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; Paiva *et al.*, 1982; 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 adversly affect the yield producing capacity, unless the latex vessel rows present in the inner hard bark contribute considerable yeild 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.*, 1987). 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 1975; 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.*, (1993). 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, 1993; Lu *et al.*, 1994; Liu, *et al.*, 1995; Heo, 1996; Carlquist, 1999a; 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 diamter 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 dicotyedonous species as reported by Chavan *et al.*, (2000).

5.2.5 Stone cells

Highly lignified sclerieds 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 1.5° - 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; Wilson, *et al.*, 1994; 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, 1992; Wu and Hao, 1993; Zhang, *et al.*, 1994; 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 (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 defence 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, defence 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 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.