent variables	Regression coefficients	*t- Stat*	R^2 Value
Latex vessels inclination in SB	1. Inclination of rays in LV free zone in SB	0.835	11.240**	0.808
	2. Sieve tube diameter	-0.135	-3.210**	
Latex vessels inclination in IHB	1. Inclination of rays in LV free zone in IHB	0.663	9.691**	0.776
	2. Sieve tube length	-0.003	-3.139**	

** Significant for $p < 0.01$

Table 24. Regression analysis on laticifer inclination in soft bark and inner hard bark (in trees having left and rightward inclination)

Dependent variable	Independent variables	Regression coefficients	*t- Stat*	R^2 Value
Latex vessels inclination to right in SB	1. Inclination of rightward rays in SB	0.259	5.778**	0.839
	2. LVs density non contiguous to rays	-0.566	-2.954**	
Latex vessels inclination to left in SB	1. Inclination of leftward rays in SB	0.576	4.115**	0.706
	2. Sieve tube diameter	0.053	3.088**	
Latex vessels inclination to right in IHB	1. Inclination of rightward rays in IHB	0.965	14.123**	0.947
	2. Number of stone cell rows in IHB	-0.234	-3.748**	
Latex vessels inclination to left in IHB	Analysis was not possible due to less number of variables			

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

Chapter 5

Discussion

5.1. Tree leaning and Girth

It has already been established that tree girth has direct relationship with various structural characters and latex yield in *H. brasiliensis* (Gomez *et al.*, 1972; Ho *et al.*, 1973; Narayanan *et al.*, 1973; Goncalves *et al.*, 1989; Lavorentic *et al.*, 1990; Koshy, 1997; Premakumari *et al.*, 1997). The present study also confirmed the positive association of tree girth with bark anatomical traits such as bark thickness, number of latex vessel rows, number of stone cell rows and total ray frequency. Inclination and orientation of laticifers in the bark observed in the present study had significant association with tree girth. In *Hevea*, the increase in the girth of the tree is attained by the meristematic activity of the cambium. Since the cambium in *Hevea* is non-storied in nature, the rate and duration of cambial activity is not only influencing girthing but also the alignment of tissue. So the correlation of girth with inclination of laticifers may be attributed to the rate of duration of meristematic activity leading to the formation of secondary phloem (bark), externally. Eventhough tree to tree variation for this trait was low, the observed clonal variability was on par with the observations on girth

as reported earlier in rubber tree (Sethuraj, 1981; Nazeer *et al.*, 1986; Premakumari *et al.*, 1986; Premakumari *et al.*, 1991; Licy *et al.*, 2003). This study revealed that tree leaning has no direct of indirect effect on any of the bark structural characters including the orientation and inclination of phloic elements.

5.2. Bark Characters

5.2.1 Bark thickness: The thickness of bark is one of the most important clonal character with respect to the distribution of laticifers and other phloic elements, in general and yield determination, in particular (Gomez and Chen, 1967; Gomez *et al.*, 1972; Ho *et al.*, 1973; Narayanan *et al.*, 1973, 1974; Gottardi, 1995; Licy *et al.*, 2003; Goncalves *et al.*, 2004). Most of the bark anatomical traits in the inner hard bark was significantly correlated with the total bark thickness such as inner hark bark thickness, number of stone cell rows in inner hard bark and also the thickness of the outer hard bark. The study confirmed that in *H. brasiliensis,* major portion of the bark was occupied by outer hard bark followed by inner hard bark. Hence in *Hevea* while considering the bark thickness, the proportion wise distribution of soft bark, inner hard bark and outer hard bark has to be taken into account.

5.2.2 Latex vessel / laticifers

5.2.2.1 Number of latex vessel rows

Latex vessels are mainly concentrated in the soft bark and inner hard bark, of which 40% is in the former and 60%, in the latter. Similar findings were also made by Bobilioff, (1920), Bryce and Gadd, (1923), Sanderson and Sutcliffe, (1929) and (Gomez *at al.*, 1972). The negative association of laticifers in soft bark with parameters like

the inner hard bark thickness, number of latex vessel rows and area occupied by stone cells in the inner hard bark revealed the intensity of sclerification leading to gradual conversion of soft bark into inner hard bark.

Girth and bark thickness have been reported as the major yield contributing traits in *Hevea* (Bryce and Campbell, 1917; Bobilioff 1920; La Rue 1921; Taylor, 1926; Rubber research institute, Malaya 1963, 1964, 1966, 1968; Gomez *et al.*, 1972; Narayanan *et al.*, 1973; Narayanan *et al.*, 1974). In this context, drastic reduction in the number of laticifer rows in the soft bark of all the clones as observed in the present study, may adversely affect the yield producing capacity, unless the latex vessel rows present in the inner hard bark contribute considerable yield in *Hevea.*

5.2.2.2 Distance between laticifer rows

Distance between laticifer rows has been reported as an important parameter in *Hevea* (Paiva *et al.*, 1982) and the average distance between two consecutive rows of laticifers had significant variation. (Gomez *et al.*, 1972; Goncalves *et al.*,1995). The present investigation also confirmed significant clonal variability. For example, majority of the clones showed high number of latex vessel rows and had less inter row distance in both soft bark and inner hard bark. This may facilitate to accommodate more number of latex vessel rows in the soft bark zone as reported by Narayanan *et al.*, (1974). Though the number of laticifer rows varied in soft bark and inner hard bark, the average distance between them did not show much variation. Narayanan *et al.*, (1974) and Gottardi (1995) reported positive correlation between girth and average

distance between latex vessel rings. The association of laticifer rows with phloic ray characters and their other significant correlations proved that the distance between latex vessel rows is one of the most influential secondary character contributing to the yield, in *Hevea.*

5.2.2.3 Latex vessel density

Gomez *et al.*, (1972) reported significant clonal differences in the density of latex vessels within a row and hence suggested this as a potential character for crop improvement programmes (Premakumari *et al.*, 1985; Abraham *et al.*, 1992; Gottardi, 1995; Reghu *et al.*, 1996).

Premakumari *et al.*, (1984) reported the negative association of ray width with latex vessel density which was in agreement with the results of present study. This may be due to the influence of ray width on the running direction of latex vessels within a ring. It was also suggested that number of connections / unit length of latex vessels was independent of latex vessel density as well as latex vessel diameter (Premakumari *et al.*, 1984). In the present study, latex vessels contiguous to rays and non contiguous to rays have been treated separately for analysis and observed that 90 % of the laticifers were distributed in the vicinity of rays and the remaining 10% were situated away from the rays. The individual latex vessels within a row were interconnected to form articulated anastomosing weave around the phloic rays. Hence it is reasonable to believe that the distribution pattern of laticifers are in tune with the orientation of phloic rays. The association of many of the bark structural characters

like ray width, height, H/W ratio, sieve tube length, number of stone cell rows with latex vessel density were also well accounted (Narayanan *et al.*, 1973).

5.2.2.4 Frequency of interconnections

Interconnections between latex vessels are formed by the dissolution of end walls of adjacent latex vessels and hence this character has been accounted as an interclonal variability trait (Premakumari *et al.*, 1996). The frequency of interconnections may be increased due to the increase in the density of latex vessels as revealed by the correlation studies. Certain other characters were negatively associated with frequency of interconnections such as the soft bark thickness, number of laticifer rows in inner hard bark, total bark thickness, girth and laticifer area index. The articulated anastomosing nature of the laticiferous system in *Hevea*, has also been correlated with the tree girth (Premakumari *et al.*, 1992).

5.2.2.5 Latex vessel diameter

Latex vessel diameter is one of the most influential character on yield in *Hevea* clones (Frey-Wyssling, 1930; Riches and Goodding, 1952; Sethuraj, 1977; Markose, 1984; Premakumari, 1992). Significant clonal variability for this charater has been recorded earlier (Gomez *et al.*, 1972; Gomez, 1982; Henon and Nicolas, 1989).

Studies conducted earlier proved the positive association of latex vessel diameter with other characters like girth, bark thickness, number of laticifer rows (Gomez *et al.*, 1972; Ho *et al.*, 1973; Narayanan *et al.*, 1973, 1974; Ho 1972; 1976; Sethuraj,

1981; Premakumari and Panikkar, 1989; Premakumari *et al.*, 1991; Gottardi, 1995). In the present investigation, the diameter of laticifers were positively correlated with soft bark thickness and laticifer area index, indicating that more thicker the soft bark, higher will be the diameter of latex vessels along with a high laticifer area index.

5.2.2.6 Laticifer area index

Tree girth, number of laticifer rows, density of laticifers and radius of laticifer are the contributing factors to ascertain laticifer area index. Hence any variation occurring in any of these factors change the laticifer area index. Clonal variability in the laticifer area index as observed in the present study was concomitant with the earlier report of Premakumari *et al.*, (1993b). This character has also been related to the running direction of laticifers (Premakumari *et al.*, 1988). The present positive correlation of laticifer area index with ray height, H/W ratio and certain other structural characters such as girth, bark thickness,number of latex vessel rows and sieve tube length invariably proved that these characters might have significant positive effect on latex yield. Hence these traits can be considered as the major yield components in *Hevea*..

5.2.3 Ray characters

5.2.3.1 Ray frequency

The principal phloic ray types observed in *Hevea* are uniseriate, biseriate and multiseriate of which multiseriate rays were the most abundant,(about 95-98%). The occurrence of more multiseriate rays in the secondary phloem has also been reported

in many other genera (Den, 1986; Varma *et al.*, 1993; Lu *et al.*, 1994; Liu, *et al.*, 1995; Heo, 1996; Carlquist, 1999; 2000). The clonal variation in ray morphology contiguous to latex vessels was not significant whereas significant variation in ray morphology was observed in those rays present in the latex vessel free zone. The total frequency of phloic rays was more in the soft bark region than in the hard bark region. Similarly, frequency of multiseriate rays increased in inner hard bark region compared to uni and biseriate rays. This increase may be due to the conversion of uni and biseriate rays to multiseriate rays during transition of soft bark to inner hard bark.

5.2.3.2 Height, width and H/W ratio of phloic rays

Phloic rays are running radially in the bark tissue and have great physiological role in the conduction of materials especially to laticifers (Hebant and Fay, 1980; Fay *et al.*, 1989). The height of rays, in most of the clones, was more in the soft bark than the inner hard bark, whereas the ray width showed a reverse trend. This may be either due to the dilation of cells of the rays during the transition of soft bark to inner hard bark or due to the fusion of ray groups as reported in Oak species (Trockenbrodt, 1994). The reduction in the H/W ratio of phloic rays in the inner hard bark zone may be due to the increase in the width of rays in this zone. The height and height/width ratio exhibited significant clonal variation. It has been reported that ray width was negatively associated with density of laticifers and this association had direct influence on the running direction of latex vessels (Premakumari *et al.*, 1985; 1988). The height and width of rays showed significant association with the length and diameter of sieve tubes as well.

5.2.4 Length and diameter of sieve tubes

Sieve tubes are important transporting elements in the secondary phloem (Bel *et al.*, 2002) and primarily meant for the assimilation of photosynthates and other substances (Schmitz and Schneid, 1989; Turgeon, 2000; Nakamura *et al.*, 2004). The presence of sieve tubes even in the early development of the primary vascularization has been reported in the phloem tissue of *Hevea* (Gomez, 1982). Long sieve tubes with distinct end walls of oblique sieve plates were observed in the present study as reported earlier in many angiosperms (Lu *et al.*, 1994; Lotova and Nilova, 1998; Magistris and Castro, 2001; Castro *et al.*, 2005). The length and diameter of sieve tubes, showed low tree-to-tree variation and highly significant clonal variation.

The present investigation proved that the length and diameter of sieve tubes had positive correlation with many of the bark structural characters especially with phloic ray dimensions. Gunnery (1935); Fernado and Tambiah (1970); Anisio *et al.*, (1998) had correlated the diameter of sieve tubes with rubber production. Narayanan and Ho (1970) and Narayanan *et al.*, (1974) reported that the diameter of sieve tubes had no relationship with any of the bark anatomical characters and yield in *H. brasiliensis*. But the present study did not confirm with the above.

Companion cells have strong association with sieve tubes both structurally and functionally (Hayashi, *et al.*, 2000; Bel, *et al.*, 2002; Bel, 2003; Nakamura, *et al.*, 2004). The pattern and arrangement of companion cells and sieve tube in *Hevea* was similar to that in various dicotyledonous species as reported by Chavan *et al.*, (2000).

5.2.5 Stone cells

Highly lignified sclereids distributed in the inner and outer hard bark zone is termed as stone cells. The hardness of bark depends on the distribution pattern and quantity of stone cells present (Bobilioff, 1918). Formation of stone cells has been reported as a clonal character in *Hevea* (Premakumari *et al.*, 1993b). The present study also showed significant clonal variability in the distribution of stone cells in the inner and outer hard bark zones. In three clones, PB 28/59, RRII 105 and RRIM 703, the area occupied by stone cells was very low indicating the low level of sclerification in these clones. The number of stone cell rows and the area occupied by stone cells in inner and outer hard bark region had significant association with many of the bark structural characteristics *Hevea.*

5.2.6 Inclination of latex vessels and phloic rays

Inclination values have established the fact that the two tissue systems, phloic rays and laticifers are aligned in the same orientation within the bark of *Hevea.* Hence the inclination values recorded were almost the same for both phloic rays and laticifers. Inclination of phloic rays and laticifers from juvenile stages also confirmed the uniform pattern of these tissue systems, as observed in the mature stage. Therefore it is assumed that the inclination of phloic elements may be a genetic character which require further investigation

The present study confirmed that both phloic rays and laticifers in six clones *viz.* RRIM 703, RRII 300, Tjir 1, PB 235 and Gl 1 were inclined towards the right and

towards the left in PB 86. But the inclination in the remaining three clones such as RRII 105, PB 28/59 and RRIM 600 depicted a mixed pattern of inclination. Certain trees of these clones had the inclination either towards the left or right or even towards both directions. These three clones also showed a tendency to change the direction of inclination mostly towards the right from soft bark to the inner hard bark region. The numerical difference in the laticifer inclination between soft bark and inner hard bark was irrelevant. This may be due to the influence of phloic rays inclination, as majority of the latex vessels are weaving around the phloic rays, within the bark.

Correlation and regression analysis have been carried out to understand the factors influencing laticifer inclination. Both these analysis conclusively proved that, the inclination of latex vessels in *Hevea* was positively influenced by the inclination of phloic rays. Certain other factors may also have some sort of positive or negative influence on latex vessel inclination. For example, in the soft bark region, sieve tube diameter had a negative effect on rightward inclination and sieve tube length had a positive effect on leftward inclination of laticifers.

The negative effect of the density of laticifers non-contiguous to rays on rightward and leftward inclination of laticifers and rays in the soft bark were also revealed through regression analysis. Correlation analysis also depicted the influence of many of the bark anatomical characters on laticifer inclination.

In this context it is pertinent to correlate the inclination of laticifers with tapping systems adopted in *Hevea* in terms of latex yield. Slope of tapping cut from upper left to lower right and vice versa was a subject of debate during the early evolution of

tapping system in *H. brasiliensis*. Petch (1911) described an increase in yield in *Hevea* when the slope of cut was given from upper left to lower right. De Jong (1916) measured the angle of inclination of latex vessels in 93 trees from unspecified clones and reported the average laticifer inclination towards the right as 3.7°. Mass (1925) made an attempt to modify the slope of tapping cut in certain seedling trees and budded trees to get maximum latex yield. Considering the economic significance of latex yield and labour of tapping, Dijkman (1951) suggested that the inclination of laticifers from vertical was the most important parameter pertaining to yield increase. Gomez and Chen (1967) considered different aspects of alignment of bark tissue and slope of tapping cut. He noticed from the recommended practice of giving 30°-45° tapping slope (upper left to lower right) for budded trees, with the concept of 3-4° rightward inclination of laticifers obtained an yield increase of 2-3%, but the length of the cut to be tapped is increased by 22%. Presently, a spiral cut from upper left to lower right, slopes at an angle of 25° for seedling tree and 30° for budded trees is followed.

The present study revealed that the inclination of laticifers varied from clone to clone towards right or left with a range of 2.60° to 8.42° and 2.51° to 4.27°, respectively. Whereas in the case of those clones which showed the mixed pattern of inclination, the range of inclination towards the right was 1.49° - 4.01°, and towards the left was 1.15° - 2.10°. In this context, the suggestions made by Gomez and Chen (1967) assumes significance. According to them, if more than half of the trees consistently displayed leftward orientation of laticifers, then right hand half spiral cut might be recommended. Hence it is suggested that the tapping practice being followed at present, needs further refinement, based on the inclination of laticifers in each clones.

5.3 Histochemical studies

Studies on the histochemical status and distribution pattern of reserve metabolites such as starch, lipids, proteins; conversion of reserve metabolites into extraneous materials like phenols and tannin in *Hevea* bark is very limited as revealed by the survey of literature. The situation was the same with respect to cell wall deposits like total polysaccharides and lignin. Starch is the end product of carbon fixation and is the tonoplast of the storage cells, probably from sucrose (Zeigler, 1964; Strafford, 1965; Czaja, 1978). A large portion of photosymthates is utilized for the growth and development of plants, a considerable fraction is used up in respiration and surplus fraction is deposited as reserve metabolites in the storage tissue which are eventually utilized for growth and respiration (Kramer and Kozlowiski, 1979). Hence in woody species, starch reserves is an important source of various kinds of organic compounds, including sucrose, which is the primary sugar that is transported in plants and regulate vascular differentiation (Shiroya *et al.*, 1962; Wetmore and Rier, 1963; Zimmermann, 1971; Giaquinta, 1980; Kozlowski and Pallardy, 1997).

In *Hevea*, the present investigation revealed the occurrence of high starch reserves in the axial parenchyma of the secondary phloem as reported earlier (Hao and Wu, 1991; Wu and Hao, 1993; Zhang, *et al.*, 1994b; Courty , *et al* 1999; Thomas *et al.*, 2002). The increased accumulation of starch in the outer hard bark region reflects the storage function. It is interesting to note that the phloic rays were devoid of starch reserves. In this context it is reasonable to believe that the phloic rays are mainly involved in the conduction and transport of photosynthates, as suggested by Savidge and Wareing, (1982)

and the metabolites conducted through them might have been diverted for the biosynthesis of rubber latex in the laticifers (Tupy, 1985), instead of storage as majority of the laticifers are distributed contiguous to phloic rays in *Hevea*. Enhanced respiratory and phosphatase activities reported in phloic rays by Hebant and Fay (1980) strongly confirm this view.

It has been reported that the rate of cell differentiation is influenced by quantity of starch reserves in storage tissues (Oribe, 2003). The present study revealed that copious quantity of starch grains were accumulated in the axial parenchyma especially in the inner hard bark regions. This may also be related to the transport of sucrose from the storage cells to the laticifers as suggested by Jacob *et al.*, (1998). This view can be further supported by the presence of numerous plasmodesmatal connections between laticifers and adjacent parenchyma cells in *H. brasiliensis* (Fay *et al.*, 1989).

The absence of starch grains in the soft bark region very near to the cambial zone, may be due to the utilization of metabolites for cell division and other cellular activities as the meristematic zone is a strong sink for sucrose (Krabel, 2000), which is the primary photosynthate being transported within the source-sink system in plants (Shiroya *et al.*, 1962; Zimmermann, 1971; Giaquinta, 1980; Kozlwski and Pallardy, 1997).

Srisuma *et al.*, (1991) reported that the variation in the quantity of cell wall polysaccharides depends on the type of cells. In the present study deposition of polysaccharides in the cell wall of all type phloic elements in *Hevea* was confirmed with histochemical evidence. The cytoplasm of certain ray cells and axial parenchyma also display localization of polysaccharides, but the intensity gradually decreased towards the outer region of bark. Accumulation of total polysaccharides on either side of the

sieve plates confirmed the translocation of such secondary metabolites through sieve plates as reported by Aloni and Peterson, (1991).

Lipids are reported to be synthesised from, starch (Higuchi *et al.*, 1967). Since the occurrence of starch and lipids in storage cells has close relationship as far as their relative amount is concerned, these two metabolites are to be viewed together for understanding their metabolism (Reghu, 1983). In the present study lipid globules were localized more in the ray cells than axial parenchyma in both soft bark and hard bark. Hasma and Subramanian (1986) reported that in *Hevea*, the total lipid constituted about 1.6% of the latex, out of which 54% was neutral lipids, 32% glycolipids and 14% phospholipids. It is interesting to note that the ray cells rich in lipids are poor in starch content and vice versa. This may be attributed to the high level of metabolic activity in phloic rays.

The cells with high protein content are likely to be highly metabolically active since some of the proteins may be enzyme proteins. The present study confirmed the presence of proteins in phloic rays, axial parenchyma and sieve tubes in *Hevea*. The localization of proteins in high quantity especially in phloic rays and sieve elements revealed the high metabolic status of *Hevea* bark.

Accumulation of phenolic compounds in plant tissues can be considered as a means of defense response (Brignolas *et al.*, 1995; Franceschi *et al.*, 2000) and against pathogen attack (Klepzig *et al.*, 1996; Krokene *et al.*, 2001). In the present investigation the increased accumulation of phenols and tannin compounds in the parenchymatous tissues towards the outer regions of *Hevea* bark clearly demonstrate high rate of conversion of reserve metabolites into extraneous materials as reported earlier by Thomas *et*

al., (1995). According to Janakowski and Golinowski (2000), nonfunctional secondary phloem having high frequency of usually sclereids accumulates large quantity of phenolic substances. The present study also confirmed the similar pattern of phenolic distribution in the inner hard bark where scleried stone cells are abundant. However, the localization of phenolics was relatively less in the outer bark zone.

Tannin compounds are derivatives of phenols (McNair, 1930) and its localization was more in the axial parenchyma. Compared to phenols, tanniferous cells were found to be more in the inner hard bark region of *Hevea* which further increased to the maximum level in the outer hard bark regions. The low level of phenols and high level of tannin deposition in the outer hard bark may be attributed to the radial conversion of available phenolics into tannin during the process of ageing and senescence of cells. In fact the outer hard bark zone of *Hevea* consists of aged tissues intermingled with stone cells and periderm, which accumulated more tannin content as reported in various other tree species by Yartseva (1984) and Chernyaeva *et al.*, (1982). The occurrence of tanniferous cells in high frequency associated with bark regeneration in *H. brasiliensis* has also been reported earlier (Thomas *et al.*, 1995). Another notable feature was the occurrence of tanniferous cells adjacent to laticifers, which confirms the earlier findings of Trancard (1979) proving the relation between tannin cells and latex vessels during metabolic conversion.

Lignins are phenolic polymers of the cell wall and their deposition is associated with mechanical strength, improved sap conduction, defense mechanisms and imperviousness to biodegradation (Helm *et al.*, 1997). Lignins are formed by oxidative polymerization of

atleast two of the three monolignols *viz.* p-coumeryl, coniferyl and cinapyl alcohols (John and Zhang, 1998). In plant system, cell wall undergo lignification process during secondary thickening (Engels and Jung, 1998). The present investigation revealed that the lignin was mainly concentrated in the stone cells distributed in the inner and outer hard bark regions. As the frequency of stone cells and phenolic accumulation are very high in the outer hard bark region, the high level of lignification may be attributed to the rate of polyphenols in lignification process in *Hevea* bark as suggested by Trancard (1979). The accumulation of polyphenols in the cell wall during lignification has been reported by various workers (Woodward and Pearce, 1988; Oven and Torelli, 1994). The low level lignification and absence of stone cells in the soft bark region may be due to the lack of the polyphenol lignin precursor in this zone.

Chapter 6

Summary

Hevea brasiliensis belonging to the family, *Euphorbiaceae* is the major source of Natural Rubber (NR). Latex is produced in the latex vessels or laticifers distributed in the bark and is exploited by the process called tapping. The present system of tapping was formulated based on the inclination of laticifers towards the right at 3-5°. But the laticifers were found to be inclined towards the right or left within the bark of Hevea. Anatomically, Hevea bark is composed of concentric layers of sieve tubes, companion cells, phloem fibres, parenchymatous tissues and network of latex vessels. Most of the bark characters were interrelated.

Detailed investigations were carried out on the structure of the bark of Hevea brasiliensis with special emphasis on alignment, orientation and angle of deviation of latex vessels. The main objectives were to study: (1) the variation in different structural characters within and between clones (2), association and interrelationship of various structural characters of bark (3), the alignment and angle of inclination of laticifers and phloic elements. (4), angle of inclination of laticifers in seedling and budded plants at the juvenile stage. (5), structural factors influencing inclination of latex vessels in the bark and (6), histochemical localization of reserve metabolites such as starch, total polysaccharides, lipids, proteins, phenols, tannins and lignin in the bark.

Ten clones of H. brasiliensis, *viz.* Tjir 1, Gl 1, PB 86, GT 1, PB 28/59, RRII 105, RRIM 600, RRIM 703, PB 235 and RRII 300, at the age of 17-21 years planted in Randomised Block Design (RBD) with three replicates and three trees per plot, were used to study the bark structural traits and histochemical investigations in the mature stage. Seedling progenies of two cross combinations and budded plants of RRII 105 and RRIM 600 at the age of 4 years were selected to investigate the inclination and orientation of laticifers in the juvenile stage.

The data generated were subjected to detailed statistical analysis *viz.* Coefficient of Variation(CV), Correlation, Analysis of variation (ANOVA) and Regression analysis.

Structurally the bark of *Hevea brasiliensis* consists of three distinguished zones *viz.* (i) the inner region contiguous to cambium called the soft bark (SB), (ii) the middle zone consisting of latex vessels and with stone cells called inner hard bark and (iii) the outer zone, made up of highly sclerified stone cells called outer hard bark. The latex vessels in *Hevea* are compound articulated, anastomosing, interconnected tubes formed in concentric rings sandwiched in between layers of other phloic elements. The latex vessels are vertically interwoven around phloic rays along the longitudinal axis of the tree.

The tree girth recorded low tree to tree variation but had significant clonal variability. Positive association of tree girth with many of the bark anatomical characters such as bark thickness, latex vessel rows, number of stone cell rows and total ray frequency, was noticed

Significant clonal variability was observed in the thickness of inner and outer hard bark. The major portion of the bark was occupied by the outer hard bark followed by inner hard bark and then by the soft bark. Correlation studies clearly indicated their close association with many of the bark characters.

Laticifer rows are mainly distributed in the soft bark and in the inner hard bark. The proportion of latex vessel rows in the inner hard bark comes about 60% of the total number of laticifer rows. Both these characters depicted tree to tree variation and significant clonal variability. Inter laticifer row distance in soft bark and inner hard bark did not show any significant difference. Clonal variation in the inter laticifer row distance was significant in the inner hard bark.

Latex vessels are running around the phloic rays in longitudinal direction. About 90% of the latex vessels in *Hevea* bark are distributed contiguous to rays indicating that ray system and laticifers are closely related in distribution. Though tree to tree variation was low, clonal variation was highly significant for these characters. As the laticifers in *Hevea* are articulated anastomosing types, the frequency of interconnections between them also showed significant clonal variation. Certain other factors like the thickness of soft bark, number of laticifer rows in the inner hard bark, girth and laticifer area index also showed negative association with frequency of interconnections. Low tree to tree variation with significant clonal variability was observed in the diameter of latex vessels. This trait showed positive association with bark thickness and laticifer area index

The present study revealed that in *Hevea*, the phloic rays and latex vessels were closely associated with respect to orientation and inclination. The alignment and inclination of phloic rays and latex vessels were found to be almost same. Out of the ten clones studied, six clones (RRIM 703, GT 1, RRII 300, Tjir 1, PB 235 and Gl 1) possessed laticifers inclined towards the right. In PB 86 the laticifers were inclined towards the left. In clones like RRII 105, PB 28/59 and RRIM 600, the inclination of laticifers were either towards right or left and even in both directions.

Correlation and regression analysis conclusively proved that the inclination pattern of phloic rays was the most influencing parameter which determines the inclination of laticifers. The frequency of rays in the latex vessel free zone in soft bark showed significant clonal variability whereas those rays contiguous to laticifers in both soft and inner hard bark did not show clonal variability. The total

frequency of phloic rays was higher in soft bark than the inner hard bark where the width of phloic rays was considerably increased in the inner hard bark.

The frequency of stone cells in the inner hard bark depicted low tree to tree variation and insignificant clonal variability. However, area occupied by stone cells in the inner and outer hard bark recorded considerable clonal variation. These characters were also closely associated with various other bark characters .

Histochemical studies revealed the localization status of reserve metabolites such as starch, lipids, proteins, phenols, tannin, cell wall polysaccharides and lignin. Starch, phenol and tannin compounds were absent in the soft bark zone, but abundant in the inner and outer in the inner hard bark region especially in axial parenchyma cells. Proteins and lipids were distributed mainly in rays. Total polysaccharides were localized in the cell wall of all phenolic elements including laticifers. Lignification was observed mainly in stone cells and also in the cell wall of those cells which undergo sclerification leading to stone cell formation.

The data on the inclination of latex vessels in this study needs to be endorsed with its effect on latex yield which will subsequently necessitate further debate on the subject especially on the tapping slope.

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Abbreviations

ANOVA	-	Analysis of variance
B	-	Frequency of biseriate rays
CD	-	Critical difference
CLV	-	Contiguous to laticifers
CS	-	Cross section
CV	-	Coefficient of variation
DC 1LVR	-	Distance from cambium to 1st latex vessel row
DR IHB	-	Distance between adjacent rows in inner hard bark
DR SB	-	Distance between adjacent rows in SB
FAA	-	Formalin acetic acid alcohol
FIC	-	Frequency of interconnections /unit area
H/W	-	Height/width ratio
HB	-	Hard bark
IHB	-	Inner hard bark
LAI	-	Total cross sectional area of latex vessels (Laticifer Area Index)
LV Dia	-	Latex vessel diameter
LV	-	Latex vessels/laticifers
L	-	Left
LVD CR	-	Latex vessel density contiguous to rays
LVD NCR	-	Latex density non contiguous to rays
LVF	-	Laticifer free zone
M	-	Frequency of multiseriate rays
MSL	-	Mean sea level
NLVR IHB	-	Number of latex vessel rows in inner hard bark
NLVR SB	-	Number of latex vessel rows in soft bark
NSR IHB	-	Number of stone cell rows in inner hard bark
OHBT	-	Outer hard bark
OHBT	-	Thickness of outer hard bark
R	-	Right
R.H	-	Ray height
R.W	-	Ray width
RBD	-	Randomized block design
RLS	-	Radial longitudinal section
RRII	-	Rubber Research Institute of India
SB	-	Soft bark
SBT	-	Soft bark thickness
SCA HB	-	Stone cell area in hard bark
SCA IHB	-	Stone cell area in inner hard bark
STD	-	Sieve tube diameter
STL	-	Sieve tube length
TBT	-	Total bark thickness
TLS	-	Transverse longitudinal sections
TLV Den	-	Total latex vessel density
TRF	-	Total ray frequency
U	-	Frequency of uniseriate rays
VR	-	Variance ratio

List of Tables

List of figures