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Chapter 14

Biochemistry and physiology of latex production

N. Usha Nair

1. INTRODUCTION

The physiology of *Hevea brasiliensis* is unique. Biosynthesis of latex, the economic product, is confined to the latex vessels which exclusively occur in the phloem region. Latex biosynthesis depends on the number, diameter and anatomical characters of latex vessel system and physiological and biochemical factors. Latex is obtained from the trees by introducing an abnormal physiological function through wounding. The capacity of the latex vessels to synthesize and regenerate latex drained during each tapping is critical and is accomplished in the interval between two successive tappings.

2. LATEX BIOCHEMISTRY

Latex is a specialized form of cytoplasm containing a suspension of rubber and non-rubber particles in an aqueous serum. Besides rubber and water, fresh latex contains carbohydrates, proteins, lipids and inorganic salts (Archer *et al.*, 1963b). Numerous other substances are also present in small quantities.

2.1 Composition

Latex can be separated into (1) a white upper layer of rubber particles, (2) an orange or yellow layer containing Frey-Wyssling particles, (3) an aqueous serum named C serum and (4) a bottom fraction containing greyish yellow gelatinous sediments by ultra centrifugation (Cook and Sekhar, 1953). The serum contains most of the soluble substances including amino acids, proteins, carbohydrates, organic acids, inorganic salts and nucleotidic materials, (Archer *et al.*, 1969). The bottom fraction consists mainly of lutoid particles and also includes varying amounts of Frey-Wyssling particles, mitochondria and other particulate components of plant cell having a density greater than that of serum.

2.1.1 Rubber hydrocarbon

The dominant particulate constituent of freshly collected latex is rubber hydrocarbon which makes up 30 to 45 per cent of the volume and occurs in sizes ranging from 0.02 to 3.00 µm with the majority in the region of 0.1 µm (Southorn and Yip, 1968; Tata and Yip, 1968). Rubber particles (Plate 40. a) are usually spherical but sometimes oval or pear-shaped (Dickenson, 1964) and are strongly protected in suspension by a film of adsorbed protein and phospholipids (Archer, 1964). This protein-phospholipid layer imparts a net negative charge to the rubber particle contributing to colloidal stability (Bowler, 1953).

2.1.2 Lutoids

Next in abundance are lutoid particles amounting 10 to 20 per cent of the volume. The lutoids (Plate 40. b,c) are sub-cellular membrane-bound bodies ranging in size from 2 to 5 µm (Southorn and Yip, 1968). The membrane encloses a fluid serum known as lutoid serum or B serum which is a destabilizer of rubber hydrocarbon.

2.1.3 Frey-Wyssling particles

Frey-Wyssling particles (Plate 40. d) are spherical and yellow coloured, larger in size and constitute one to three per cent of latex by volume. Diameter of the particles ranges from 4 to 6 µm and each particle is enclosed in a typical double membrane (Dickenson, 1964; 1969).

2.1.4 Carbohydrates

Quebrachitol (methylinositol), sucrose and glucose are the major soluble carbohydrates in latex (Low, 1978). Quebrachitol is the most concentrated single component in the serum phase.

The concentration of quebrachitol varies with clones and ranges from one to three per cent of whole latex. It is a major contributor to the osmotic pressure of the cytosol (d'Auzac and Jacob, 1989). The concentration of sucrose in latex also varies with clones and is influenced by exploitation techniques.

2.1.5 Proteins

The total protein content of fresh latex is approximately one per cent of which about 20 per cent is adsorbed on rubber particles, an equal quantity found in the bottom fraction and the remainder in the serum phase (Archer *at al.*, 1963b). The major protein found in serum phase is α-globulin (Archer *et al.*, 1969; Hahn *et al.*, 1971). The adsorbed proteins and phospholipids on the rubber particle impart a net negative charge thereby contributing to the colloidal stability of latex. The isoelectric point of rubber particles in fresh latex varies from 4.0 to 4.6 and the variation has been ascribed to the presence of more than one protein on the rubber particle. The quantity of the adsorbed proteins on rubber particles is a clonal characteristic (Bowler, 1953).

The major protein on the rubber surface is not identical with the major C serum protein α-globulin (RRIM, 1982). Two enzymes, isopentenyl pyrophosphate polymerase (Archer *et al.*, 1963a; Lynen, 1967) and rubber transferase (Archer *et al.*, 1963b; 1966; McMullen and McSweeney, 1966; Lynen, 1967; Archer and Cockbain, 1969) are found to be associated with the rubber surface.

C serum contains nearly half the enzymes detected in latex. Enzymes of the glycolytic pathway (Bealing, 1969; d'Auzac and Jacob, 1969) as well as enzymes for rubber biosynthesis (Archer and Audley, 1967) are found in C serum. Twenty seven enzymes were separated by electrophoresis of which, 17 were shown to exist in multiple forms (Jacob *et al.*, 1978).

Proteins in the bottom fraction are the soluble proteins in lutoid serum (B serum) and in the lutoid membrane. Hevein is the major protein in B serum which accounts for about 70 per cent of the water soluble proteins in the bottom fraction (Archer *et al.*, 1969). It is a low molecular weight anionic protein (about 5000 Da), having about five per cent sulphur (Archer, 1960; Tata, 1975a; 1975b), with a single chain of 43 amino acids, rich in cysteine and glycine. It is involved in the coagulation of latex by bringing together rubber particles (Gidrol *et al.*, 1994)

Lutoids from young latex vessels contain a protein deposited in the form of bundles of microfibrils each having probably a double helical structure (Archer *et al.*, 1963b). This microfibrillar protein, which has a lower isoelectric point than hevein, comprises about 40 per cent of the total protein of lutoid particles in young latex vessels but is absent in older vessels. Two basic proteins, a major one identical with hevamine A, a cationic protein (Archer, 1976) and a minor one have lysozyme and chitinase activities (Tata, 1980; Tata *et al.*, 1983). The basic proteins of bottom fraction are involved in the mechanism of latex flow.

2.1.6 Lipids

Lipids of fresh latex consist of fats, waxes, sterols, sterolesters and phospholipids. Lipids associated with the rubber and non-rubber particles in latex play a vital role in the stability and colloidal behaviour of latex. There is distinct clonal variation in the amount of lipids extractable from rubber cream and bottom fraction (Nair *et al.*, 1993). Total lipids constitute about 1.6 per cent of latex, of which 54 per cent constitute neutral lipids, 33 per cent glycolipids and 14 per cent phospholipids (Hasma and Subramaniam, 1986). Tryglycerides and sterols are the main components of the neutral lipids of rubber

particles. Lutoid stability as indicated by bursting index, is negatively correlated with the phospholipid content (Sherif and Sethuraj, 1978). The constituents of the phospholipids are mainly phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylinositol (PI).

2.1.7 Other constituents

Most of the classic amino acids have been reported in latex. Nucleotides contained in latex are important as co-factors and are intermediates in biosynthetic processes. Low molecular weight thiols, which are the main reducing agents in latex, include glutathione and cysteine. Ascorbic acid is also a very important reducing agent in latex. These two reducing components are involved in the redox potential of latex. Malic acid and citric acid make up 90 per cent of organic acids in latex. Total concentration of inorganic ions in fresh latex is about 0.5 per cent, the major ions being potassium, magnesium, copper, iron, sodium, calcium and phosphate (Archer *et al.*, 1963b).

2.2 Rubber biosynthesis

Rubber is composed of isoprene units linked together to form a polymer (Bouchardat, 1875). The individual steps in the synthesis of rubber from sucrose are well established (Lynen, 1969). Biosynthesis of rubber can be divided into three stages : (1) generation of acetyl-coenzyme A (acetyl-CoA); (2) conversion of acetyl-CoA to isopentenyl pyrophosphate (IPP) via mevalonic acid; and (3) polymerization of IPP to rubber. Sucrose in latex is the primary source of acetate and acetyl-CoA essential for the biosynthesis of rubber. Acetate forms the basic precursor of rubber biosynthesis in all rubber plants (Bonner, 1949; Arreguin *et al.*, 1951; Bandurski and Teas, 1957; Park and Bonner, 1958). Using acetate, labelled with ^{14}C it was proved that latex used as a medium can convert it to labelled rubber, indicating the presence of the entire enzyme system necessary for the conversion of acetate to rubber. The pool of acetyl-CoA in latex is conceivably generated from sucrose and *Hevea* latex contains all the enzymes required for the metabolism of sucrose to pyruvate (d'Auzac and Jacob, 1969; Bealing, 1969).

2.2.1 Formation of hydroxymethylglutaryl-CoA

The enzymes required for the conversion of acetate and mevalonate to rubber have also been detected in latex (Lynen, 1969). It has generally been assumed that the source of acetyl-CoA is pyruvate from glycolysis (Lynen, 1969). The conversion of pyruvate to acetyl-CoA in latex has not been proved and is considered to be the unknown step in rubber biosynthesis (d'Auzac and Jacob, 1969). Other possible sources of acetyl-CoA are via β oxidation of fatty acids or via the metabolism of amino acids especially leucine (Backhaus, 1985). Two molecules of acetyl-CoA condense to form acetoacetyl-CoA. Acetoacetyl-CoA condenses with another molecule of acetyl-CoA resulting in β-hydroxy-β-methylglutaryl-CoA (HMG-CoA).

2.2.2 Formation of mevalonate

The discovery of mevalonic acid as an intermediary has been decisive in formulation of general pathways for the synthesis of isoprenoid compounds (Wagner and Folkers, 1961). Mevalonic acid is derived from HMG-CoA in a nicotinamide adenine dinucleotide phosphate-linked reduction (NADPH) which also occurs in *Hevea* latex

(Archer and Audley, 1967). The activity of the enzyme HMG-CoA reductase in latex is surprisingly low and may be a limiting factor in rubber biosynthesis (Lynen, 1969). Three genes hmg1, hmg2 and hmg3 have been discovered for the rubber tree of which hmg1 gene is considered responsible for rubber biosynthesis (Kush *et al.*, 1990). Mevalonic acid synthesis is considered to take place on the endoplasmic reticulum (Archer, 1980).

2.2.3 Conversion to isopentenyl pyrophosphate

Conversion of mevalonate to isopentenyl pyrophosphate (IPP) requires two preparatory phosphorylations leading to 5-phospho and 5-pyrophospho mevalonic acid (Archer and Audley, 1967). The enzymes required for these conversions, mevalonate kinase and phosphomevalonate kinase respectively, are present in latex (Lynen, 1969). All the other enzymes required for the conversion of HMG-CoA to IPP are also found in the latex serum (Archer, 1980).

2.2.4 Polymerization to rubber

The mechanism of polymerization of IPP has been elucidated in relation to terpene biosynthesis (Lynen *et al.*, 1959). Two steps are involved in the process : (1) isomerization of IPP to dimethylallyl pyrophosphate (DMAPP) by a shift of the double bond by IPP isomerase and (2) condensation of DMAPP with IPP by rubber cis-polyprenyl transferase (Archer *et al.*, 1963a; McMullen and McSweeney, 1966; Archer and Audley, 1967; Archer and Cockbain, 1969; Madhavan and Benedict, 1984), to give a molecule each of pyrophosphate and geranyl pyrophosphate (C 10). This C 10 molecule has allelic structure and repeats the condensation, with another molecule of IPP. The propagation, repeated several times, results in the formation of natural rubber with high molecular weight. The stereo-specificity of rubber transferase enzyme in latex ensures a cis configuration for each double bond.

Chain initiation can also be observed. Originally, it was thought that chain initiation had to be via DMAPP, but recent evidence suggests that rubber initiation can proceed from the trans compounds geranyl pyrophosphate (C 10), farnesyl pyrophosphate (C 15) or geranyl geranyl pyrophosphate (C 20) implying that one of the terminal ends of the rubber molecule can be trans (Archer *et al.*, 1982). In *Hevea* latex IPP isomerase activity required for the formation of DMAPP has been detected in serum (Barnard, 1964; Archer and Audley, 1987). The conversion of IPP takes place on the surface of existing rubber particles and thus is predominantly a chain extension process on already existing rubber chains which carry allyl pyrophosphate end groups (Archer and Audley, 1967). Rubber transferase forms an integral part of the outer layer surrounding the rubber particle and acts as a conduit for the growing chain. By repeated Z-additions of IPP the enzyme produces a tail of increasing length (Paterson-Jones *et al.*, 1990). It has been assumed that the growing hydrocarbon chain diffuses into the interior of the rubber droplet and that the hydrophilic pyrophosphate end groups remain in the serum phase where it will interact with IPP, bound to the active site of rubber transferase. The pathway of rubber biosynthesis is given in Figure 1.

Hevea rubber differs from the majority of isoprenoid compounds in two respects. It has a high molecular weight which varies from a lakh to several millions (Schulz and Mula, 1960) and the geometric configuration of double bonds is exclusively cis (Bunn, 1942; Golub *et al.*, 1962).

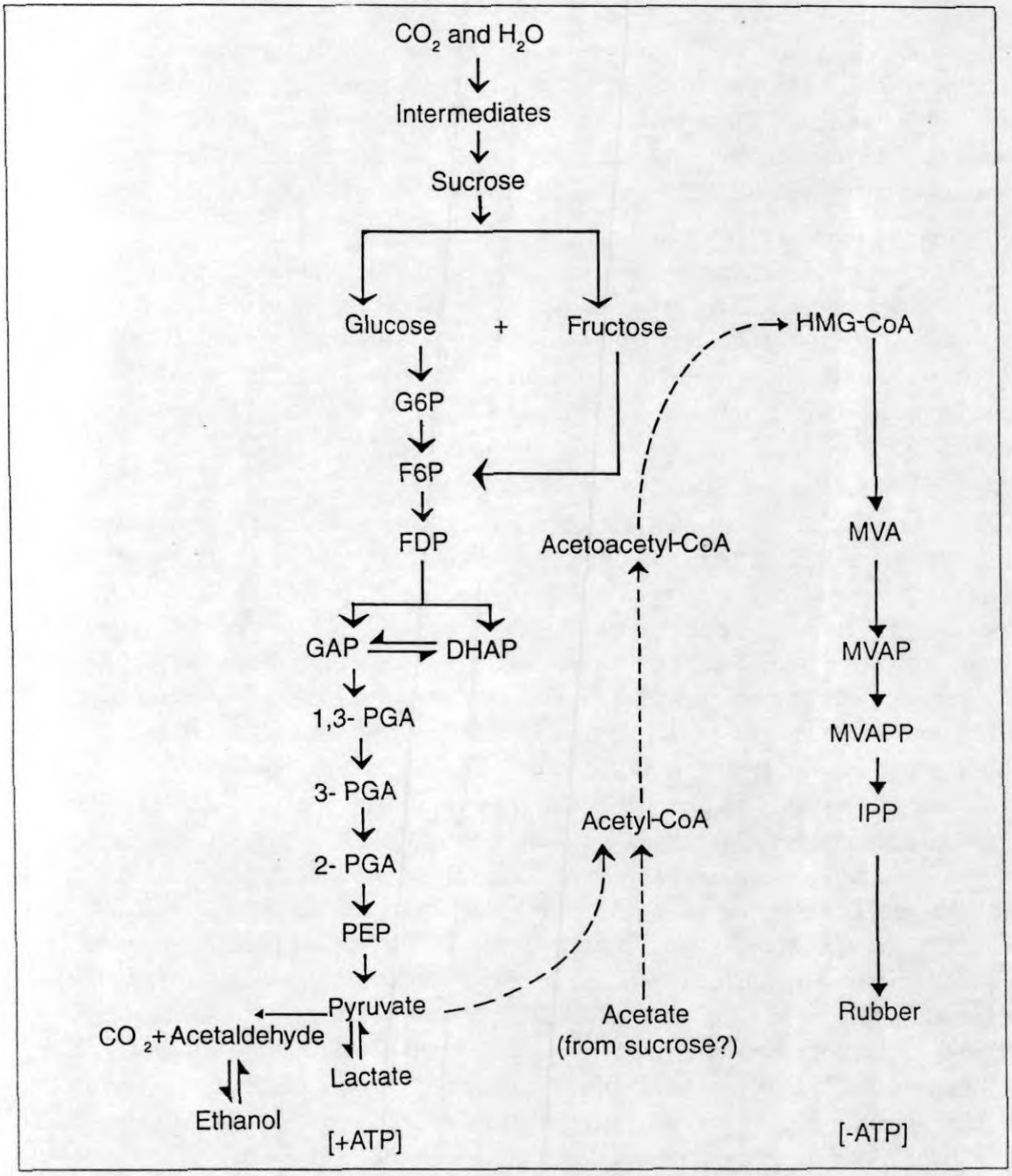

Fig. 1. Pathway of rubber biosynthesis*

G6P	–	glucose-6-phosphate	PEP	–	phosphoenol pyruvate
F6P	–	fructose-6-phosphate	ATP	–	adenosine triphosphate
FDP	–	fructose-1, 6-diphosphate	Acetyl-CoA	–	acetyl-coenzyme A
GAP	–	glyceraldehyde-3-phosphate	Acetoacetyl-CoA	–	acetoacetyl-coenzyme A
DHAP	–	dihydroxyacetone phosphate	HMG-CoA	–	β-hydroxy-β-methylglutaryl-Coenzyme A
1,3-PGA-1–		3-phosphoglyceric acid	MVA	–	mevalonic acid
3-PGA	–	3-phosphoglyceric acid	MVAP	–	mevalonic acid 5-phosphate
2-PGA	–	2-phosphoglyceric acid	MVAPP	–	mevalonic acid 5-pyrophosphate
IPP	–	isopetenyl pyrophosphate			

* Adapted from Moir, 1969

3. PHYSIOLOGY OF LATEX FLOW

Rubber yield is directly determined by the volume of latex obtained through tapping, the two important attributes being dry rubber content (DRC) and total volume of latex. Yield, in turn, is linked with latex flow characteristics of the tree.

3.1 Mechanism of latex flow

Latex vessels are filled with viscous latex under hydrostatic pressure usually between 10 and 15 atmospheres in the early morning. When the vessel is cut, the pressure at the location of the cut is released and latex exudes. This expulsion results in the displacement of latex along the length of latex vessels owing to the strong forces of cohesion existing in the liquid phase. A fall in pressure in the latex vessels follows and consequently water from the surrounding tissues enter the latex vessels owing to gradients in water potential. This dilution, termed dilution reaction, makes the latex less viscous, resulting in an enhanced flow rate. As a result the total solids and DRC of the latex decline immediately after tapping but show some recovery before the flow ceases. The rubber content is restored to its usual level by synthesis between tappings. The turgor pressure gradually recovers as the latex flow slows down and reaches normalcy when the flow ceases.

3.2 Latex vessel plugging

The discovery of the existence of an inherent clotting mechanism within the latex vessels responsible for the cessation of latex flow (Boatman, 1966; Southorn, 1968) was a major breakthrough in understanding the mechanism of latex flow. Latex contains destabilizing factors located in the lutoid particles. The lutoid particles are ruptured during latex flow releasing the destabilizing substances which in turn flocculate the rubber particles in the vicinity.

The dilution reaction following tapping alters the osmotic concentration of the latex. The lutoids are damaged both by osmotic effects and by mechanical shearing forces. The greater degree of damage to lutoids in early flow fractions has been attributed to these phenomena (Pakianathan *et al.*, 1966). Subsequently the lutoids which suffered damage aggregate with rubber particles within the vessels to form flocs near the cut ends thus leading to latex vessel plugging (Pakianathan *et al.*, 1973).

Lutoid damage leads to release of lutoid serum which contains hevein, a lectin-like protein. Hevein forms bonds between rubber particles by fixing to the N-acetyl-glucosamine (GlcNAc) groups borne by a 22 kDa receptor protein localized on the surface of the rubber particles (Gidrol *et al.*, 1994).

3.3 Factors influencing latex production

Important physiological requirements of a rubber tree with high productivity are high net assimilation rate for growth and high ratio of partition of assimilates into rubber (Templeton, 1969). Therefore, any high yielding tree must have capacity for a high rate of girthing so as to produce a large trunk and thus ensuring high potential yield. Rate of girth increment on tapping is comparatively less in clones of higher productivity than those of lower productivity, indicating that rubber formation and growth process compete with each other. Formation of rubber is a high energy absorbing process which could lead to depression of growth after tapping (Bonner, 1967; Chua, 1967).

A peculiar situation in *Hevea* is that the yield of latex from a tree is determined not only by the inherent factors and environment but also by the exploitation methods (Sethuraj, 1985). The yield of rubber from a tree on every tapping is determined by the volume of latex and the percentage of rubber it contains (DRC). Effect of the major factors on yield of rubber obtained from a day's tapping can be derived from the formula :

$$y = \frac{F.l.C_r}{p}$$

where y is quantity of rubber obtained from a tree each time it is tapped,

F is the average initial flow rate per cm of tapping cut during the first 5 min after tapping

l is the length of the cut

p is the plugging index, a measure of the extent of latex vessel plugging, calculated from the formula :

$$P = \frac{(\text{volume in 5 min})/5 \times 100}{\text{total volume}}$$ and

C_r is the rubber content (percentage by weight).

The three major components (F, p and C_r) are influenced by the inherent characteristics, exploitation systems and environment.

The anatomical, physiological and biochemical sub-components influencing the major components (Sethuraj, 1992) are initial flow rate (number of latex vessel rings, diameter and other anatomical characters of latex vessels and turgor pressure at the time of tapping), plugging index (rubber particle stability as determined by the composition of the protecting film, lutoid particle stability and the composition of lutoid membrane, flocculation potential of lutoid serum, antagonizing effect of C serum on lutoid serum activity, dilution reaction on tapping, drainage area, whole tree water relations and mineral composition of latex) and rubber content (rubber biosynthetic capacity and level of exploitation).

3.4 Latex yield limiting factors

Two primary factors limiting production are latex flow which governs the quantity of latex collected at tapping and latex regeneration between two successive tappings. Important biochemical parameters connected with latex production are total solid content (TSC), bursting index, sucrose, pH, inorganic phosphorus (Pi), magnesium and thiols (RSH).

3.4.1 Total solid content

Rubber constitutes over 90 per cent of the TSC of latex and under certain conditions TSC reflects the biosynthetic activity. A decrease in DRC indicates inadequate regeneration which can become a limiting factor in production. On the other hand, a high TSC can limit production by making flow difficult (Van Gils, 1951).

3.4.2 Bursting index

Bursting index is a measure of lutoid stability. A high bursting index of lutoids indicates a high destabilization of latex and an early plugging (Jacob *et al.*, 1989).

3.4.3 Sucrose

Sucrose is the initial molecule involved in rubber synthesis. A high sucrose content in latex may indicate a good loading of latex vessels and an active metabolism. It may also indicate low metabolic utilization of sucrose and low productivity. Excessive exploitation can cause a decrease in sugar content (Jacob *et al.*, 1989).

3.4.4 pH

The extent of latex regeneration between tappings depends on the metabolic activity of laticifers. Some of the enzymes which play key role in latex metabolism are sensitive to variations in pH, which thus is a metabolic regulator.

3.4.5 Inorganic phosphorus

Inorganic phosphorus reflects the energy metabolism and is necessary for the production of nucleic acids required in rubber biosynthesis. Direct correlation between Pi content of latex and production has been established in many clones (Subronto, 1978; Eschbach *et al.*, 1984).

3.4.6 Magnesium

Magnesium is an activator as well as inhibitor of many enzymes involved in latex metabolism and therefore, has a complex link with production (Jacob *et al.*, 1989).

3.4.7 Thiols

Thiols trap toxic forms of oxygen thereby protecting the membranes of latex organelles. They are also activators of key enzymes in latex (d'Auzac *et al.*, 1982).

3.5 Physiological diagnosis

Physiological characteristics of latex could be used to assess the production capacity of clones despite differences of environment and age (Eschbach *et al.*, 1983; 1984; Jacob *et al.*, 1986; Prevot *et al.*, 1986). Higher metabolic activity is generally associated with high Pi, RSH and pH levels.

It is desirable that latex of an ideal clone has low bursting index and medium solid content. Stable latex has low bursting index and is characterized by low magnesium and high phosphorus and thiol contents. Medium total solid contents facilitate good latex flow.

Physiological parameters help to characterize clones as having active, slow or intermediate metabolism. Studies on RSH, Pi and sucrose content form the basis of physiological diagnosis of the latex producing system (latex diagnosis) of the tree as affected by tapping. Latex diagnosis makes it possible to recommend appropriate exploitation system taking into consideration the metabolic status of the clone, for optimum production.

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