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 overwhelmingly 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 Chelidanum. 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; Trecul, 1865; 1866; 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).

Laticifers

- Articulated
  - Anastomosing (Companulaceae, Caricaceae, Compositae, Euphorbiaceae (in part) and Papavvaraceae

- Non-articulated
  - Branched (Apocynaceae, Asclepiadaceae, Euphorbiaceae (in part) and Moraceae

- Unbranched (Apocynaceae, Eucommiaceae, Moraceae and Urticaceae (in part)
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; Panikkar, 1974; 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).


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).
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; Paiva, 1982; 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 (Bobilloff, 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., (1986) 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., 1972) 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 (Bobilloff, 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 G1 and 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 interconnections 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, 1975, 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 \( \mu \text{m} \) to 26.87 \( \mu \text{m} \), whereas Gomez (1982) recorded the diameter within the range of 21.60 - 29.90 \( \mu \text{m} \) 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 RR11 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., (1993a). 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 diamensions 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 (Fernando 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 dicotyledenous 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 into 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.