CARBOHYDRATE METABOLISM AND IONIC BALANCE IN THE LATEX PRODUCTION MECHANISM IN *HEVEA BRASILIENSIS*

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By

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I certify that the thesis entitled **Carbohydrate metabolism and ionic balance in the latex production mechanism in** *Hevea brasiliensis* is an authentic record of the original research work carried out by Sheela. P. Simon in the School of Biosciences, M.G. University, Kottayam and at Rubber Research Institute of India, Kottayam under the joint supervision of Dr. G. Muraleedhara Kurup (Professor, School of Biosciences) and Dr. K.R Vijayakumar (Joint Director, Rubber Research Institute of India, Kottayam) .

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This is to certify that the thesis entitled " **Carbohydrate metabolism and ionic balance in the latex production mechanism in *Hevea brasiliensis*** " is an authentic record of the original research work carried out by Mrs. Sheela.P.Simon, Scientific Assistant, Plant Physiology Division, Rubber Research Institute of India, Kottayam. The work has been jointly supervised and guided by myself and Dr. K.R. Vijayakumar, Joint Director (Research), RRII, Kottayam during the period 1997-2002. The work presented in this thesis has not been submitted for any other degree or diploma earlier. It is also certified that Mrs. Sheela. P.Simon has fulfilled the course requirements and passed the qualifying examination for the Ph.D degree of Mahatma Gandhi University.

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DECLARATION

I hereby declare that the thesis entitled **Carbohydrate metabolism and ionic balance in the latex production mechanism in *Hevea brasiliensis*** is a bonafide record of the research work carried out by me at the School of Biosciences, M.G.University, Kottayam and Rubber Research Institute of India, Kottayam, under the joint supervision of Dr. G. Muraleedhara Kurup, Professor, School of Biosciences, M.G.University, Kottayam and Dr. K.R.Vijayakumar, Joint Director (Research), Rubber Research Institute of India, Kottayam. I further declare that the thesis has not been previously formed the basis for the award of any degree.

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SHEELA P. SIMON

CONTENTS

Symbols and Abbreviations

ATP	-	Adenosine 5' triphosphate
ATPase	-	Adenosine tri phosphatase
ANSA	-	1-Amino 2- naphthol Sulphonic acid
BTP histidine	-	Bis Tris Propane histidine
BI	-	Bursting index
Ca^{2+}	-	Calcium ion
C	-	Celsius, centigrade
CO_2	-	Carbon dioxide
cm	-	centimetre
$CuSO_4$	-	Copper sulphate
CD	-	Critical difference
DTNB	-	5,5' Dithio-bis-2-Nitro benzoic acid
DRC	-	Dry rubber content
et al	-	*et alibi*, and others
etc	-	*et ceteri* and the other
EDTA	-	Ethylene diamine tetra acetic acid
GSH	-	Glutathione (reduced)
g	-	gram
HCl	-	Hydrochloric acid
IFR	-	Initial flow rate
kD	-	kilo Dalton
M	-	Molar
Mg	-	Magnesium
Mg SO_4	-	Magnesium sulphate
Mg Cl_2	-	Magnesium chloride
MES	-	(2-(N-Morpholino)ethane sulphonic acid
mg	-	milli gram
ml	-	milli litre
mM	-	milli molar
nm	-	nanometre
NR	-	Natural Rubber
NAD	-	Nicotinamide adenine dinucleotide
NADP	-	Nicotinamideadeninedinucleotide phosphate
NADPH	-	Nicotinamide adenine dinucleotide phosphate (reduced)

pNP-β-D-Glu.NAc	-	p.Nitrophenyl B-D-N.acetyl glucosamide
N	-	Normal
ns	-	not significant
OD	-	Optical density
Pi	-	Phosphorus (inorganic)
PI	-	Plugging index
PAGE	-	poly acrylamide gel electrophoresis
PEP	-	Phospho enol pyruvate
KH_2PO_4	-	Pottassium di hydrogen ortho phosphate
KOH	-	Pottassium hydroxide
PPi	-	Pyro phosphate
PK	-	Pyruvate kinase
RNA	-	Ribonucleic acid
rpm	-	Revolutions per minute
RSH	-	Reduced thiols
RRII	-	Rubber Research Institute of India
RRIM	-	Rubber Research Institute of Malaysia
Na_2CO_3	-	Sodium carbonate
NaOH	-	Sodium hydroxide
SE	-	Standard Error
SOD	-	Superoxide dismutase
H_2SO_4	-	Sulphuric acid
TPD	-	Tapping Panel Dryness
TSC	-	Total solid content
Tris	-	Tris hydroxymethyl amino methane
TCA	-	Trichloroacetic acid
TP	-	Turgor pressure
UV	-	Ultra violet
UDP	-	Uridine di phosphate
UDPG	-	Uridine di phosphate glucose
viz	-	videlicet, namely
%	-	percentage
µ	-	micro
µl	-	microlitres

CHAPTER 1

Introduction and Review of Literature

Natural rubber (cis- 1,4 polyisoprene), a polymer of very high molecular weight is mostly obtained from the latex of *Hevea brasiliensis* (Rubber tree). In *Hevea* rubber is synthesized on the surface of rubber particles suspended in latex, which constitute the cytoplasm of laticifers. Latex is obtained by wounding the bark of the tree by a process termed tapping. During tapping, laticifers are severed and latex flows out of the tree, which contains 30-50% cis 1, 4-poly isoprene.

Fresh latex is a poly disperse system in which rubber particles and organelles like lutoids and Frey Wyssling particles are suspended in an aqueous cytosol. These components can be easily isolated by differential or density gradient centrifugation (Cook and Sekhar, 1953).

The rubber hydrocarbon molecule has a molecular weight of 10^5-10^7 consisting of long chains of the monomer, isoprene C_5H_8. Rubber particles are spherical or pear shaped and the size usually ranges from 5nm-3μm. It is surrounded by a membrane composed of proteins and lipids (Cockbain *et al.*, 1963).

Although rubber is the major component of *Hevea* latex, there are a number of other isoprenoid substances in latex including isoprenoid alcohols (Archer and Audley, 1987), sterols and wax alcohols (Altman, 1948), tocopherol (Dunphy *et al.*1965), Ubiquinone (Wittle *et al.,* 1967) and carotenoids (Eaton and Fullerton., 1929).

The C-serum is a mixture of glucids, polyols, minerals, organic acids, aminoacids, nitrogeneous bases, proteins, reducing agents and miscellaneous solutes (Archer *et al.*, 1969).

There are two major non-rubber particles in latex, lutoids and Frey wyssling particles. Lutoid particles amount 10-20% of the volume of latex. They are single membrane bound microvacuoles with typical lysosomal characteristics (Pujarniscle 1968). The membrane consists of proteins and lipids including neutral lipids, phospholipids, phosphatidic acid and glycolipids.(Hebant 1981). Lutoids have a serum of acidic pH ~5.5 and accumulate various proteins which include hevein, chitinase, β1-3 glucanase, lysozymes and divalent cations such as Ca^{2+}, Mg^{2+}, $Cu2^{+}$ etc (d` Auzac 1995). The release of these contents into the C-serum imparts a major role in the coagulation of latex after flow. Hevein is the most important protein for latex coagulation,(D'Auzac 1995).

The other non-rubber constituent is the Frey Wyssling particles, which are bound by double membrane and are yellow or orange in colour due

to the presence of carotenoids (Frey Wyssling 1929). This particle contains large amount of poly phenol oxidase.

In Hevea brasiliensis rubber is synthesized on the surface of particles suspended in latex. Natural rubber from Hevea consists of long chains of cis-poly isoprene which are synthesized through mevalonate pathway (Keckwick 1989) from acetyl CoA derived from glycolysis. (Jacob, 1970; Tupy, 1973). Numerous investigations have shown that acetate is the simplest initial precursor of isopentenyl pyrophosphate (Bandurski and Teas, 1957) and hence of rubber.

1.1 BIOSYNTHESIS OF RUBBER FROM SUCROSE

Being a non-photosynthetic tissue, the productivity of the sink will depend on photosynthate supply by the source leaves and on long distance translocation, allocation and efficiency of utilization of mobile sugars for laticiferous tissue metabolism and rubber formation. Photosynthates are translocated to latex vessels as sucrose in *Hevea*, which is the usual transport form of sugars in higher plants, and this sucrose is the main precursor of acetate (Lynen 1969). Carbohydrate supply to the laticifers is one of the main factors which limits latex production. (Backhaus, 1985; Tupy, 1985). Latex vessels are the factory where the rubber is synthesized. Production potential of a clone depends on production without stimulation which determines its initial

metabolic activity , sucrose concentration in laticifers , conversion of sucrose to rubber (Gohet *et al.*, 1997).

There are two findings that showed this transport. First, sucrose is the only sugar, which could be found in the latex, providing the activity of invertase (Tupy and Resing, 1968) and second when radio labelled sucrose, fructose or glucose were applied on the bark, most of the radioactivity of latex was found as sucrose irrespective of the source label. Various chromatographic studies have shown that sucrose is the main glucid in latex and therefore very probably the main transfer sugar.

Glycolysis can be considered as the major catabolic process of sugars in latex. There are two phases in the conversion of sucrose to cis-1,4 polyisoprene.

1. Conversion of sucrose into acetate. It simultaneously provides energy in the form of ATP and reducing power in the form of NAD (P) H.
2. Synthesis ofcis-1,4 polyisoprene. It requires ATP, reducing power in the form of NAD(P)H and acetate or Acetyl-CoA for the building of isopentenyl pyrophosphate.

Carbohydrate breakdown is the obvious source of ATP, Acetyl-CoA and NADPH required for rubber biosynthesis (Lynen 1969). Glycolysis generates the energy required for the synthesis of rubber and produces NADH or NADPH for isoprene biogenesis (Lynen 1969).

Enzyme activities linked to sugar metabolism other than glycolysis are the pathways from pyruvate to acetyl-CoA, the pentose phosphate pathway, enzymatic reactions involved in the galactose metabolism, quebrachitol synthesis and sucrose (neo) synthesis (Jacob *et al.*, 1989).

The principal control points in sucrose catabolism are

(a) Invertase activity

(b) Hexose phosphorylation

(c) Phosphoenol pyruvate metabolism

(d) Control by external and internal factors

The external and internal factors are regulated by pH, sucrose level, effect of tapping, stimulation of latex production using chemicals, climate and sucrose catabolism in relation to latex flow and production.

The sequence from sucrose to Acetyl-CoA is entirely cytosolic and certain stages control its activity. One of the main enzyme involved in carbohydrate metabolism is the invertase (Tupy, 1973; Jacob, 1970). Invertase plays an important role in controlling carbohydrate metabolism and latex production. (Tupy and Resing, 1968,1969; Tupy,1969.) and the most striking regulatory factor of invertase is latex pH and latex sucrose content (Tupy, 1969;Yeang et al 1986). This enzyme is extremely pH dependent with an optimum pH 7.3-7.5 and molecular weight is ~590,000kD. Variations in physiological pH considerably influence the activity of this enzyme. Mg^{2+} and Cu^{2+} present in the cytosol are inhibitors (Primot, 1975; Conduru

Neto *et al.*,1984; Tupy, 1973.) and Pi, NH_4^+, RSH and Cl^- are activators (Jacob *et al.*,1982). Significance of latex pH for invertase activity, latex production and effect of stimulation was studied by Tupy (1969). Tupy (1969&1973) demonstrated the correlation between invertase activity and latex production. Tupy, 1973., Jacob, 1970 and Conduru Neto *et al.*, 1984 reported that invertase was one of the key enzyme in carbohydrate metabolism and it can be a limiting factor for production.

Glucidic catabolism supplies the reduced cofactors which generate cell reducers (reduced glutathione, ascorbic acid, tocopherols) which can with the help of enzyme systems such as catalase, superoxide dismutase,peroxidase oppose the harmful effects of various forms of toxic oxygen on the degradation of membranes and decompartmentalization of cells (Chrestin *et al* ., 1984; Gohet, 1997).

Enzymes involved in the synthesis of sucrose are pyrophosphate: 1: fructose 6-phosphate phospho transferase (PP-PFK); UDPG pyrophosphorylase and sucrose synthase (Tupy and Primot, 1982). These enzymes can also effectively slow down the glycolytic activity (Tupy 1988a).Presence of sucrose synthase in latex has been suggested by Jacob1970. Sucrose catabolism in latex has been shown to be dependent on sucrose synthase activity (Tupy,1979; Tupy and Primot, 1982). The molecular weight of sucrose synthase was reported to be 380,000kD. Tupy and Primot ,(1982) studied the comparison of total activities

of invertase and sucrose synthase (synthetic reaction) in latex cytosol of different *Hevea* clones and cultures.

Isoprene synthesis is the major synthetic process in latex since over 90 % of the dry matter is cis-1, 4 polyisoprene. The synthesis of rubber is now known (Lynen, 1969., Kekwick, 1988, 1989 and d' Auzac, 1997). Polymerisation of isopentenyl pyrophosphate by prenyl transferase , assisted by the rubber elongation factor ,gives rise to the long chains of cis-polyisoprene (rubber) (Chrestin *et al.*, 1997).

1.2. Factors influencing metabolism of laticifers

Metabolism of laticifers depends on various factors.

1.2.1. Sugar loading

Sucrose loading of laticifers is an extremely important phenomenon in latex production. Since plasmadesmata are absent from laticiferous vessels, sugars can enter only by crossing the plasmalemma (De Fay *et al.*,1989). An inter membrane transport process is involved in this process which require energy.It operates at laticifer plasmalemma level and involves a co-transport of H^+-sucrose energized by an electrochemical proton gradient set up by an ATPase proton pump (Lacrotte *et al*, 1988a, b., Sentenac and Grignon, 1987., Thibaud *et al.*, 1988;Lacrotte,R.1991).

1.2.2. Sucrose supply

The supply of sucrose to laticiferous tissue and its utilization is a crucial point and could be a limiting factor in the production of

rubber (Backhaus,1985., Tupy,1985). Latex is relatively rich in sucrose (1-50mM) whereas glucose, fructose and raffinose are present in small quantities (d' Auzac and Pujarniscle, 1959., Bealing, 1969., Low, 1978).These sugars are easily catabolised in latex.

1.2.3. Availability of sucrose in laticiferous tissue

The sucrose content of latex is the result of carbohydrate loading to laticiferous tissue andutilisation of sucrose for respiration and rubber biosynthesis. In a healthy tree, availability of sucrose in latex is an important factor in the metabolic activity of the laticifers and of production (Tupy, 1988b). Sucrose catabolism supplies the acetate molecules, which initiate isoprene chain and provide the biochemical energy for the functioning of the laticifers (Jacob *et al.*, 1988b).A high sucrose content in latex may indicate an active metabolism and high productivity (Tupy and Primot,1976) or a low metabolic utilization and low productivity. Positive and significant correlations have established between sugar content and latex production by Jacob *et al.* (1986).

Sucrose availability for metabolic processes in latex producing bark depends on a number of factors such as clonal properties, level of irradiance, soil moisture status, and bark treatment with stimulants and system of tapping. Conventional tapping due to removal of secondary phloem tissue negatively affects long distance sucrose translocation and the level of latex sucrose decreases as the surface of consumed bark above the tapping cut increases.

1.3. Factors related to mechanism of latex flow and production

Latex production of Hevea is controlled by factors related to the mechanisms of latex flow and regeneration. Some of these factors are tapping systems, stimulants and climatic conditions. (Jacob et al,1989).

1.3.1. Effects of tapping intensity on sucrose availability and its and its utilization for rubber biosynthesis

The economic product of *Hevea* is a rubber containing cytoplasm, which is continuously formed in an artificially created sink from which it is withdrawn at short regular intervals of time. When a tree is newly brought into regular tapping, the first initial tapping on full spiral cut bring about a drastic decline in sucrose content in the latex vessels (Tupy, 1973). A strong decrease in sucrose was also seen when the d/3 system of tapping is applied. There are significant clonal differences in the effect of initial tapping on the level of latex sucrose (Tupy, 1979). A high sucrose content in latex may indicate an active metabolism and high productivity(Tupy and Primot,1976) or low metabolic utilization and low productivity.

Sucrose availability in latex vessels obviously decreases with an increased intensity of exploitation. Tupy (1982) studied the level and distribution of latex sucrose in different areas of bark and the effect of

exploitation intensity. They demonstrates the sucrose distribution pattern and the lowest sucrose levels in more or less distant areas of bark below the tapping cut as a result of high intensities of tapping. Similar sucrose concentrations were found in latex samples taken by punctures in the drained area as in latex collected from the tapping cut. Vijayakumar *et al* (1991) studied the variations in biochemical components of latex in trees tapped intensively. It was concluded that the induction of high level of sugars in the latex might be an indication of faster induction of Tapping Panel Dryness (TPD), a major syndrome encountered in rubber plantations.

Carbohydrates are supplied to latex vessels as sucrose and it is used in rubber synthesis. Carbohydrate utilization in glycolysis obviously provides substrates and energy for other life functions of latex producing tissue including active transport processes between cells and cell compartments (d' Auzac, 1997).

Inadequate sucrose availability for basic biological functions of latex vessels may, in the long run, result in premature latex vessel degeneration and tapping panel dryness. This is indicated by observation that high tapping panel dryness is associated with more or less continued low levels of sucrose in latex. (Gohet *et al* .,1997) .

Other biochemical parameters varies according to different tapping frequencies. Phospholipids and total sugars showed an increase in intensively tapped trees 1/2S d/0.5 6d/7 when compared to 1/2S d/2 6d/7 system of

tapping.(Vijayakumar et al., 1991). Trees tapped with d/2 system produced higher bottom fraction, thiol, Pi and lower yield than d/4 system of tapping (Do Kim Thanh,1996). Enzymes associated with carbohydrate metabolism and pH regulation in trees with low frequency tapping system has not been studied so far.

1.3.2. Effect of climatic conditions on sucrose availability and its utilization in latex production

Seasonal variation in the level of latex sucrose results from the variations in irradiance and photosynthetic activity and from physiological changes related to wintering. The period of wintering is characterized by a striking rise in sucrose level during the process of refoliation, which is possible to explain by mobilization of stored carbohydrates from the bark and trunk parenchyma. The latex yield falls at the time of wintering suggests that some seasonal physiological mechanism limiting sucrose utilization in latex metabolism (Tupy, 1973). Decrease in invertase, which was a key enzyme in glucidic catabolism(Tupy 1973) observed during the wintering month. A fall in pH occurred with the dry season and leaf fall. Seasonal variation also play a considerable role in latex composition.(Van De Sype 1985).There was depressive effect of defoliation and leafing on production.(Martin,1969; Van de sype,1985). Variations in enzymes involved in carbohydrate metabolism during peak yielding season, defoliation, refoliation and stress period in our climatic condition has not been studied so far.

1.3.3. Influence of stimulation with ethrel on the metabolism of laticifers

It is a common practice in rubber plantation to apply an ethylene generator such as Ethrel to the tapping panel in order to stimulate latex production. The productivity of *Hevea* depends on the duration of flow after tapping and latex regeneration capacity between two tappings. Hormone treatment has a favorable effect on these two limiting factors. The duration of flow was increased (Dejonge 1955) and increases the total volume of latex during tapping (Ho and Paardekooper, 1965). In high yielding trees low frequency tapping system with stimulation can reduce the cost of production of natural rubber (Karunachamy *et al.*,2001).

Ethrel intensify the influx of sucrose from apoplast .It is a sign of activation of intra-laticiferous metabolism (Chrestin,1984;). The level of sucrose in latex is increased by various stimulants (d'Auzac and Pujarniscle, 1961; Tupy 1973) and that also against a concentration gradient. The long-term effect of stimulation resulted in the lowering of latex sucrose (Tupy, 1973; Tupy and Primot, 1976). Application of yield stimulant decreases the latex sucrose in the long run was also evidenced by Low and Gomez (1982) and Tupy (1973). Eschbach *et al*, (1984) showed that in four clones they studied, the relative increased production due to stimulation is directly proportional with the sink effect of sucrose in latex. Sucrose is the basis of the production of cis-polyisoprene in latex and the sucrose content of latex can in

many cases be correlated with the amount of rubber produced (Tupy and Primot, 1976).

Factors increasing sucrose catabolism such as activation of invertase by an increase of latex pH stimulate latex flow. The initial events occurring after bark treatment with stimulants, seems to include activation of electrogenic proton extrusion enhancing transport of solutes into latex vessels and increased cytoplasmic pH which in turn stimulates glycolysis and generation of metabolic substrates and energy (Koshy, 1997).

In addition, after stimulation, the mechanisms involved in latex regeneration are modified. One of the main effects of stimulation is the activation of the mechanism connected to the rapid alkalinisation of the laticiferous cytosol and acidification of lutoids. This alkalinisation influences the metabolic processes, which depends on the pH (d'Auzac and Pujarniscle, 1960., Tupy, 1969., Tupy, 1973., Tupy and Primot, 1976; Jacob *et al* .,1979). Alkalinisation of the pH of the cytosol appears to be the determining factor in the activation of the metabolism and particularly as regards the synthesis of cis-polyisoprene.

It is very probable that the walls of laticiferous vessels have no functional plasmodesmata it must be concluded that sucrose is absorbed actively by the laticiferous vessels (d'Auzac *et al* 1982). An enhanced sucrose transport would require an increased generation of an electro chemical potential gradient of protons. Stimulation of an electrogenic proton excretion from plant cells by various hormones seems to be a widely occurring

phenomenon, probably resulting from the activation of an ATP driven proton pump (Raven1977). Exogenous ethylene might directly or indirectly activate the operation of an ATPase proton pump located in the laticiferous plasmalemma and which can set up the proton motive force necessary to power a proton- sucrose symport towards the inside of the laticiferous vessels. Intensifying of ATPase is caused partly by activation of enzyme synthesis ,an increase in the cytosol activator content and an increase in availability of ATP(Gidrol and Chrestin.,1984).

In addition to invertase, various key enzymes are very sensitive to pH. The enzyme PEP Carboxylase (Jacob *et al.*, 1979) that is particularly important since it produces strong acids such as malate and citrate. These enzymes are also sensitive to inhibiting solutes present in the cytosol including magnesium, calcium, citrate etc. Pyruvate kinase is another important enzyme .In addition, thiol group molecules such as glutathione in latex enhance the action of invertase and pyruvate kinase.

Thiols have a regulatory role in the metabolic processes. They participate in the rubber biosynthesis processes as a factor for latex stability. Stimulation always causes a considerable decrease in RSH contents which is most marked after 24 hours of treatment (Prevot *et al.*, 1986; Lacrotte *et al.*, 1988a). Thereafter the thiol content tends to return to the normal level (Prevot *et al*, 1986; Commere *et al.*, 1990; Gohet 1997).

The metabolism of clones with sugar rich latex is usually slow and requires stimulation to ensure effective use of its carbohydrates for regeneration and vice-versa . Supplying of sugar is an active metabolism (Lacrotte *et al.*, 1985). It can be intensified by stimulation which induces a sink effect at tapping panel (Lacrotte *et al.*, 1985; Tupy 1973). Treatment of ethrel may therefore be a way of removing the limiting aspect of sugar supply. Weakness or absence of this sink effect after stimulation is a signal that probably indicates a serious deficiency with regard to the carbohydrate reserves of the tree and hence regeneration processes in the laticifers. (Tupy, 1973; Eshbach *et al.*, 1984; Gohet *et al.*, 1997).

The variations in the enzymes invoved in carbohydrate metabolism and the enzymes that regulate pH under low frequency tapping system with stimulation in clone RRII 105 have not been studied.

1.4. Clonal variations in carbohydrate metabolism and pH balance

Various parameters related to rubber biosynthesis varied among clones.Clonal variations in sucrose , Pi, thiols were studied by Usha Nair *et al.*,2001., Koshy,1997. Many researchers showed that high yield was always accompanied by a high pH , thiols, RNA, sucrose contents and invertase activities in the cytosol. (Eschbach *et al.*, 1984; Jacob *et al.*, 1987; Koshy, (1997).

1.5. Factors involved in the latex regeneration mechanism in *Hevea* latex

The factors which play an important role in the regulation of latex regeneration and consequently on the rubber biosynthesis are

1. The cytosolic pH.
2. Magnesium
3. Inorganic phosphorus
4. Thiols
5. Availability of sucrose
6. Redox potential
7. Total solid content

1.5.1. pH

pH measured in fresh latex is that of cytosol compartment. Most of the rubber regeneration processes takes place in this compartment (Lynen, 1969). Invertase, which is the key enzyme involved in glycolysis is extremely sensitive to physiological variations in pH. The importance of cytosolic pH for latex production was evidenced by Primot *et al.*, (1978), and Koshy, (1997).

Highly significant positive correlations have been shown between latex pH and production (Coupe, 1977; Eschbach *et al.*, 1984 and Koshy, (1997). A low latex pH will correspond to a weak glucidic catabolism, weak isoprene

synthesis and hence low production and vice versa. Regulation of cytosol pH is by a biochemical pH stat and by a biophysical pH stat, which bring cytosol-lutoid exchange of protons (Jacob *et al.,* 1983; Chrestin *et al.,* 1985). pH - production correlation was refined in particular by Coupe *et a.,,* (1977). It was shown that the increase in pH after stimulation was parallel with production. The regulation of the cytosol pH is of prime importance. It is based on a biochemical pH-stat mechanism involving the functioning of PEP carboxylase, the production of strong acids malate and citrate and their degradation (Jacob *et al.*, 1979) or sequestration in the vacuo- lysosomal compartment.

A biophysical (bio-osmotic) pH stat system actively transfers protons produced by the metabolism either to the lutoids or to the apoplast. One ATPase located on the tonoplast and another that is very probably located on the plasmalemma extrudes protons and help to maintain a favorable cytosol pH. At the same time, these ATPases create a proton motive force which energizes the flow of solutes such as sucrose, ions etc from the apoplast into the cytosol and the out flow of solutes such as citrate, magnesium, calcium and Pi from the cytosol into the lutoids . Regulation of invertase by pH in situ is evidenced by a highly significant positive correlation between invertase activity and pH in the cytosol. (Yeang et al .,1984).

1.5.2. Magnesium (Mg^{2+})

Magnesium is an activator of the numerous enzymes in latex. For e.g. ATPases (Chrestin *et al*, 1985); Transferases (Skilletor and Kekwick, 1971; Jacob *et al.*, 1981); phosphatase (Jacob *et al.*, 1986) and PEP carboxylase (Jacob *et al.*, 1977 &1981). Magnesium is also an inhibitor of other enzymes such as invertase (Primot, 1977) and acid phosphatase (Jacob and Sontag, 1974). Alkaline pyrophosphatase in cytosol requires magnesium in order to function. Subronto *et al.*, (1978) demonstrated a significant inverse correlation between magnesium content and production. However Eschbach *et al.*, (1984) demonstrated a positive correlation between magnesium and production.

1.5.3. Inorganic phosphorous (Pi)

The Pi content in latex reflects its energy metabolism. It makes a considerable contribution to glucidic catabolism and isoprene synthesis. Pi is released insitu from the hydrolysis of phosphorylated molecules and pyrophosphate produced during the lengthening of polyisoprene chain by rubber transferase (Lynen, 1969). The hydrolysis of pyrophosphate is caused by a specific pyrophosphatase in the cytosol (Jacob *et al.*, 1986) and a pyrophosphate-fructose 6-phosphate phospho transferase (Prevot *et al.*, 1987).

Eschbach *et al.*, (1984) and Subronto *et al.*, (1978) revealed a direct correlation between Pi content of latex and production of certain clones. Stimulation also increases Pi content of latex (Eschbach *et al*, 1984). Latex Pi content tends to decrease during wintering (Van de Sype, 1985).

The activity of the pyrophosphatase in latex has an imperative need for Mg^{2+} and depends to a great extent of the Mg/PPi ratio. The regeneration of cell material between two tapping implies intense synthesis at all cell levels in the laticiferous cells and above all very active isoprene anabolism, which generates PPi in the cytosol compartment. Alkaline pyrophosphatase in cytosol hydrolyses pyrophosphate (Rauser,1971) formed during isoprene synthesis and prevent accumulation of PPi, must have an important role. There are certain favorable conditions for the pyrophosphatase functioning. The optimum pH of the enzyme is similar to the cytosol pH(7.5). The enzyme has a relatively strong affinity for PPi. Pyrophosphatase activity may be limited by the availability of Mg^{2+}. Mg^{2+} can be chelated by anion group molecules such as citrate or malate. The concentration of these organic acids in the cytosol can be fairly high and variable (Ribaillier 1972). They can therefore reduce by competition, the amount of magnesium available to a sufficient extent to slow down the pyrophosphatase significantly and hence indirectly control it. There is an acid pyrophosphatase activity in lutoid. Some amount of this enzyme is present in cytosol .It is probable that the alkaline pyrophosphatase (E.C.3.6.1.1) plays an important metabolic role. The PPi content is a function of various factors such as stimulation and tapping frequency.

1.5.4. Thiols (RSH)

Thiols play an important role in the protection of membranes of latex

organelles and thus promoting colloidal stability and flow of latex (Chrestin *et al.*, 1984). Thiols are also potential activators of key enzymes such as invertase (Jacob *et al.*, 1982) and pyruvate kinase (Jacob *et al.*, 1981) in latex.

Thiol deficiency would affect cell decompartmentation leading to malfunctioning of laticifers and decreased production. Several authors have demonstrated a direct correlation between thiol concentration and production (Eschbach et al., 1984; Jacob *et al.*, 1984; Prevot *et al.*, 1986).

1.5.5. Sucrose

A high sucrose content in latex may indicate a good loading of the laticifer cell, which may be accompanied by an active metabolism (Tupy and Primot, 1976). High sugar content in latex may also indicate a low metabolic utilization of sucrose and low productivity (Prevot *et al.*, 1986). Sucrose tends accumulate when insitu regeneration is complete and when laticiferous metabolism slows down.

Variations in sugar content are used in latex diagnosis to estimate the physiological condition of rubber trees (Jacob *et al.*, 1988). They also indicate the functioning typology of latex bearing systems according to their clonal origin (Serres *et al.*, 1988). A high sucrose content in latex may indicate good loading of laticifer cell, which may be accompanied by an active metabolism (Tupy and Primot, 1976). A high sugar content in latex may also indicate low metabolic utilization of this sugar and finally low production Insitu sugar

availability depends partly on sucrose supply to the laticifers and partly on sucrose utilization by the latex generating metabolism (Gohet et al.,1997).

In previously untapped bark the sucrose content in latex decreased dramatically until 4 th day and progressively increased for the next 6 days to reach initial values. In tapped bark, no changes in the level of latex sucrose were observed in the first 12 hrs whereas sucrose catabolism was enhanced (Tupy and Primot, 1982). Over a longer period, the sugar contents in the latex decreases as a result of stimulation (Low and Gomez, 1982; Tupy, 1969, 1973; Tupy and Primot, 1976).

Sucrose is the principle sugar in latex cytosol (Bealing, 1969; Bolle Jones, 1953; Tupy and Resing, 1969). The sucrose content of latex cytosol of unexploited trees generally varies between 30 and 60mM (Bealing and Chua, 1972; Tupy, 1973&1985). The introduction of regular tapping every three days results in a drastic fall of sucrose content in latex during initial taps, although the production is very low (Tupy, 1973). Over exploitation by tapping or over stimulation leads abnormally low DRC values, a sign of inadequate regeneration of latex. A latex sugar content of less than 3mM is an alarm signal. The sugar reserves of the tapping panel are exhausted and the sucrose supply from the phloem is inadequate.

1.5.6. Redox potential (RP)

A large number of oxidation-reduction takes place in the laticifers. Poly isoprene synthesis involves oxidative catabolism (glycolysis) which

provide biochemical energy and reducing power and anabolic processes connected with energy using reducing reactions leading to production of rubber (Lynen, 1969). A reducing redox potential indicates the intactness and functional effectiveness of the lutoids and a favorable environment for isoprene synthesis and production

1.5.7. Total solid content (TSC)

TSC reflects the biosynthetic activity of laticifers. A low value indicates decreased insitu isoprene regeneration, which is a limiting factor in production. Positive correlation between TSC and production was reported by Prevot *et al.,* (1986). In the case of intensive exploitation there is a decrease in TSC, which reflects inadequate latex regeneration between two tappings. High TSC value indicate effective regeneration which may inturn disturb the latex flow by increasing latex viscosity.

1.6. Latex regeneration metabolism and isoprene synthesis

Ninety percent of latex regeneration metabolism is geared towards isoprene synthesis. Renewal of the rubber lost during tapping before the next tapping operation is a key yield-limiting factor. The successive reactions that begin with sucrose and end with the elongation of isopentenyl pyrophosphate, which is the monomer of the polyisoprene chain, are now known.

The main factor limiting glycolysis in the laticifer system is the invertase functioning, the initial reaction of this catabolic pathway. Lynen

(1969) has described isoprene anabolism from sucrose to rubber. The invertase stage is therefore probably the essential link in the metabolic activity responsible for insitu regeneration and its regulation. Insitu latex renewal, especially isoprene synthesis, requires a great deal of energy

Glycoproteins , especially their carbohydrate portion plays very important role in many biological functions. In some pathological conditions alterations in amount of specific monosaccharides have been found (Djurdjic and Mandic,1990).Variations in carbohydrate components like fucose, hexose, sialic acid in B-serum has not been studied so far.

Krishnakumar *et al.*, (1999) studied the biochemical composition of soft bark tissues affected by TPD (Tapping panel dryness syndrome) and showed that TPD affected tissues contained comparatively higher levels of sugars and indicated that lack of availability of sucrose was not the cause for TPD. Gohet *et al.*; (1997) studied the relation between clone type, latex sucrose content and the occurrence of Tapping panel dryness.

Lacrotte *et al.*, (1984) demonstrates the existence of a proton pump ATPase on the plasmalemma of laticiferous cells which is sensitive to ethylene and it is the controlling part of the carbohydrate supply to the laticifers because sucrose is of prime importance for the laticiferous metabolism and for rubber production.

Inorganic pyrophosphate is released during numerous reactions linked with anabolic processes. In *Hevea*, between two consecutive tapping, high

metabolic activity is observed in the laticiferous vessels to regenerate cellular material and especially polyisoprene, the synthesis of which releases PPi (Lynen, 1969; Jacob,*et al.*,1988). The accumulation of PPi can inhibit key steps in the synthesis of rubber, hence in latex the enzyme activities capable of removing PPi must be efficient to avoid accumulation of this molecule and the consequences of such accumulation.

However PPi does not accumulate in the cytosol compartment where rubber is produced. PPi content in cytosol is less than 0.1mM. The cytosol contains two enzymes, which use energy released by PPi hydrolysis. A soluble inorganic pyrophosphate D-fructose 6-phosphate1-phosphotransferase (E.C.2.7.1.90) and also a pyrophosphatase

A cytosol specific alkaline pyrophosphatase (E.C.3.6.11) was demonstrated by Jacob *et al.*, 1989. This enzyme displays narrow specificity and Mg^{2+} dependence. It preferred PPi as substrate and it can also hydrolyze polyphosphates. The activity of the pyrophosphatase in latex depends to a great extent on the Mg^{2+}/PPi ratio. The optimum of this ratio for pyrophosphatase can vary from 1-10. The optimum pH of this enzyme is similar to that of cytosol pH. Alkaline pyrophosphatase activity is one factor, which can prevent accumulation of PPi and its consequences; it also enables the turn over of inorganic phosphorous indispensable to energetic metabolism.

An acid pyrophosphatase activity occurs simultaneously in *Hevea brasiliensis* latex. It appears to be caused essentially by the acid lutoid phosphatase (E.C.3.1.3.2). It is not a specific enzyme but a hyrolase with a large range of substrates and is not Mg^{2+} dependent (Jacob and Sontag, 1974).

The involvement of latex pH in metabolic regulation and therefore in rubber production was studied extensively by Tupy (1969). Cytosolic alkalinisation leads to a marked activation of numerous pH dependent enzymes from glucidic catabolism and isoprene anabolism such as invertase, pyruvate decarboxylase, PEP carboxylase and resulting in a regenerative biosynthesis of cis-polyisoprene within the laticiferous cells (Jacob, 1970).

Highly significant direct relationships were shown between pH of cytosol, lutoids, and transtonoplastic ΔpH on the one hand and rubber production on the other hand (Coupe and Lambert, 1977).

1.7. Phospholipids, glycolipids and latex production

Latex is a hydrosol in which the dispersed particles are protected by a film of proteins and lipids(Hebant,1981). Glycolipids and phospholipids associated with rubber and non-rubber particles in latex plays a vital role in the stability and colloidal behaviour of latex. The concentration and distribution of lipids between the rubber cream and bottom fraction had been studied by Ho *et al*, (1975). Usha Nair *et al* (1993) studied the clonal

variations in lipid content of different clones and their implication in latex production. Greater stability of lutoids in the high yielding clones may be associated with a higher content of phospholipids and triglycerides in their lutoid membrane. The colloidal stability of latex was shown to be related to the lipid content of rubber particles (Sherief and Sethuraj, 1978). Lutoid stability as indicated by bursting index was found to be negatively correlated with the phospholipid content of the bottom fraction of latex (Sherief and Sethuraj, 1978). The stability of lutoid plays an important role in latex flow and in latex production. The major proportion of phospholipids present in lutoid membrane is phosphatidic acid which gives a highly electronegative charge on the membrane which was responsible for the colloidal stability to the latex. Clonal variations in leaf and latex lipid composition was studied by Molly Thomas *et al.,* (1990) and reported that these parameters may be used as early prediction parameters for yield characteristics. The relation between phospholipids and ionic balance in lutoids and cytosol has not been studied so far.

1.8. Membrane enzymes involved in proton transport

The pH of latex could be regarded as a selective criterion in clonal typology. (Eschbach, 1984). D'Auzac (1975&1977) showed the existence of an ATPase bound to the lutoid membrane (tonoplast). This tonoplastic ATPase is an electrogenic H^+ pump as demonstrated by Marin (1983) and Cretin *et al.*,

(1982). Another enzyme NADH cytochrome c-reductase (E.C.1.6.99.3)was able to translocate protons from intravacuolar medium into cytosol.

These two enzyme systems are antagonistic in nature. The ATPases operates as an electrogenic pump, which enables influx of cytosol protons to the lutoid compartment. The tonoplastic ATPase has an optimum activity at pH 6.8. However, the NADH Cytochrome c-reductase allows an outflow of protons from the lutoids to the cytosol. These enzymes have an optimum pH of 7.2. The functioning of these two enzymes can have the effect of a fine control of the pH. The ATPases also creates a proton motive force, which is able to regulate the transtonoplast fluxes of various solutes and in particular accumulation of citrate, Mg^{2+}, Ca^{2+} and lysine in lutoids. Both of these enzymes which are located at the lutoid tonoplast are involved in the control of proton exchanges between cytosol and vacuolar compartment in latex cells.

Movement of protons generated by these two tonoplasic H^+ pumps could be used as the driving force for secondary transport systems in order to detoxify the laticiferous cytosol and to ensure a maximum regenerative biosynthesis of cis-poly isoprene (Gidrol and Chrestin, 1984).

Physiological parameters involved in latex production were not only linked to the pH of latex cytosol. It is also associated positively with the transtonoplastic pH gradient (the difference in pH across the membrane separating the cytosol from the intra vacuolar medium) and negatively with the intravacuolar pH (Coupe and Lambert, 1977).

A pyrophosphatase (Siswanto *et al,* 1994) whose hydrolytic activity aids that of a cytosol pyrophosphatase (Jacob *et al,* 1989) may also perform a classical pump function for proton influx into lutoids.

Ethylene activates ATPase and pyrophosphatase causing acidification of the lutoid serum and coupled with alkalinasation of the cytosol resulting in acceleration of cytosol metabolism (Gidrol and Chrestin, 1984). No definite information is available regarding the variations in enzymes involved in proton transport under different situations such as seasonal, tapping systems and with stimulation.

1.9. Enzymes involved in latex production through coagulation

Hevein (one of the most predominant proteins in latex) is found in the lutoid particles of latex (Gidrol et al.,1994). Upon tapping, lutoids burst open due to the difference in turgor pressure (Buttery and Boatman, 1967). Rubber particles are the site of biosynthesis of natural rubber, which occurs in the cytosol only (Archer *et al.*, 1969). Under normal conditions hevein does not interact with the rubber particles insitu due to its compartmentalization in the lutoids. In tapping panel dryness syndrome, the bursting of lutoid particles inplanta has been reported (Chretin, 1989). The bursting of lutoids may allow the rubber particles and hevein to interact resulting in planta coagulation of latex (Gidrol, *et al.,* 1994). Hevein freed by the bursting of lutoid bodies is present in high concentration together with Ca^{2+} in the latex cell cytosol. This lectin creates multivalent bridges between rubber particles, through its binding

to the N-acetyl glucosamine moiety of the 22kDa-receptor protein located at the surface of rubber particles. This stage is associated with low chitinase activity and low level of free N-acetyl glucosaminidase in the latex cytosol. Rubber particles are agglutinated and the latex is coagulated. The binding of N-acetyl glucosamine to hevein is Ca^{2+} dependent. The binding of Ca^{2+} could provoke changes in the conformation of hevein, which in turn are important for the stabilization of the saccharide binding sites. It is possible that Ca^{2+} favor dimerization of hevein inplanta, allowing in turn binding of N-acetyl glucosamine. Furthermore, the binding of Ca^{2+} likely confers to hevein a high degree of stability protecting it against hydrolysis by proteolytic enzymes present in lutoids.

Lutoids are known to have an acidic pH~5.5 and highly buffered content and to be very rich in Ca^{2+} (1.5mM) as compared with C-serum (0.2mM) (Ribaillier *et al*, 1971). The bursting of lutoids leads to the release of divalent cations along with hevein and provokes the acidification of the immediate vicinity where the process of coagulation starts. Previous physiological and biochemical studies by d'Auzac, 1989 on effect of divalent cations and pH on coagulation and latex flow in response to stimulation supports this hypothesis.

The presence of chitinase in latex (Melinda N Martin, 1991) inhibits coagulation by removing N-acetyl glucosamine moiety from the 22kDa receptor protein in one hand and by increasing the concentration of free

N-acetyl glucosamine in the latex which block hevein binding sites. N-acetyl glucosaminidase (E.C.3.2.1.36) which catalyses the hydrolysis of the β-N-acetyl glucosaminyl linkages exists in carbohydrates and glycolipids and glycoproteins. Roger-Giordani *et al* (1992) reported the presence of this enzyme in latex. It is a 92kD protein consists of two 46kD subunits and has a glycoside content of 16%. This enzyme inhibits latex coagulation (Chrestin,*et al* 1997).

In contrast to the above findings on destabilizing effect of hevein, (Ukun *et al* 1999) presented evidence that hevein has a stabilizing effect on suspension of rubber particles. The results showed that hevein content of lutoids in high yielding clones was higher compared to low yielding clones. The hevein content,N- acetyl glucosaminidase and proton pumping enzymes involved in ionic balance in different clones has not been reported so far.

1.10. Carbohydrate metabolism and latex flow

Latex pH and actual invertase activity in successive latex fractions after tapping exhibit a decline and are very low at cessation of flow (Chong, 1981; Tupy, 1973). Yield response to ethephon greatly depends on sucrose availability in latex vessels determining the possibilities for an enhancement of invertase activity after treatment (Tupy, 1973). In trees with very low latex sucrose the stimulation of yield can be very low or null (Tupy and Primot, 1976).

Control of the internal metabolism of latex cells by intracellular pH and the ionic composition of the cytosol has a predominant impact on rubber production (Jacob *et al*, 1986). An increase of cytosolic pH enhancing invertase and accelerates sugar utilization. The rubber biosynthesis includes the transformation of PEP into pyruvate and acetate (Lynen, 1969). PEP carboxylase is activated similarly as invertase about 10 times by an increase of physiological pH from 6.5-7.2. (Tupy, 1973). pH sensitivity of PEP carboxylase may be involved in the regulation of cytosolic pH. Regulation of invertase by pH insitu is evidenced by a highly significant positive correlation between invertase activity and pH in the cyosol. Sucrose utilization was also highly significantly correlated with pH

There were significant clonal differences in latex pH and sucrose level, and an association of low pH with relatively high sucrose, which is a consequence of low invertase activity at, lowers pH (Tupy, 1973; Gohet *et al.*, 1997). Regular tapping activates the metabolism of latex vessels and lowers latex sucrose levels

As it is evident from this review, no definite information is available relating carbohydrate metabolism, associated enzymes and ionic balance in high and low yielding clones , in different seasons, under different tapping frequencies and effect of stimulation under low frequency tapping system. With this objective the following studies were carried out in clone RRII 105 (most popular and high yielding Indian clone).

1. Variations in the physiological and biochemical parameters associated with latex production in clone RRII 105 under d/2 system of tapping.
2. Seasonal variations in carbohydrates and related enzymes during peak yielding, defoliation, refoliation and dry period.
3. Variations in carbohydrate components and enzymes in trees tapped under low frequency and with stimulation.
4. Ionic balance (Variations in pH) and latex production in high and low yielding clones.
5. Variations in carbohydrate metabolism in trees affected with Tapping panel dryness syndrome.

CHAPTER

MATERIALS AND METHODS

2.1. Location

All the studies were conducted in *Hevea brasiliensis* located at Rubber Research Institute of India, Kottayam ($9^0$32'N,$76^0$36'E,73M).

The study consists of six experiments viz

1. Seasonal variations in parameters associated with latex production
2. Seasonal variations in carbohydrates and enzymes
3. Clonal variations in carbohydrate metabolism and pH regulation
4. Effect of tapping frequency on carbohydrates and ionic balance
5. Effect of stimulation on factors involved in carbohydrate metabolism an ionic balance.
6. Biochemical changes associated with Tapping Panel Dryness

2.2.1 Plant material for experiment 1, 2, and 4

32 trees of clone RRII 105 with uniform yield and girth planted in 1988 and opened for tapping in 1997 were selected for the study. Out of these, 16 trees were tapped under ½ S d/2 6d/7 system of tapping and the remaining were tapped under 1/2S d/4 6d/7 system of tapping.12 trees of uniform yield from each group were selected for physiological and biochemical measurements.

For comparing seasonal variations trees under d/2 system of tapping were used. Two seasons , peak season (Sep- Oct- Nov 1999)and stress season (Feb- Mar- Apr. 2000) were used for seasonal variation study. For tapping frequency experiments, trees tapped under d/2 and d/4 system of tappings were used.

2.2.2. Plant Material for experiment 3

Trees of Clone RRII 105, RRIM 600, HP 20 and RRII 38 planted in 1988 in the farm of Rubber Research Institute of India, Kottayam, were taken as the materials for the study. The trees were opened for tapping in 1996. The tapping system was ½ S d/2 6d/7.

2.2.3. Plant material for stimulation experiment

36 *Hevea* trees of clone RRII 105 with uniform girth and comparable yield were selected for this study. The trees were under ½ S d/4 6d/7 tapping system. The following treatments were imposed with six replications.

T1 - ½ S d/4 6d/7 (un stimulated control)

T2 - ½ S d/4 6d/7 + 2.5 % Ethephon

T3 -1/2 S d/4 6d/7 + 2.5% Ethephon and the tapping system changed to ½ S d/2 6d/7

T4 -1/2S d/4 6d/7 + 2.5% Ethephon and tapping rest for 2 days

T5 -1/2S d/4 6d/7 + 2.5% Ethephon and tapping rest for 6 days

T6 -1/2S d/4 6d/7 + 2.5% Ethephon and tapping rest for 10 days

T7 -1/2S d/4 6d/7 + 2.5% Ethephon and tapping rest for 14 days

The study was conducted during October-2000.

2.3. LATEX COLLECTION AND SEPARATION OF DIFFERENT FRACTIONS FOR BIOCHEMICAL ANALYSIS

Fresh latex was collected (flow between 5 and 30 minute) in ice-cooled containers and centrifuged immediately at 23,000 rpm for 45 minutes at 4°C (Sorvall OTD 55 B Ultracentrifuge). The upper rubber phase was removed and the middle serum (C-serum, representing the cytosol) was collected using a syringe and used for various biochemical estimations.

The bottom fraction (lutoid) was removed and washed 3-4 times in 0.4M mannitol and subjected to repeated freezing and thawing to rupture the lutoids and liberate the serum inside the lutoids. This was centrifuged at 20,000 for 30 minutes to remove the lutoid membrane fragments and the supernatant (b-serum) was collected and used for biochemical analysis.

2.4. DRY RUBBER YIELD

Dry rubber yield was calculated using the formula

Dry rubber yield = DRC× Total volume (ml)/100 and represented in g $tree^{-1}tap^{-1}$

2.5. DRC

Dry rubber content expressed as a percentage was determined by gravimetric method after acid coagulation and oven drying (80^0 C for 72 hrs) of 10 g latex.

2.6. LATEX PH

The pH of latex samples was measured using a digital pH meter immediately after collection. Before measurement, the temperature of the samples was adjusted to 25^0C.

2.7. C-AND B-SERUM PH

pH of c-and b-serum was measured using a pH meter immediately after centrifugation and separation of fractions from latex.

2.8. AMOUNT OF C-SERUM, RUBBER CREAM AND BOTTOM FRACTION

A known volume of latex was centrifuged at 23,000 rpm at 45 minutes at 4^0C .The latex was separated into three fractions rubber cream,c-serum and bottom fraction (lutoids). Fractions were separated and collected.. The volume of c-serum was measured in milliliters. Rubber cream and bottom fraction were weighed and expressed as mg/gm fresh weight of latex.

2.9. BURSTING INDEX (BI) OF LUTOIDS

Bursting index of lutoids was determined by the method of Ribaillier, (1968).

Reagents

1. 0.8M Acetate buffer pH 5.0

27.128g of anhydrous sodium acetate was dissolved in 250ml of distilled water.9.2 ml of glacial acetic acid was diluted to 200ml with distilled water.The two solutions were mixed in the ratio 2:1 and adjusted the pH to 5.0.

2. 0.8M Sodium paranitrophenyl phosphate

2.105g of sodium paranitrophenyl phosphate was dissolved in 100ml acetate buffer at pH 5.0.

3. 0.5% Triton X100

0.5ml of Triton X100 was mixed with 0.5ml of 1N NaOH and made up to 100ml with distilled water.

4. 0.6M Mannitol

21.84g mannitol was dissolved in 200ml distilled water.

5. 2N Trichloroacetic acid

32.678g of trichloro acetic acid was dissolved in 100ml distilled water.

6. 1N Sodium hydroxide

40g sodium hydroxide was dissolved in 1000ml distilled water.

7. PAT (For total acid phosphatase).

50ml p-nitro phenyl phosphate was added to 100ml 0.5% Triton X100 and 245ml water. Kept at 20^0C

8. PAL (For liberated acid phosphatase)

50 ml p-nitrophenyl phosphate was added to 200ml 0.6M mannitol and 145ml water. Kept at 20^0C.

9. P-nitrophenol standard

10mg p-nitro phenyl phosphate was dissolved in 25ml distilled water. From this 1ml was diluted to 25ml for working standard.

Procedure

Liberated Control (LC):

2.9ml PAL solution and 1ml 2N TCA were taken in test tubes and 100µl latex was added to it. Mixed well and the resultant mixture was filtered using whatman No. 1 filter paper.

Test Control (TC) :

2.9ml PAT solution was pipetted into test tubes and added 1ml TCA and 100µ l latex. Filtered using whatman No.1 filter paper.

Test PAT: (TT)

2.9ml PAT solution was pipetted in test tubes and 100µl latex was added. The reaction mixture was incubated for 10' and the reaction was stopped by adding 1ml 2N TCA.The mixture was filtered using whatman No.1 filterpaper and took the supernatant.

Test PAL (LT)

2.9ml PAL solution was pippeted in test tubes and 100µ l latex was added. The reaction mixture was incubated for 10' and the reaction was stopped by adding 1ml 2N TCA. The mixture was filtered using whatman No.1 filter paper and collected the supernatant.

Estimation

0.5 ml of the supernatant was added to 0.5 ml 1N NaOH and 4.0 ml water. The p-nitrophenol liberated was measured spectrophotometrically at 410 nm.

The total acid phosphatase activity (PAT) = (TT-TC)

It represents the entire potential of the enzyme in the latex. The activity is measured in the presence of Triton X100 that induces the complete lysis of the lutoids and the liberation of the enzymes that they contain in soluble form.

Free acid phosphatase activity (PAL) = (LT-LC)

It takes into account the enzyme accessible to the substrate while the lutoids are perfectly intact. This measurement is carried out in isotonic medium (0.4M mannitol), during generally short periods (10-30 min) in order that there may not be any destabilization of organelles during the measurement. This measurement

mainly represents the activity of the phosphatase liberated by the lutoids. The ratio of the two-phosphatase activities i.e. free and total is a measure of the integrity of the lutoids.

BI = LT-LC / TT-TC x 100 (expressed as %)

2.10. ESTIMATION OF THIOLS, INORGANIC PHOSPHOROUS AND SUCROSE IN LATEX

Extraction

About 1 gm latex was extracted with 2.5 % trichloro acetic acid (TCA) and made up to 10.0ml with 2.5% TCA. Filtered the solution using Whatman No.1 filter paper.

The filtrate was used for the measurement of thiols, inorganic phosphorous and sucrose.

2.10.1. Estimation of Thiols in latex (Boyne and Ellman, 1972)

The thiol groups of RSH react with DTNB (Dithiobis 2-nitro benzoic acid) to form TNB, which strongly absorbs at 412 nm. The R-SH groups become quickly oxidized especially in alkaline medium and so the analysis was done within 24 hours of latex collection.

Reagents

1. 0.5 M Tris(hydroxymethyl aminomethane) solution

   6.06 gm Tris was dissolved in 100 ml of distilled water

2. 10 mM DTNB (Dithio-bis-2-nitro benzoic acid)

   79.4 mg DTNB (mol.wt 396.36) and 140.3 mg EDTA (Ethylene diamine tetraacetic acid disodium salt) was dissolved in ~10.0ml water and

adjusted the pH to 6.5 with 0.5M Tris and made up to 20.0 ml in a volumetric flask and stored in refrigerator.

Glutathione standard (GSH)

25.0mg reduced glutathione was made up to 25 ml with 2.5% TCA in a volumetric flask. 1.0ml of the above solution was diluted to 25ml with 2.5%TCA and used as working standard.

Estimation

0.1ml 10mMDTNB solution and 2ml 0.5 M Tris were added to 2ml TCA extract and shake well. Standards were also treated in the same way. Read the optical density of test and standards at 412nm.

2.10.2 ESTIMATION OF INORGANIC PHOSPHOROUS IN LATEX

(Tausky and Shore, 1953)

Reagents

1. Sulphomolybdic acid reagent:

In a 1000ml standard flask added 100g ammonium molybdate , 278ml concentrated sulphuricacid and 700ml distilled water. Made up the volume to 1000ml with distilled water. Kept in fridge.

2. **Ferrous sulphate solution:**

At the moment of experiment 5.0 gm ferrous sulphate was dissolved in 50.0ml distilled water. To this added 10.0ml of the above sulphomolybdic acid reagent and made up the volume to 100ml with water.

3. Standard: KH2PO4 (~ 200 microgram/ml 2.5%TCA).

Estimation

Pipetted out 0.5 ml of the TCA extract and made up to 2.0ml with 2.5 % TCA. Pipetted out 0.2, 0.4, 0.6, 0.8 and 1.0 ml standards and made up to 2.0ml with 2.5 %TCA.A blank was also prepared without test or standard. Added 2.0ml ferrous sulphate reagent to all the tubes and read the standards and tests at 740nm after 10 minutes.

2.10.3 ESTIMATION OF INORGANIC PHOSPHOROUS IN C AND B SERUM.

(Tausky and Shore, 1953)

To 25 µl of c and b serum, added 5.0ml 2.5% Trichloroacetic acid to precipitate the proteins and centrifuged at 5000 rpm for 15 minutes. Supernatent was collected and used for Pi estimation. To 2.0ml of supernatant added 2.0 ml ferrous sulphate solution. Read the optical density at 740 nm after 10 minutes along with the standards.

2.10.4. Estimation of Sucrose in latex (Scott and Melvin, 1953)

Sucrose content was estimated by the method of Scott and Melvin, (1953).

Reagents

1. Anthrone reagent

0.1g Anthrone was dissolved in a mixture of 29ml water and 100ml conc. Sulphuricacid under ice cold conditions and kept in ice bath.

2. Sucrose standard (200**µg/ml**).

Estimation

0.1ml 2.5% TCA extracts were pippeted out in test tubesand made upto 0.5ml with 2.5%TCA.Kept the tubes in ice bath and added 3.0 ml cold anthrone reagent. Standards were also treated the same way. A blank was prepared by adding all reagents except sample or standard. All the tubes were heated in a boiling water bath for 15 minutes. Cooled the tubes in a water bath. Read the optical density at 627 nm.

2.10.5. ESTIMATION OF SUCROSE IN C-SERUM

To 25 µl c-serum added 5.0 ml 2.5% trichloroacetic acid and centrifuged at 5000rpm for 15 minutes. Take 0.1 ml supernatent and made up the volume to 0.5 ml with water. Standards and blank were also taken. All the tubes were kept in ice bath. Added 3.0 ml cold anthrone reagent and heated in a boiling waterbath for 15 minutes. Cooled and read the optical density at 627 nm.

2.11. LIPID EXTRACTION FROM LATEX, RUBBER CREAM AND BOTTOM FRACTION

Lipids were extracted from latex, rubber cream and bottom fraction by the method of Hasma and Subramaniam, (1986).

2.11.1. Lipid extraction from latex

About 1gm latex was extracted with chloroform – methanol (2:1) and kept for 24 hours at room temperature. It was filtered and the residues washed with 2:1 choroform-methanol 3-4 times and added 10.0ml-distilled water. The aqueous layer was removed with a pasteur pipette. The washed lower layer of chloroform

was evaporated to dryness and the residue dissolved in a known volume of chloroform.

2.11.2. Lipid extraction from rubber cream

Fresh latex was collected (flow between 5 and 30 minute) in ice-cold containers and centrifuged immediately at 16,000 rpm for 45 minutes at 4°C (Sorvall OTD 55 B Ultracentrifuge). The upper rubber phase was removed and suspended in distilled water. Stir well and filtered through muslin cloth. ~1gm filtrate was used for extraction of lipids. The remaining procedure for lipid extraction was carried out as in the case of latex.

2.11.3. Lipid extraction from bottom fraction

About 1 gm bottom fraction collected after the centrifugation of latex was extracted with chloroform-methanol (2:1) and the remaining procedure was same as that of latex.

2.12. ESTIMATION OF PHOSPHOLIPIDS

Phospholipids in latex, rubber cream and bottom fraction was estimated by the method of Gutfinger and Letan,(1978).

Reagents

1. 4% Ammonium molybdate

   4g Ammonium molybdate was added to 100ml distilled water. Heated gently to dissolve the contents.

2. 5N H_2SO_4

3. ANSA Reagent:

Mix thoroughly 0.1gm of 1-Amino 2- naphthaleine 4- sulphonic acid with 1.2 g of sodium bisulphite and 1.2 g of sodium sulphate.

4. Standard KH_2PO4

Estimation

Pipette out 5ml portion of lipid extract into a Kjeldahl flask. Evaporated off the solvent in a water bath .Added 2ml of 5ml H_2SO_4 and digested till the black colour disappears, added 2 drops of 2N HNO_3. Cool and add 2ml of H_2O and heated in a boiling water bath exactly for 5'. A blank containing 1ml of H_2O and 1ml of 5N H_2SO_4 was taken..To all the tubes added 1ml of 4% ammonium molybdate solution. Then added 0.1 ml of ANSA reagent. Made up the volume to 10 ml with water. Keep exactly for 10'. Read the optical density at 670nm along with the standard..The amount of phospholipids was determined by multiplying the amount of phosphorous with the conversion factor 25.9.

2.13. ESTIMATION OF GLYCOLIPID

Glycolipids in latex, bottom fraction, and rubber cream were estimated according to the method of Dubois et al (1956).

Reagents

1. 5% Phenol

   5g phenol was dissolved in 100ml distilled water.

2. Concentrated sulphuric acid

3. Galactose standard

Estimation

After evaporation of chloroform extract, samples containing 10-20µg of sugars were taken and added 1ml of water and 1ml of 5% aqueous phenol. This was followed by the rapid addition of 4ml of concentrated sulphuric acid to ensure maximum heating of the mixture. The contents of the tubes were thoroughly mixed and allowed to stand at room temperature for 15 minutes.. The intensity of the colour was read at 490nm. Galactose was used as the standard.

2.14. ESTIMATION OF SIALIC ACID B SERUM

Sialic acid in b-serum was estimated by the method of Leonard Warren, (1963).

Reagents

1. 0.5M Sodium sulphate

   14.2g sodium sulphate was dissolved in 200ml distilled water.

2. 0.2M Sodium metaperiodate in 0.5M Phosphoric acid

   854mg sodium meta periodate was dissolved in 20ml 0.5M phosphoricacid.

3. 10.0 %Sodium arsenite in 0.5M sodium sulphate solution

   10g sodium arsenite was dissolved in 100ml 0.5M sodium sulphate solution.

4. 0.6% Thiobarbituric acid in 0.5M sodium sulphate

   600mg thiobarbituric acid was dissolved in 100ml 0.5M sodium sulphate solution.

5. Cyclohexanone

Estimation

100 µl serum was added to 0.1 ml sodium metaperiodate. Mixed thoroughly and stand at room temperature for 20 min. Added 1.0 ml sodium arsenite solution and the tubes were shaken vigorously. After 2 min the tubes were shaken again to assure the complete discharge of yellow brown colour. 3.0 ml of thiobarbituric acid solution were added and the contents were mixed vigorously and heated in a boiling water bath for 15 min. Tubes were cooled in tap water for 5 min and 4.0 ml cyclohexanone were added. The tubes were shaken vigorously and centrifuged briefly. Top clear cyclohexanone phase was removed and read the optical density at 532 and 549 nm. The amount of sialic acid was expressed in µ moles .

2.15. ESTIMATION OF PROTEIN BOUND HEXOSE AND FUCOSE IN B-SERUM

Protein bound hexose and fucose in b-serum was measured according to the method of Djurdjic and Mandic, (1990)

Reagents

1. Saline (0.9% Sodium chloride)

   900mg sodium chloride was dissolved in 100ml distilled water.

2. Ethanol
3. Concentrated Sulphuric acid
4. Cold mixture

   6parts of conc. H_2SO_4 was mixed with 1part distilled water.

5. CPS reagent

0.075g phenol was dissolved in 100ml conc. H_2SO_4. To this solution added 1g cystein- hydrochloride

6. D-galactose standard

7. Fucose

8. **Estimation**

The determination of protein bound hexoses and fucose in serum was performed in 100 µl serum samples diluted with saline (1: 5). The proteins were precipitated by addition of 5.0 ml ethanol and to the precipitate added 1.0 ml of water and 5.0 ml of cold mixture. After heating exactly for 3 min in a boiling water bath the mixture was immediately cooled and 1.0-ml of CPS reagent was added. The obtained mixture was kept in an ice bath for 60 min and OD was measured at 398 nm for fucose and 498nm for hexose. The calibration curves for the determination of hexose were made using standard solutions of D-galactose and L-fucose in the same concentration range.

2.16. ESTIMATION OF SUCROSE SYNTHASE ACTIVITY IN C- SERUM

Activity of Sucrose synthase in c-serum was determined by the method of Tupy, J.(1969).

Reagents

1. 50mMHEPES buffer pH 7.5

298mg HEPES was dissolved in 25ml distilled water . The pH was adjusted to 7.5.

2. 1µmole UDP Glucose

15.25mg UDPG was dissolved in 25ml distilled water.

3. 15mM Magnesium chloride

76.125mg MgCl2 was dissolved in 25ml HEPES buffer pH7.5.

4. 2μM Fructose

9mg fructose dissolved in 25ml distilled water.

5. 1N NaOH

4g NaOH dissolved in 100ml distilled water.

6. Anthrone reagent

1g anthrone was dissolved in an ice cold solution containing 100ml conc.H2SO4 and 29ml distilled water. Kept in ice.

7. Sucrose standard

200μg/ml **HEPES** buffer

Assay

Sucrose synthase was assayed by measuring sucrose synthesis from fructose and UDP Glucose. Enzyme solution (25μl) was incubated for 15 min at 30^0C with 100μl 2μmol fructose and 100μl 1μmol UDP Glucose and 100**ul** of HEPES buffer pH 7.5 containing 15mM $MgCl_2$. The reaction was terminated after 15 minutes by the addition of 120μl of 1N NaOH. Heated the mixture in a boiling waterbath for 10 min to destroy the unreacted fructose. Cooled the mixture and added 3.0ml cold anthrone reagent and heated in a boiling water bath for 10 minutes and read the optical density at 625 nm along with sucrose standard. Controls without enzyme were also run along with tests and standards.

2.17. ESTIMATION OF INVERTASE ACTIVITY IN C-SERUM

Activity of invertase inc-serum was determined by the method of Tupy, (1973).

Reagents

1. 50mM phosphate buffer pH 7.4
2. 50mM sucrose

   1.71g sucrose was dissolved in 100ml distilled water.

3. Glucose standard
4. Copper reagent A

   25g of anhydrous sodium carbonate, 25g sodium potassium tartarate (Rochelle salt), 20g sodium bicarbonate, 200g sodium sulphate (anhydrous) was dissolved in 800ml distilled water and diluted to 1 litre. Stored the reagent at room temperature.

5. Copper reagent B

   15% $CuSO_4.7H_2O$ containing one or two drops of concentrated H_2SO_4/100ml.

6. Arsenomolybdate colour reagent.

Dissolved 25g ammonium molybdate in 450ml distilled water, add 21ml of concentrated H_2SO_4 and mixed 3.0g $Na_2HAsO_4.7H_2O$ dissolved in 25ml water, mixed and placed in an incubator at 37^0c f or 24-48 hours.

Assay

For measuring invertase activity, 25µl of enzyme solution was incubated for 15' at 30^0C with 100µl of 50mM sucrose and 300µl of 50mM phosphate buffer

pH 7.4. The reaction was stopped by adding 0.5ml reagent prepared by mixing 25 parts of copper A and 1 part copper B.

Place the tubes in a boiling waterbath for 20'. Cool the tubes under a running tap. Add 1ml arsenomolybdate reagent. Dilute the mixture to 25ml. After 15' measure the OD at 500nm. Glucose is used as standard.

2.18. ATPASE ACTIVITY OF LUTOIDS

Activity of ATPase was determined by the method of Xavier Gidrol *et al*, (1988).

Reagents

1. 50mM Hepes-Mes Tris (pH 7.0)
2. 300mM mannitol
3. 5mM Mg SO_4
4. 0.1mM ammonium molybdate
5. 5mM ATP

Preparation of lutoid fraction and assay of ATPase.

The fresh latex was collected in glass vessels held in melting ice. The first 20.0ml were discarded in order to avoid possible bacterial contamination and damaged particle content. The fresh latex was then immediately centrifuged at 35000 g for 20 min at 4^0C. The supernatent serum (cytosol) and the polyisoprenoid particle fractions were discarded. The pellet resuspended in 5 volumes of a 50 mM Hepes-Mes-Tris (pH 7.0), 300mM mannitol buffer form the crude lutoid fraction. The crude lutoid fraction was washed three times with the same buffer. The sediment obtained by the centrifugation of the lutoid suspension at 35000g for 10minutes at 4^0c was resuspended in the ATPase buffer (50mM Hepes –Mes-Tris pH 7.0,

300mM mannitol, 5mM MgSO4, 0.1mM ammonium molybdate). Assay was performed in 2.5ml assay buffer with 10% lutoid. The reaction was started by the addition of 5mM ATP at pH 7.0. The incubation time was 10 min at 26^{0}C under continuous stirring. Then enzymatic hydrolysis of ATP was stopped by adding ice cold TCA at a final concentration of 0.5 mM. The Pi released was measured spectrophotometrically.

2.19. PYROPHOSPHATASE ACTIVITY OF C-SERUM

(Jacob et al., 1989)

Reagents

1. 50mM Tris-Maleate pH 7.0
2. 5mM Magnesium chloride
3. 1mM sodium pyrophosphate
4. 100µM Ammonium molybdate

Procedure

100µl c-serum was incubated in a reaction mixture containing 2.5ml Tris-maleate buffer and 0.2ml magnesium chloride. The reaction was initiated by adding 1.0ml 1mM sodium pyrophosphate. After 30 minutes at 30^{0}C, the reaction was stopped by adding 100µl ammonium molybdate. The Pi released was measured at740nm.. The pyrophosphatase activities were expressed as µmoles of Pi liberated/min/mg protein.

2.20. PYROPHOSPHATASE ACTIVITY OF LUTOIDS (Siswanto *et al*, 1995)

Reagents

1. 25mMBTP -histidine buffer pH 7.6
2. 5mM Magnesium sulphate
3. 1mM sodium pyrophosphate
4. 100µM Ammonium molybdate

The bottom fraction (lutoids) obtained after centrifugation of latex samples were washed twice in 25mM Tris- MES pH 7.6 containing 5mM magnesium chloride and 0.33M mannitol. The lutoids were then suspended in BTP-histidine buffer containing 2.5mM magnesium sulphate and homogenized. The mixture was centrifuged at 20,000rpm for 30 minutes.

The assay medium consists of 2.5 ml BTP-histidine buffer, 0.1ml magnesium sulphate and 0.1ml supernatant. The reaction was started by adding 0.1ml sodium pyrophosphate. After 30 minutes at 30^0C, the reaction was stopped by adding 100µl ammonium molybdate. THE Pi released was measured. The pyrophosphatase activities were expressed as µmoles of Pi liberated/min/mg protein.

2.21. N- ACETYL GLUCOSAMINIDASE IN B-SERUM (Roger Giordani, *et al*, 1992).

Reagents

1. 0.1 M Succinate buffer pH 6.0
2. 0.02%Sodium azide

3. 2.5 mM p-nitrophenyl β-D- N acetyl glucosaminide (pNP-β-D-GlcNAc)
4. 0.2 M Sodium carbonate (Na_2CO_3)

Assay

The standard incubation medium used to study the enzyme activity contained 0.1M succinate buffer pH 6.0 (2.5 ml), 0.05ml sodium azide 0.2 ml pNP-β-D-GlcNAc and 50 µl b-serum at 30^0C. After incubation adding 0.25 ml of 0.2 M Na2 CO3 stopped the reaction and the absorbance of p-nitrophenol formed was read at 400 nm and compared with a control sample containing only substrate. The specific activity of enzyme was defined as the amount of p-nitrophenol liberated/min/mg protein.

2.22. CHITINASE ACTIVITY IN B- SERUM

(Pedraza-Reyas, Lopez- Romero (1991).

Reagents

1. 50 mM phosphate buffer
2. 1mM Magnesium chloride
3. Chitin azure

Assay

100µL B-serum was mixed with 1.0 mg chitin azure in 0.5 ml 50 mM phosphate buffer pH 6.5 with 1mM $MgCl_2$. The reaction mixture was incubated at 30^0C with gentle shaking for 48 hours followed by centrifugation at 14000 g for 4 min. The absorbance at 575 nm of the supernatant fluid was then examined. Chitinase activity is defined as the amount of protein that results in an increase of 0.01 A_{575} units under the above conditions.

2.23. HEVEIN CONTENT OF B-SERUM (Ukun *et al.*, 1999)

Lyophilised b-serum was suspended in water to which 0.5g/l sodium thionite was added. After centrifugation, solutions were 100% saturated with ammonium sulphate and centrifuged again. Precipitates were dissolved in small volumes of 0.2 M acetic acid and submitted to gel-filtration on a column of Sephadex G-25. ~5mg protein was applied to the column and eluted with1.2 M acetic acid. The fractions were collected at the rate of 3.0 ml/minute and optical density was measured at 280 nm.

2.24. ESTIMATION OF PYROPHOSPHATE

Reagents

1. 40Mm ammonium molybdate
2. 5N Sulfuric acid
3. Triethanolamine

Reagent: 4.0ml ammonium molybdate, 1.0 ml 5N sulfuric acid and 50 µl triethanolamine.

The volume of C-serum was adjusted to 2.0 ml so that sample it becomes 0.5 N w.r.t Sulphuric acid and add 1.0ml reagent. Kept for 15 minutes, centrifuged if there is any precipitate. To the supernatant added 0.15 ml 1M mercapto ethanol and mixed. The optical density was measured at 700 nm after 15 minutes.

2.25. Estimation of total protein in C-and B-serum

Total soluble proteins in C- and B- serum were estimated by the method of (Lowry *et al*, 1951).

Reagents

1. A: 2% Sodium carbonate in 0.1 N NaOH
2. B: 0.5% Copper sulphate in 1% sodium potassium tartarate
3. Alkaline copper reagent C: 50.0 ml A + 1.0 ml B
4. Diluted folins reagent (1: 2)
5. 10.0% Trichloroacetic acid
6. Bovine Serum Albumin standard

Estimation

Proteins were precipitated from the serum (25µl each) by adding 5.0ml 10.0% TCA and centrifuged. The precipitate was dissolved in a known volume of 0.1 N NaOH. 0.5 ml sample and standards were then made upto 1.0 ml with 0.1N NaOH. A blank containing 1.0 ml 0.1N NaOH was taken. To all the tubes added 5.0-ml alkaline copper reagent. Kept for 10 min. Added 0.5ml diluted folins reagent. Mixed well and the tubes were kept for 30 min. The optical density was measured at 660 nm in a UV visible spectrophotometer. Bovine serum albumin was used as standard.

2.26. Extraction of bark samples for biochemical analysis

Bark samples were extracted with 80 % alcohol for sugars, 2.5 % TCA for inorganic phosphorus, 0.1 M phosphate buffer pH 7.8 for invertase sucrose synthase, hexose and fucose.

CHAPTER-3

RESULTS

3.1 VARIATIONS IN PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS ASSOCIATED WITH LATEX PRODUCTION.

Results of the study conducted in clone RRII 105 under ½ S d/2 6d/7 tapping system are presented in this chapter. Analysis of variance was carried out to study the seasonal differences in dry rubber yield, dry rubber content, total thiols, sucrose and inorganic phosphorous in latex. Regression analysis of these parameters with yield was worked out using the pooled data of one year. Independent t test was carried out to study the seasonal variations in biochemical parameters

3.1.1 Monthly variations in yield and other physiological parameters related to yield

Monthly variations in yield and other physiological parameters of newly opened trees of clone RRII 105 are presented in figures 1-5. Maximum dry rubber yield was observed during peak season (Figure 1). Maximum sucrose content in latex was observed during the month of March. (Figure- 3). All the months in peak season showed high in organic phosphorus. (Figure 4)

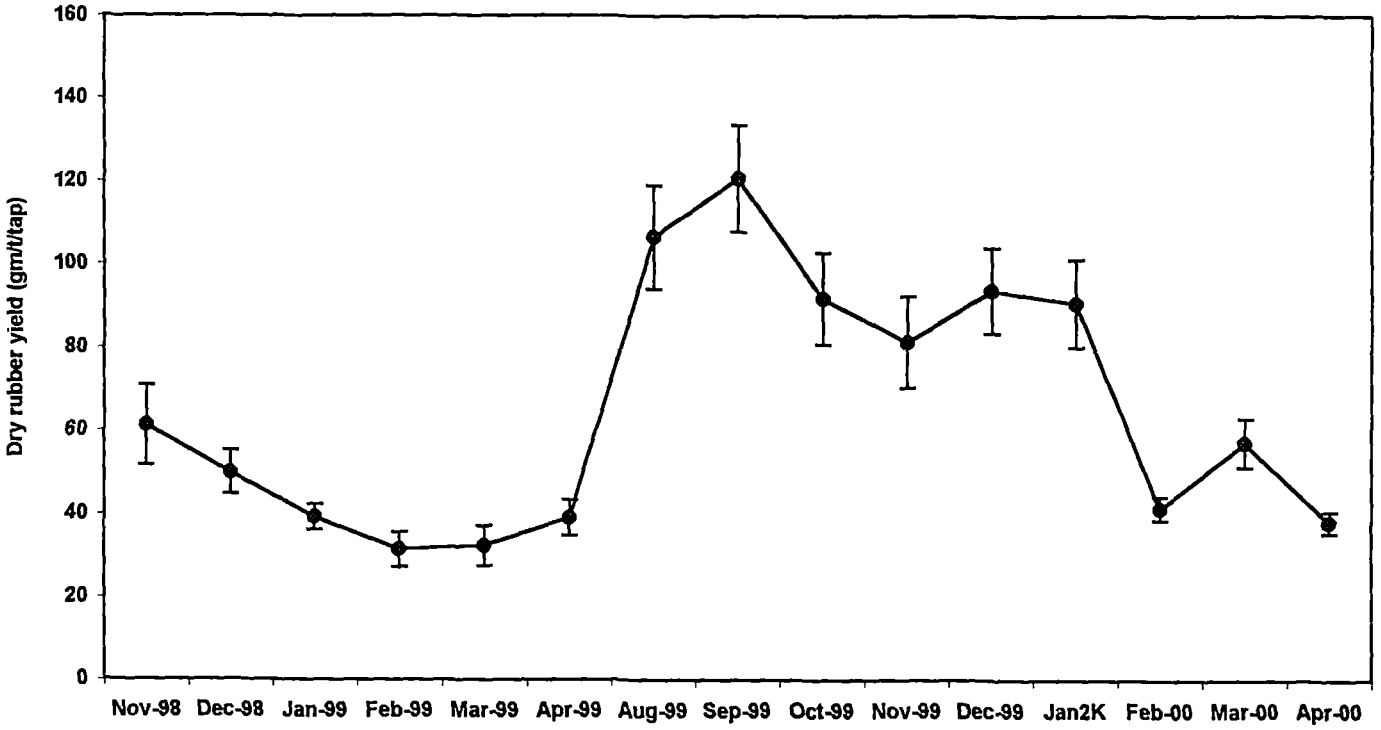


Figure-1 Monthly variations in dry rubber yield of newly opened trees of clone RRII 105 under 1/2 S d/2 6d/7 tapping system

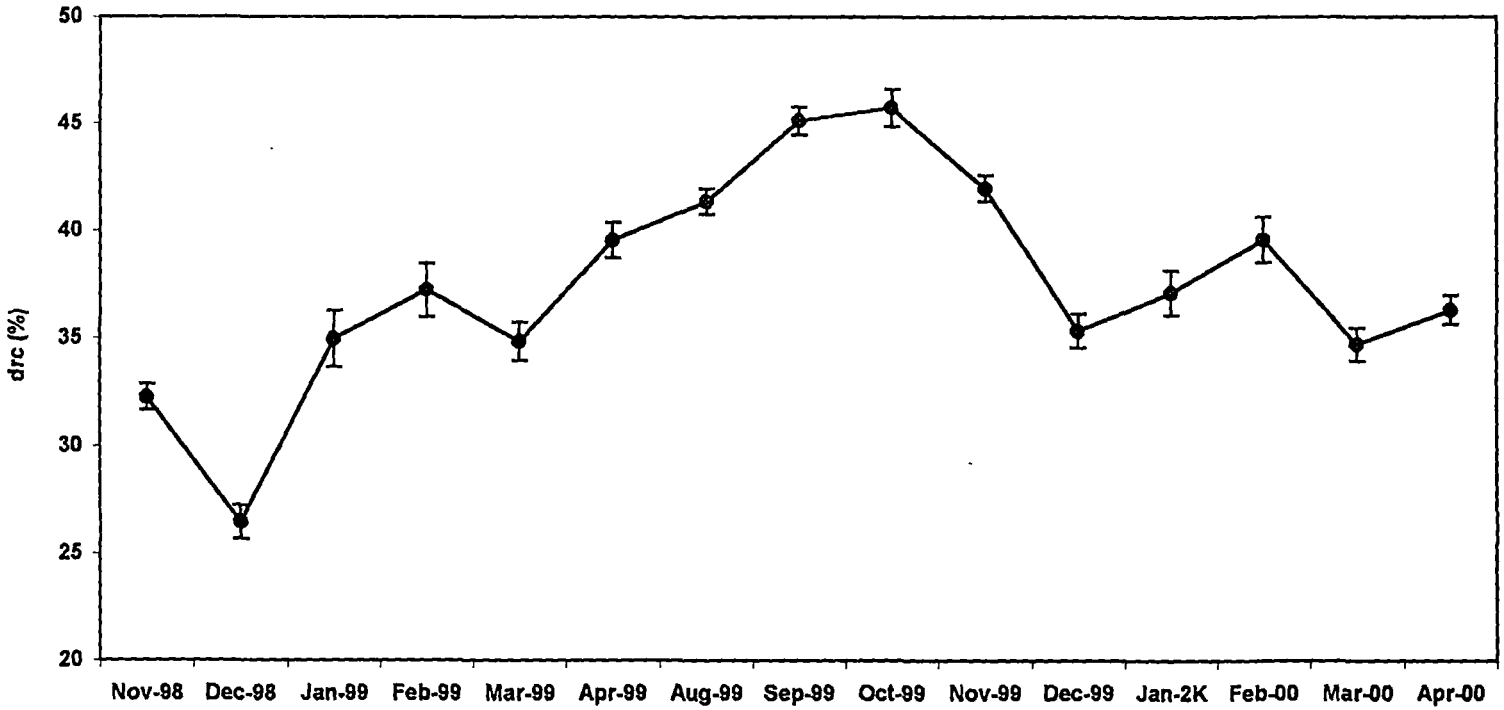


Figure 2 Monthly variations in dry rubber content in newly opened trees of clone RRII 105 under 1/2 S d/2 6 d/7 tapping system

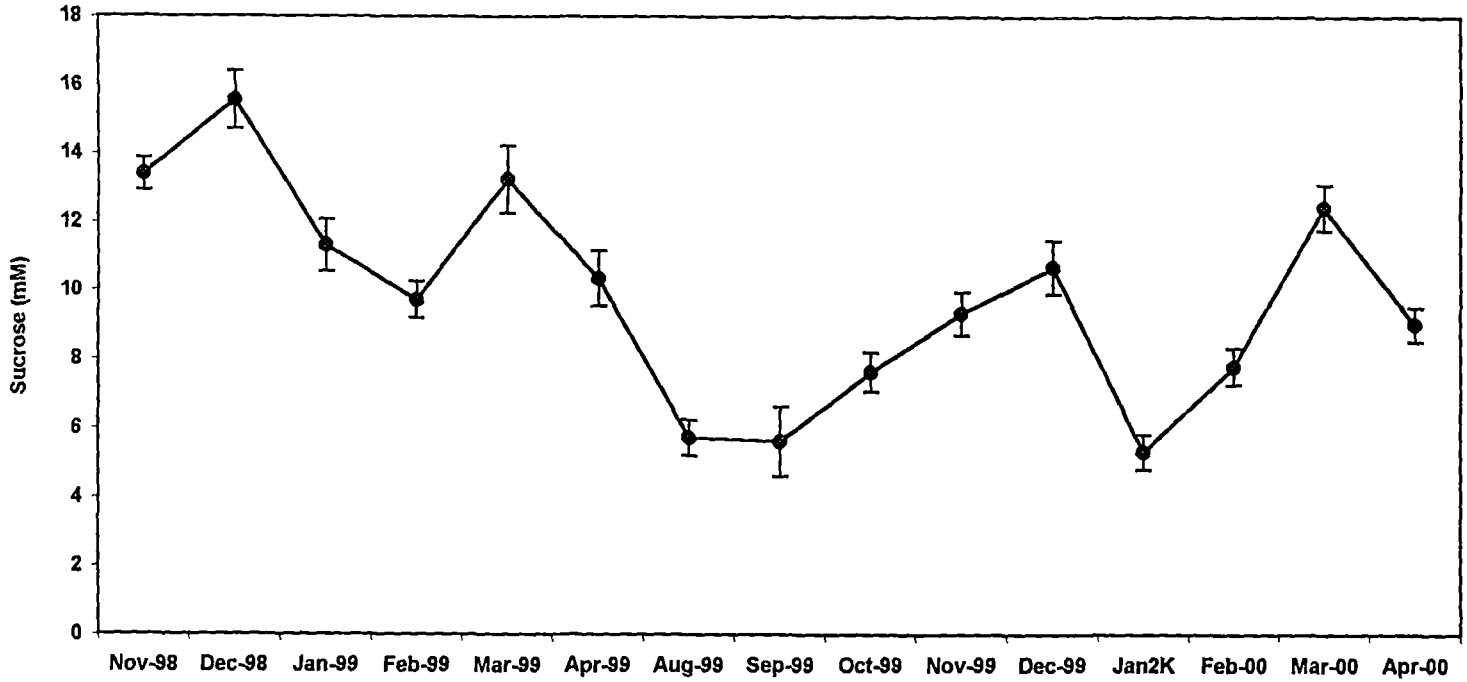


Figure 3 Monthly variations in latex sucrose in newly opened trees of clone RRII 105 under 1/2 S d/2 6d/7 tapping system

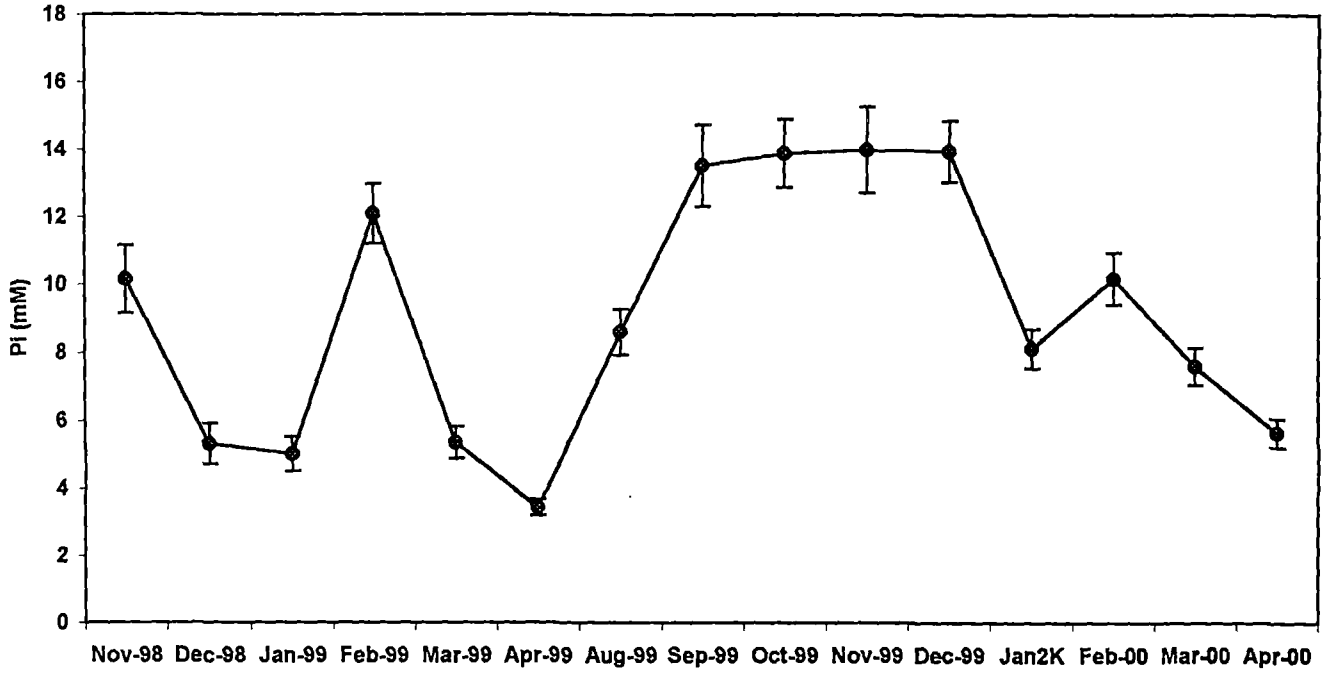


Figure 4 Monthly variations in latex inorganic phosphorous in newly opened trees of clone RRII 105 under 1/2 S d/2 6 d/7 tapping system

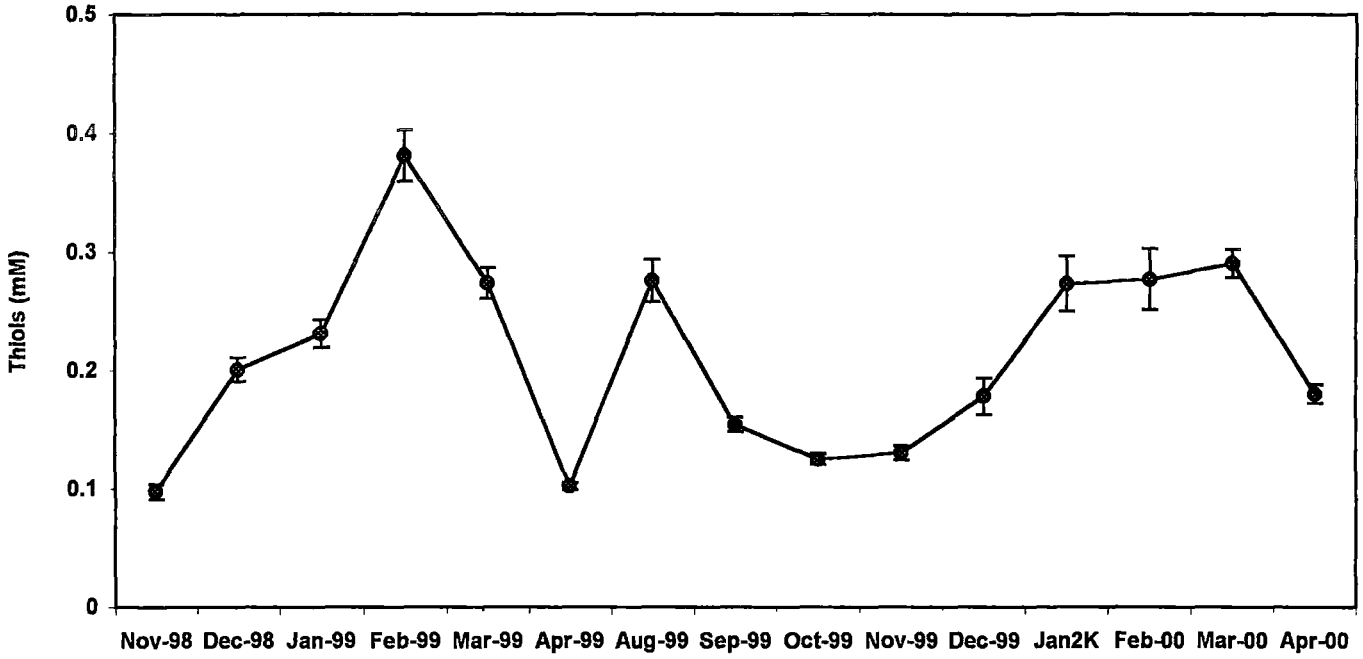


Figure-5 Monthly variations in latex thiols in newly opened trees of clone RRII 105 under 1/2 S d/2 6d/7 tapping system

3.1.2. Seasonal variations in yield and other physiological parameters related to yield in clone RRII 105 under ½ S d/2 6d/7 system of tapping

Seasonal variations in yield and physiological parameters are presented in Table-1.Inorganic phosphorous and sucrose in latex, drc, dry rubber yield were found to be significantly higher during peak season when compared to stress season. Thiols were significantly lower during peak season.

Table-1. Seasonal variations in dry rubber yield and related physiological and biochemical parameters in clone RRII 105 under 1/2S d/2 6d/7 system of tapping.

Parameter	Peak season	Stress season	Significance
Yield (g tree^{-1} tap^{-1})	102.3	56.4	15.1 **
Dry rubber content (%))	43.03	35.29	3.09 *
Sucrose (mM)	8.22	9.74	1.28 *
Inorganic phosphorus (mM)	13.95	9.25	1.9 *
Thiol (mM)	0.15	0.25	0.03 **

** CD (P=0.01) * CD (P=0.05)

3.1.3. Relationship between yield and other physiological parameters related to yield

The results are presented in Table-2 .Regression analysis showed a positive correlation between yield, Pi,and Thiols and Sucrose showed a negative correlation

Table-2. Correlation between yield and physiological parameters (pooled data of one year).

Variable	Coefficient	Standard error	T-Stat
Constant (Yield)	10.942	82.071	0.1333
Thiols in latex	166.80	71.687	2.326 *
Inorganic phosphorus in latex	3.484	1.049	3.318 **
Sucrose in latex	-5.361	2.717	-1.972 *

3.1.4. Seasonal variations in bursting index , pH and enzymes involved in proton transport

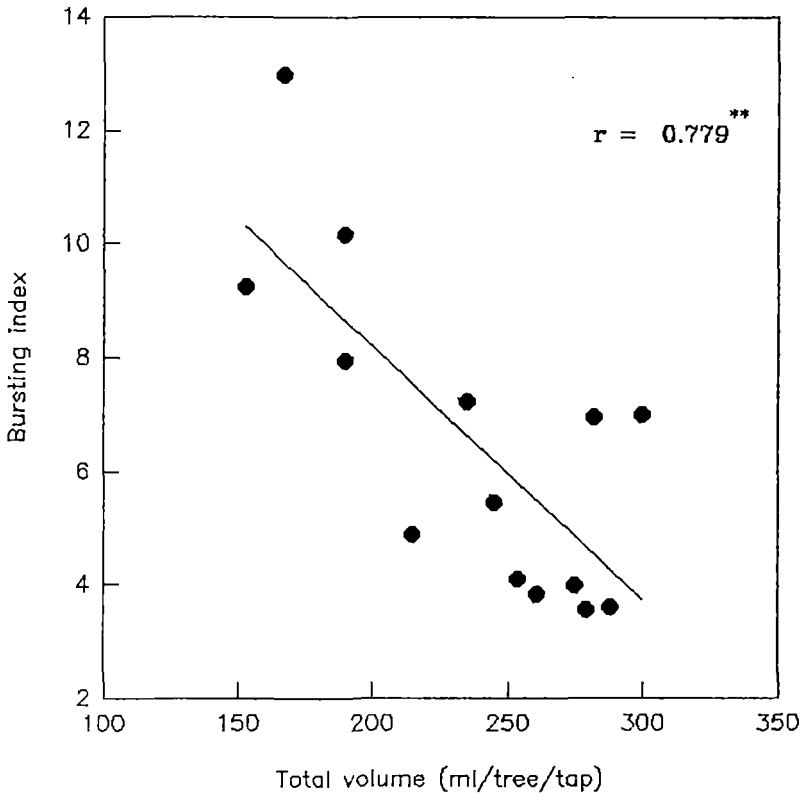
The results are presented in Table 3. The bursting index of lutoids showed an increase during stress when compared to peak season. Latex and c-serum pH showed a decrease during stress period. There was no significant difference in b-serum pH. Pyrophosphatase activity and ATPase activities in lutoids were high during peak season when compared to stress.

Table-3. Seasonal variations in bursting index and pH in clone RRII 105 under 1/2S d/2 6d/7 system of tapping.

Parameter	Peak season	Stress season	Significance
Bursting Index	5.79±0.38	13.9±0.82	*
Latex pH	7.15±0.062	6.07±0.085	*
C-serum pH	6.89±0.065	6.5±0.066	*
B-serum pH	5.58±0.059	5.66±0.07	ns
ATPase(lutoid) (μmole Pi min^{-1} $mgprotein^{-1}$)	3.91±0.36	2.83±0.27	*
Lutoid pyrophosphatase (μmole Pi min^{-1} mg $protein^{-1}$)	4.44±0.41	3.99±0.37	ns

*significant at $p \leq 0.05$ ns- not significant

A negative correlation between total volume and BI was observed (Fig-6).



Figure–6 Relationship between total volume and bursting index in clone RRII 105 under 1/2 S d/2 6 d/7 tapping system (peak yielding season)

3.1.5. Seasonal variation in parameters related to energy metabolism and related enzyme (pyro phosphatase in C-serum) in clone RRII 105 under 1/2S d/2 6d/7 system of tapping

The result of this experiment are presented in Table 4. Iorganic phosphorus and pyrophosphatase in C- serum showed a significant increase in peak season and pyrophosphate in C-serum showed a decrease during peak season (Table4).

Table-4. Seasonal variations in biochemical parameters associated with latex production in clone RRII 105 under 1/2S d/2 6d/7 system of tapping.

Parameter	Peak season	Stress season	Significance
C-serum phosphorus (mg/ml)	0.48±0.025	0.37±0.019	*
C-serum pyrophosphate (mM)	3.27±0.15	4.41±0.02	*
C-serum pyrophosphatase (μmole Pi min^{-1} mg $protein^{-1}$)	13.82±0.28	12.96±0.20	*

*significant at $p \leq 0.05$ ns- not significant

C- serum Pi showed a positive and C- serum PPi showed a negative correlation with yield (Fig-7&8)

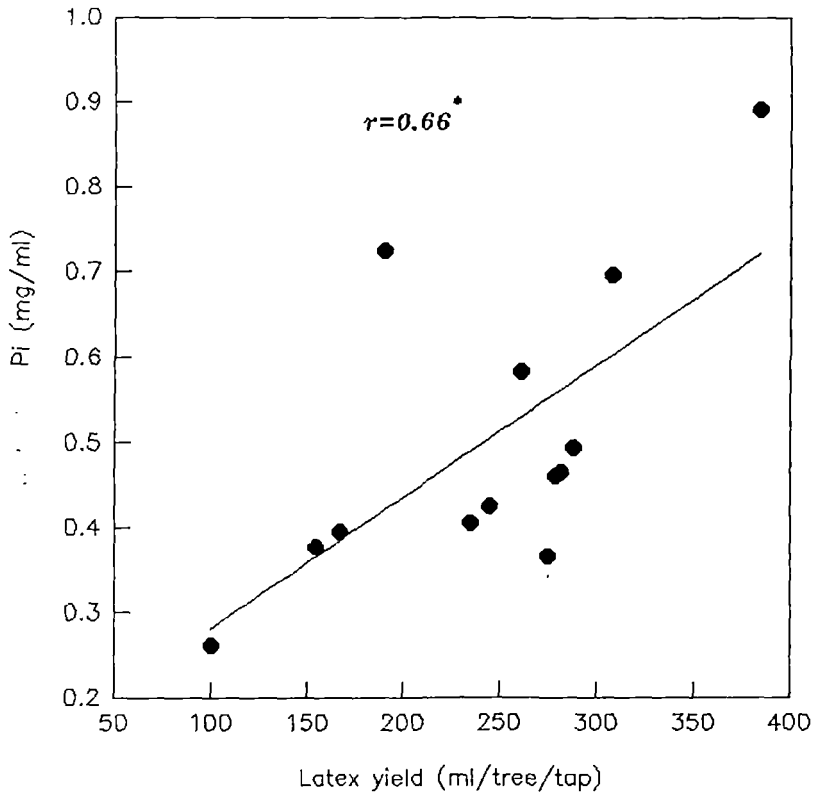
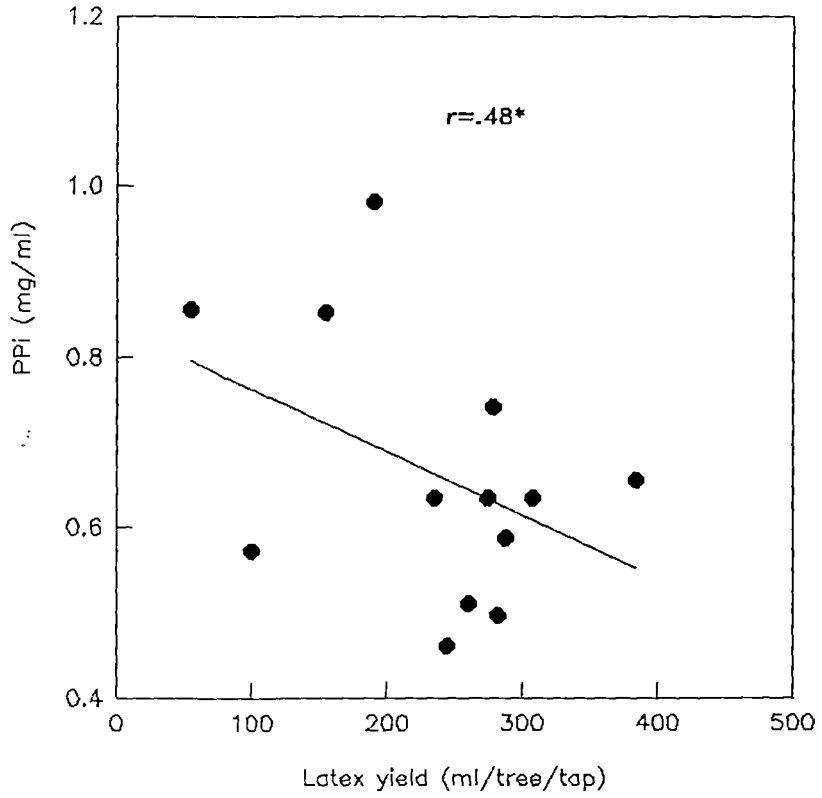


Figure-7 Relationship between latex yield and c-serum Pi in clone RRII 105 under 1/2 S d/2 6d/7 tapping system (peak yielding season)



Figure–8 Relationship between latex yield and C-serum PPI in clone RRII 105 under 1/2 S d/2 6 d/7 tapping system (peak yielding season)

3.1.6. Seasonal variations in phospholipids, glycolipids and carbohydrate components in clone RRII 105 under1/2S d/2 6d/7 system of tapping.

Glycolipids in latex and phospholipids in latex and bottom fraction showed an increase during peak period. Glycolipids in rubber cream and bottom fraction and phospholipids in rubber cream showed no difference. Fucose and hexose in B-serum showed an increase during peak season when compared to stress period. Sialic acid shows an increase during stress season. (Table 5).Hexose and fucose showed a positive correlation with total volume and yield (Fig-10 &11) . A positive correlation was observed between phospholipids in bottom fraction and yield.(Figure 9).

Table-5. Seasonal variations in phospholipids ,glycolipids carbohydrate components in clone RRII 105 under 1/2S d/2 6d/7 system of tapping.

Parameter	Peak season	Stress season	Significance
Latex Glycolipids (mg/g dry wt.)	12.69±0.53	7.6±0.68	*
Latex phospholipids (mg/g dry wt.)	6.47±0.19	3.18±0.36	*
Rubber cream glycolipids (mg/g dry wt.)	5.51±0.38	5.45±0.74	ns
Rubber cream phospholipids (mg/g dry wt.)	2.02±0.24	2.29±0.33	ns
Bottom fraction glycolipids(mg/g dry wt.)	38.73±2.97	38.61±1.37	ns
Bottom fraction phospholipids (mg/g dry wt.)	20.54±1.98	13.31±0.92	*
b-serum fucose (mg/ml)	5.599±0.556	4.49±0.294	*
b-serum hexose (mg/ml)	7.92±0.,3015	6.0±0.442	*
Sialic acid in B-serum (µmoles)	0.01428±.00106	0.02025±.0022	*

*significant at $p \leq 0.05$ ns- not significant

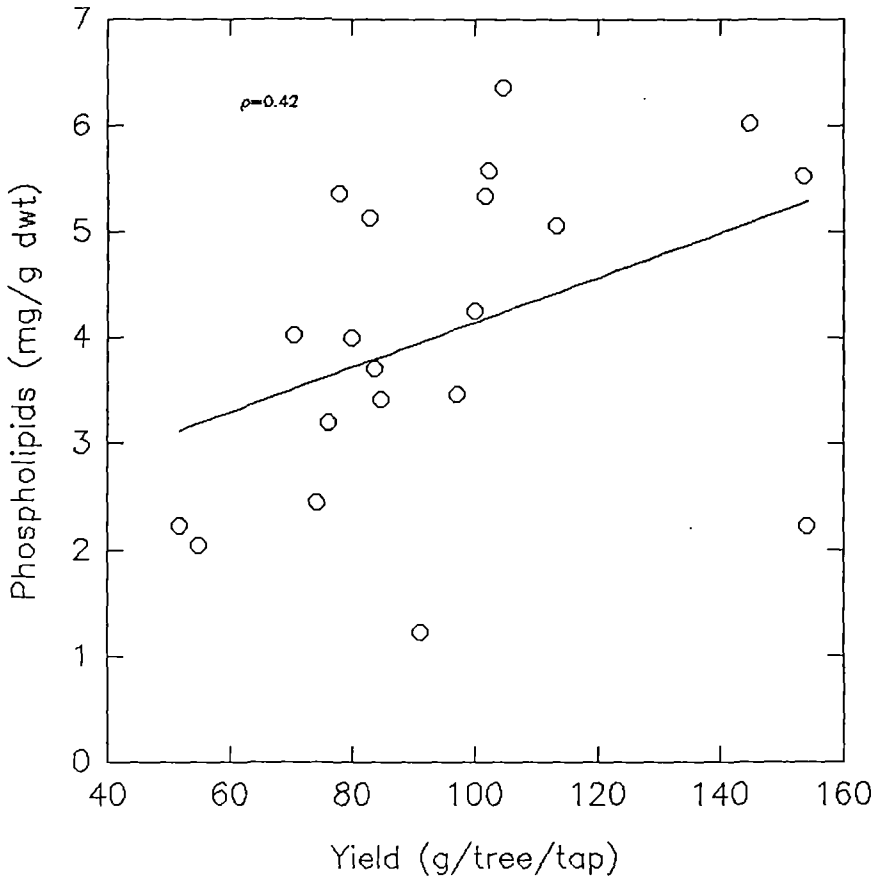


Fig-9 Relationship between yield and phospholipids in clone RRII 105 under 1/2 S d/2 6d/7 tapping system

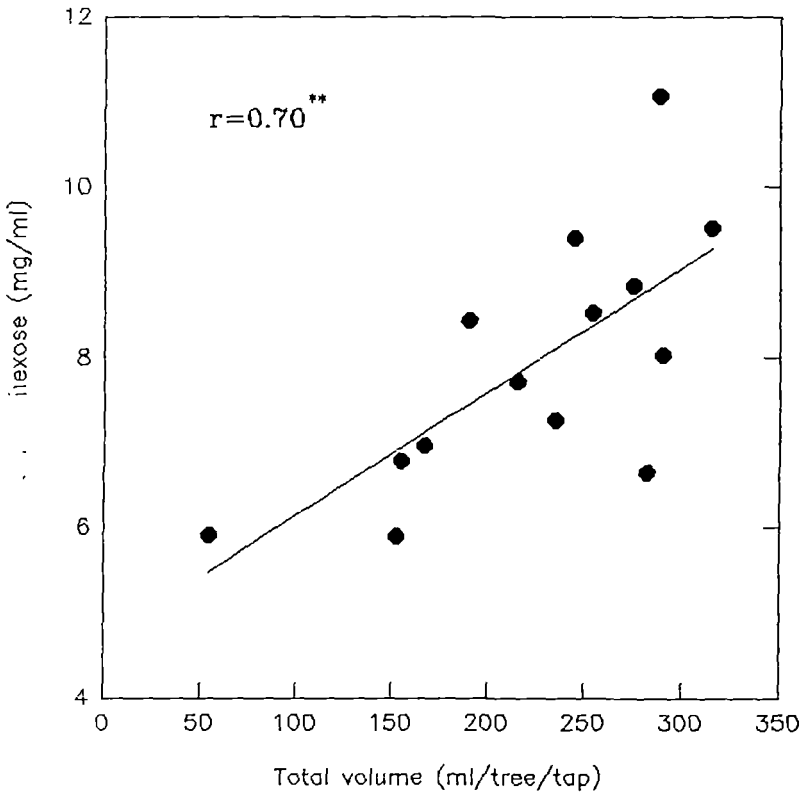


Figure-10 Relationship between latex yield and β-serum hexose in clone RRII 105 under 1/2 S d/2 6 d/7 tapping system (peak yielding eason)

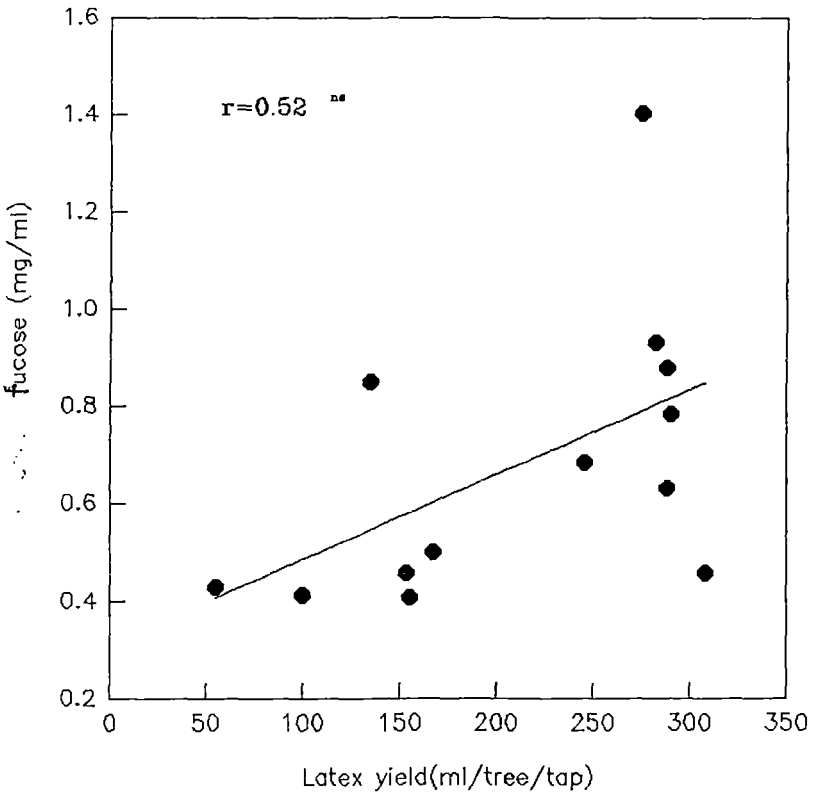


Figure-11 Relationship between latex yield and B-serum fucose in clone RRII 105 under 1/2 S d/2 6 d/7 tapping system (peak yielding season)

3.2. Seasonal variations in carbohydrates and related enzymes associated with latex production in clone RRII 105 under 1/2S d/2 6d/7 system of tapping

Results of seasonal variations in carbohydrate and associated enzymes are shown in Table- 6. Yield was minimum during summer and maximum during peak season (i..e September.-November). When defoliation and refoliation period was considered yield was minimum during defoliation. Latex sucrose during refoliation was lower than that during defoliation. Latex sucrose during summer was more than that of peak season. Trend of C-serum sucrose was similar to latex sucrose. Invertase activity was low during defoliation when compared to refoliation period. Maximum invertase activity in C-serum was observed during peak yielding period followed by refoliation, defoliation and summer. Sucrose synthase in C-serum during summer was maximum and minimum on defoliation.

A negative correlation was observed between sucrose in latex and yield. (Fig -12).Invertase was positively correlated with yield and total volume (Fig- 13&14)

Table-6. Seasonal variation in carbohydrates and related enzymes in cloneRRII 105 under 1/2S d/2 6d/7 system of tapping system parameters

Parameters	Peak	Defoliation	Refoliation	Summe
Yield (gm tree^{-1} tap^{-1})	104.35±6.98	67.52±3.95	87.77±4.82	43.31±2
Sucrose in latex (mM)	8.22±0.67	10.32±0.98	6.89±0.54	9.74±0.
Sucrose in C-serum (mM)	14.464 ± 5.08	8.74±0.43	7.94±0.55	20.96±
Invertase in C-serum (nmole sucrose liberated /min /mg protein)	176.3±8.68	73.69±5.89	135.2±3.77	67.82±8
Sucrose synthase in C-serum(nmole sucrose formed /min/mg protein)	40.39±4.12	11.24±1.35	40.36±2.73	119.35±

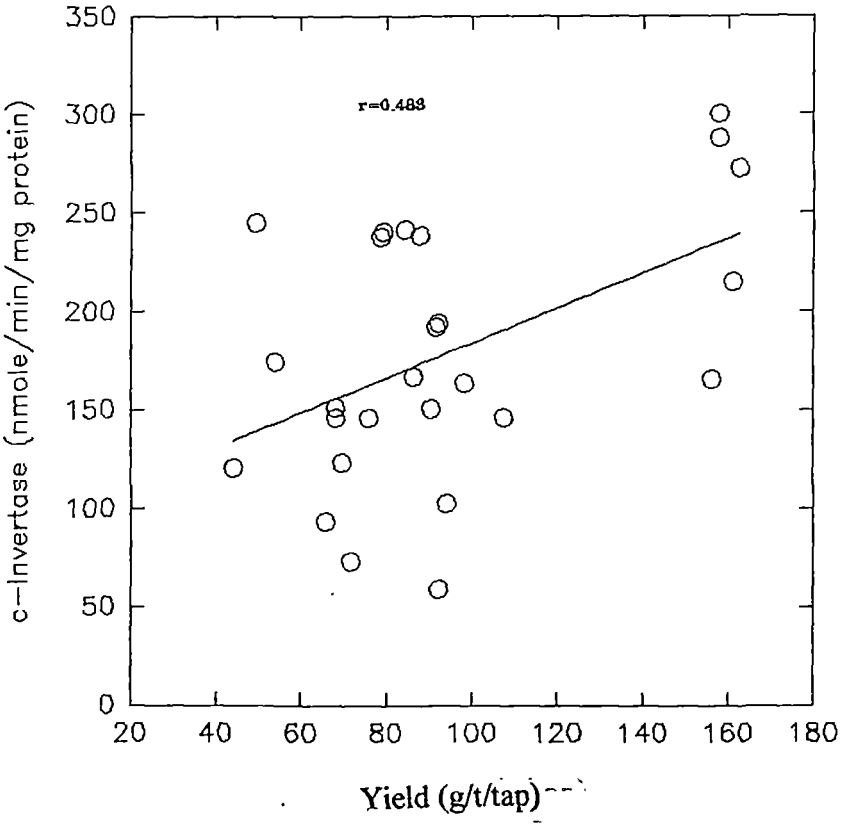


Fig- 14. Relationship between yield and invertase in C- serum in clone RRII 105 under ½ S d/2 6d/7 tapping system (Peak season)

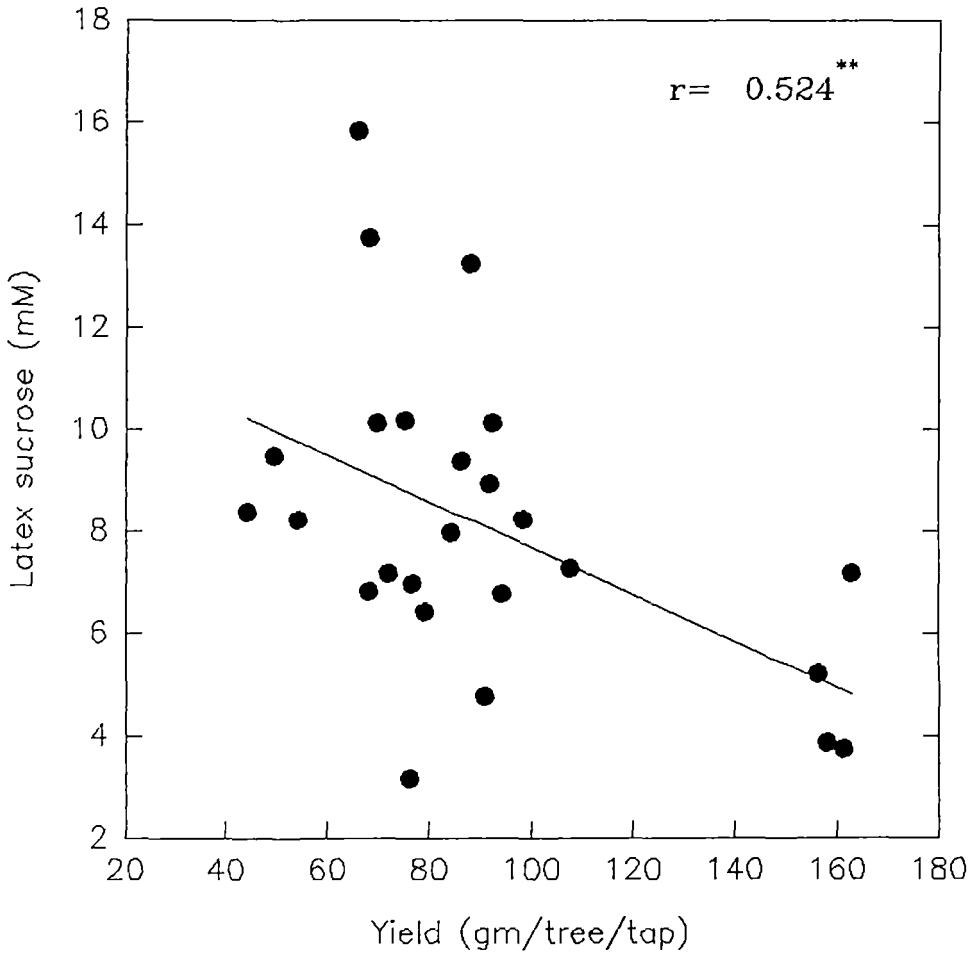


Figure-12 Relationship between yield and latex sucrose in clone RRII 105 under 1/2 S d/2 6d/7 tapping system (peak yielding season)

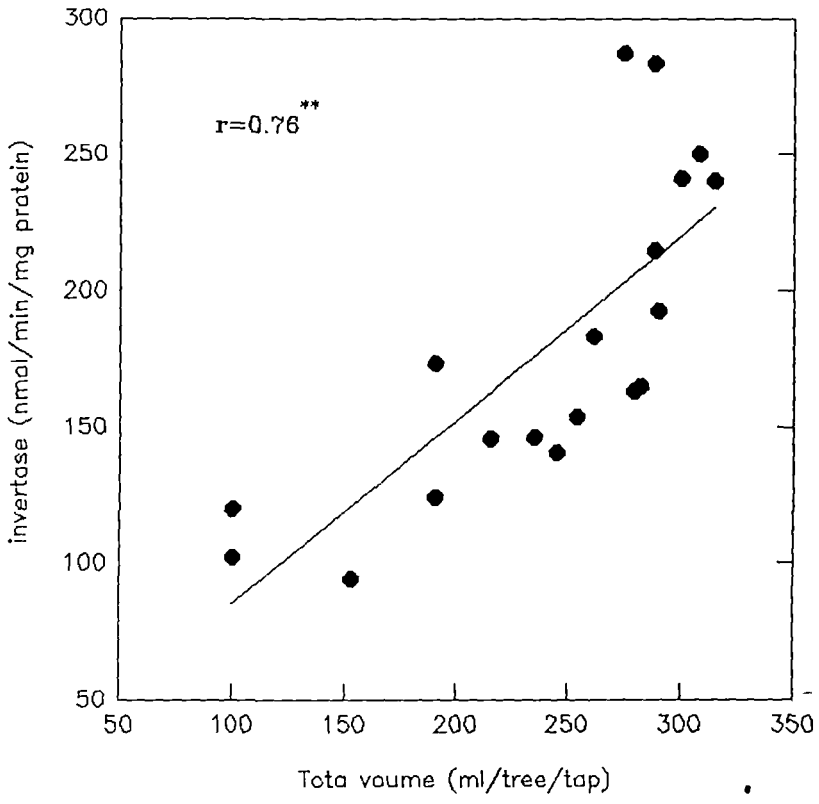


Figure-15 Relationship between total volume and c-serum invertase in clone RRII 105 under 1/2 S d/2 6 d/7 tapping system (peak yielding season)

3.3. Clonal variations in parameters associated with latex production

Results of the study carried out in high and low yielding clones were presented in this chapter. Co variance analysis was used to compare the clonal variation.

3.3.1. Clonal variations in yield and carbohydrate metabolism

Results are presented in Table 7. Yield was significantly high in high yielding clones compared to low yielders. DRC showed no significant difference. No consistant pattern in C-serum sucrose was observed. Sucrose in latex was high in low yielders when compared to high yielding clones. C-serum invertase was significantly high in high yielding clones when compared to low yielders. C-serum sucrose synthase showed no significant difference between high and low yielders. Results are presented in Table 7.

3.3.2. Clonal variations in pH and ATPase , an enzyme involved in proton transport (pH regulation).

Variations in pH and ATPase were shown in Table 8. Latex pH showed no significant difference between high and low yielding clones. C-pH was significantly high in high yielding clones. B-pH was low in high yielding clones. ATPase in lutoid was high in high yielding clones when compared to low yielders.

Table-7. Clonal variations in carbohydrates and related enzymes

Category	Clones	Yield (g/t/tap)	DRC (%)	C-serum Sucrose (mM)	Latex Sucrose (mM)	C-serum invertase (n mole suc/min/mg protein)	C-serum sucrose synthase (n mole suc/min/mg prot
High yielding	RRII 105	69.98	33.02	19.56	6.63	81.52	28.31
	RRIM 600	53.95	33.08	9.54	4.26	83.46	24.02
Low yielding	HP-20	35.74	38.03	17.71	9.03	43.28	28.06
	RRII 38	39.69	36.06	8.88	10.23	43.18	21.33
CD (p=0.05)		13.56	4.63	5.31	2.88	5.38	11.96

Table-8. Clonal variations in pH and lutoidic ATPase

Category	Clones	Latex pH	C-serum pH	B-serum pH	Lutoid ATPase (m mole Pi/min/mg protein)
High yielding	RRII 105	6.91	6.698	5.51	5.62
	RRIM 600	6.84	6.486	5.34	4.2
Low yielding	HP-20	6.79	6.41	5.66	2.96
	RRII-38	6.81	6.356	5.73	2.33
CD (p=0.05)		0.08	0.067	0.252	1.39

3.3.3. Clonal variations in parameters related to energy metabolism

Variations in inorganic phosphorous in latex, C-serum, and B-serum were presented in Table 9. Latex Pi was high in high yielding clones and low in low yielding clones. C-serum Pi and B-serum Pi also showed the similar pattern. N-acetyl glucosaminidase in B-serum was high in high yielding clones. Pyrophosphatase in C-serum was high in high yielding clones when compared to low yielders.

3.3.4 Clonal variations in hevein

No consistant variation was observed in the hevein content of B-serum between clones (Fig-15). Among the four clones the low yielder HP-20 showed a higher hevein content in B-serum. The other low yielder RRII 38 showed the same trend of high yielding clones. Hevein is a clonal character.

Table-9. Clonal variations in Pi (Latex, C-serum, B-serum), PPase in C-serum and N-Acetyl glucosaminidase in B-serum.

Category	Clones	Latex PimM	C-serum Pi mM	b-serum PimM	C-serum pyrophosphatase µmolePi/min/mg protein	N-Acetyl Glucosaminidase mg p. nitro phenol/min/mg protein
High yielding	RRII 105	7.52	7.37	75.47	14.9	0.675
	RRIM 600	5.38	7.17	61.26	12.5	0.517
Low yielding	HP 20	2.56	4.53	29.23	10.6	0.422
	RRII 38	3.24	3.49	59.75	10.96	0.289
	CD	3.23	1.88	12.79	1.96	0.151

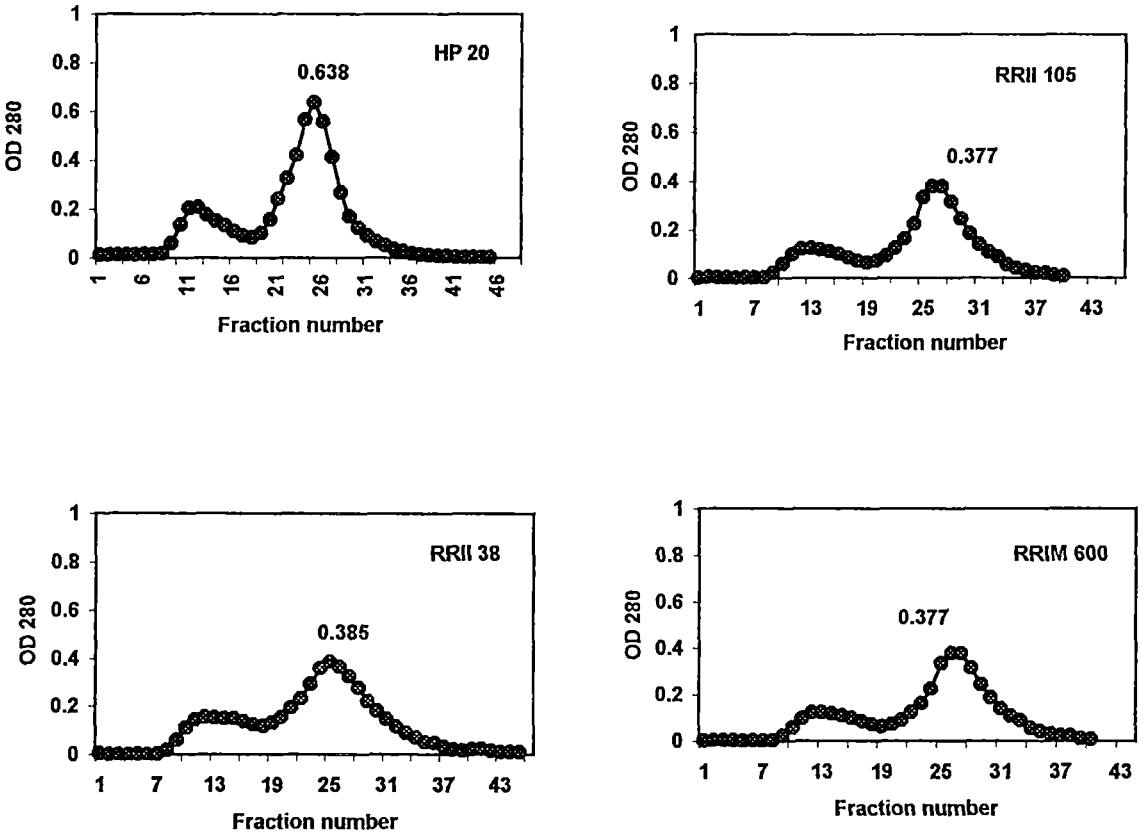


Fig15 Hevein content of B-serum of different clones under d/2 system of tapping. (Peak season). Protein content 6.0 mg on a sephadex G-25 column (3x50 cm). Elution with 1.2 M acetic acid. Fractions of 3.5 ml were collected.

3.4. Physiological and biochemical parameters related to carbohydrate metabolism and ionic balance under ½ S d/2 6d/7 and ½ S d/4 6d/7 tapping system in clone RRII 105.

The results of various parameters related to latex production are presented in this chapter. The data of peak yielding period of 1999 was used. Co variance analysis was used for comparing yield , sucrose and thiols.Independent t test was used for comparison.

3.4.1. Monthly variations in yield and physiological parameters in clone RRII 105 under ½ S d/2 6d/7 and ½ S d/4 6d/7 tapping system.

The monthly variations in yield, drc, sucrose, thiol and Pi are presented in figures 16-20. Both d/2 and d/4 system of tapping showed distinct monthly variations in all these parameters.

3.4.2. Effect of tapping frequency on parameters related to carbohydrate metabolism

Results are shown in Table 10. Yield was significantly high in d/2-tapped trees when compared to d/4 tapped trees during the peak yielding season of 1999. Tapping treatments have no significant effect on sucrose content in latex, and c-

serum. Carbohydrate components also showed no significant difference between d/2 and d/4 tapped trees. c- serum invertase showed an increase in d/2 tapped trees. C-serum sucrose synthase showed a decrease.

Table-10 Effect of tapping frequency on carbohydrates in clone RRII 105.

Parameters	High frequency 1/2 S d/2 6d/7	Low frequency 1/2 S d/4 6d/7	Significance
Yield (g $tree^{-1}$ tap^{-1})	95.27	67.31	10.3 **
Sucrose in latex(mM)	9.03	8.46	1.09 ns
Thiols in latex(mM)	0.205	0.132	0.066 *
c-serum sucrose (mg/ml)	5.07±0.55	4.1±0.65	ns
ß-serum fucose (mg/ml)	6.64±0.09	7.97±0.08	ns
ß-serum hexose (mg/ml)	8.03±0.35	8.15±0.38	ns
DRC (%)	43.3±0.45	41.5±0.67	*
Invertase (C-serum) nmole sucrose/ min/mg protein	211.49±14.02	138.79±7.11	*
Sucrose synthase (C-serum) nmole sucrose/ min/mgprotein	78.03±5.71	118.98±9.13	*

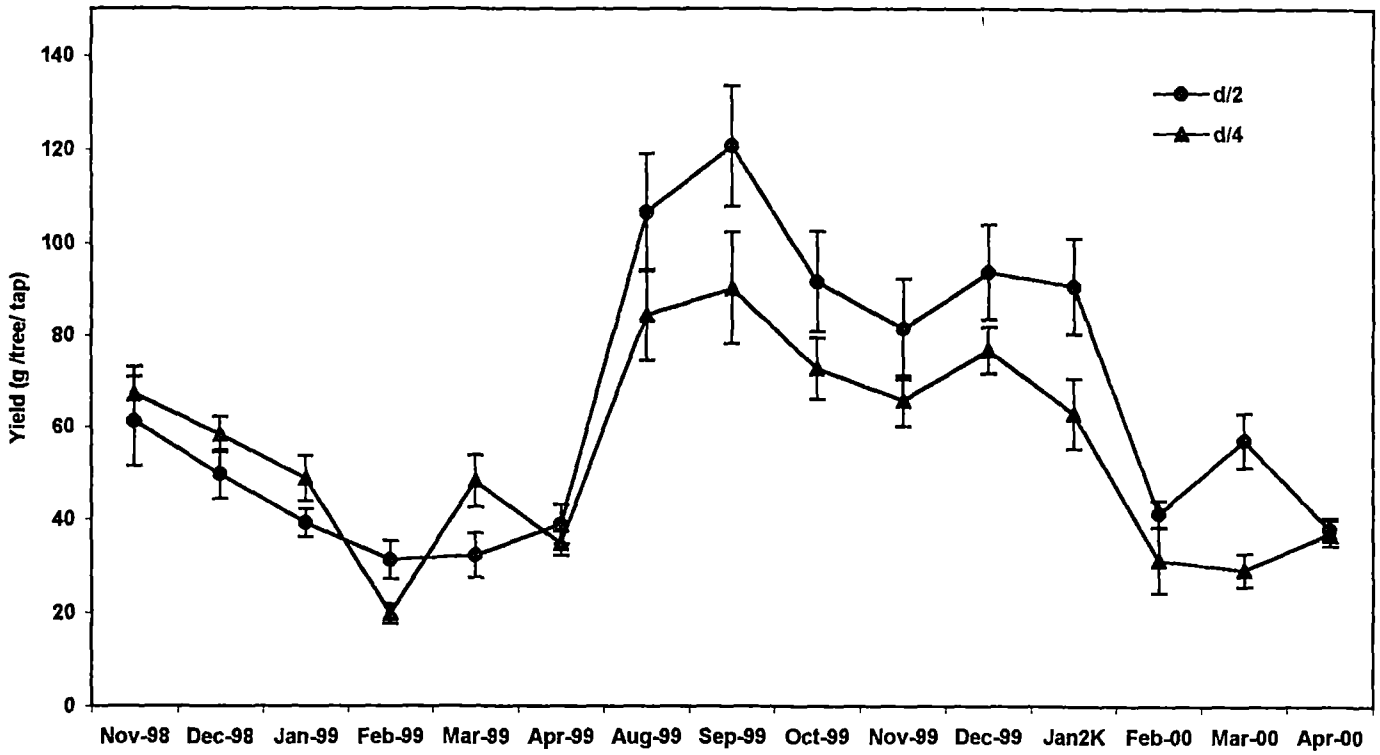


Figure-16 Monthly variations in dry rubber yield in newly opened trees of clone RRII 105 under 1/2 S d/2 6d/7 and 1/2 S d/4 6d/7 tapping systems

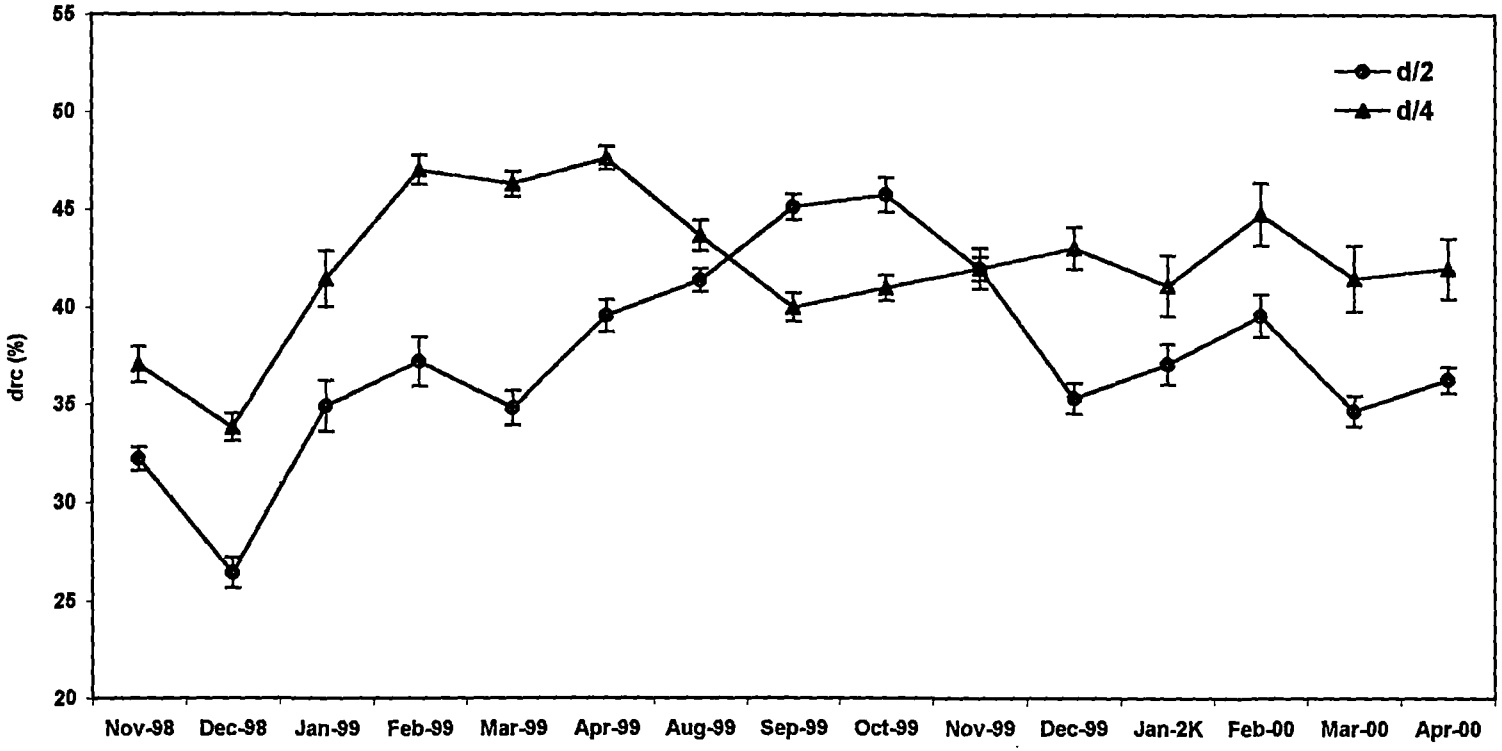


Figure-17 Monthly variations in dry rubber content in newly opened trees of clone RRII 105 under 1/2 S d/2 6 d/7 and 1/2 S d/4 6 d/7 tapping systems

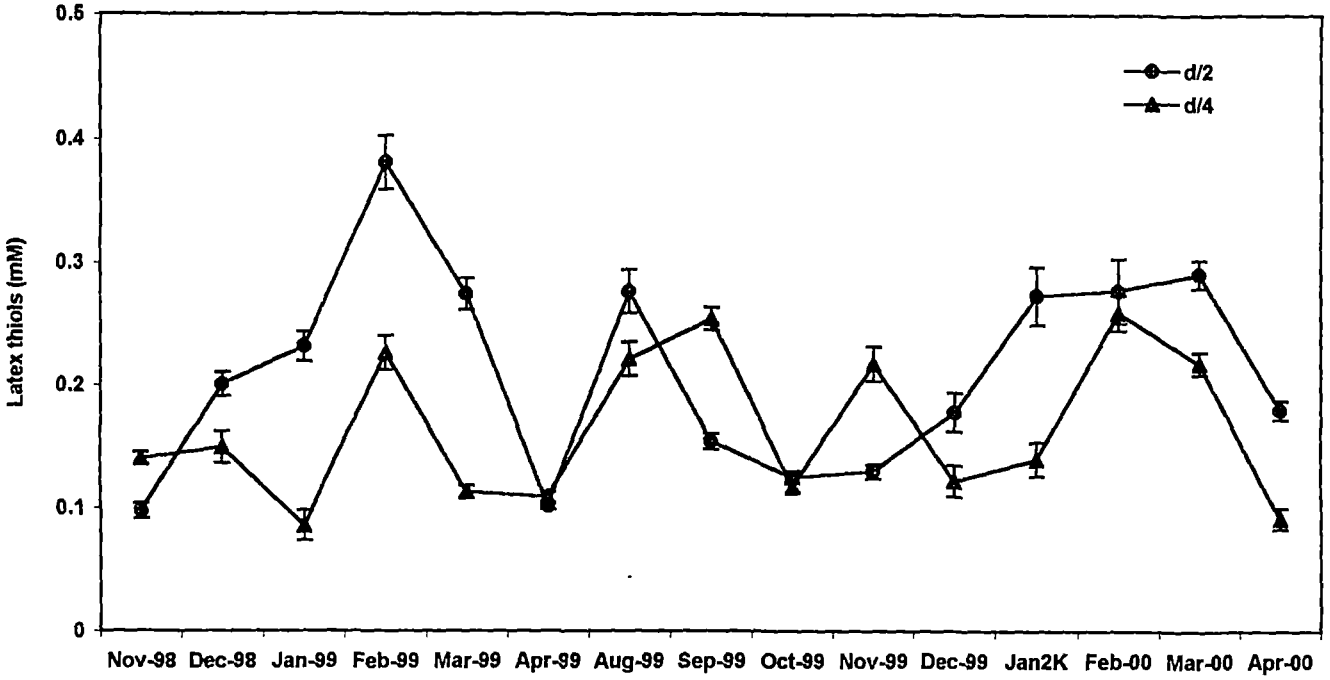


Figure 19 Monthly variations in latex thiols in newly opened trees of clone RRII 105 under 1/2 S d/2 6 d/7 and 1/2 S d/4 6 d/7 tapping systems

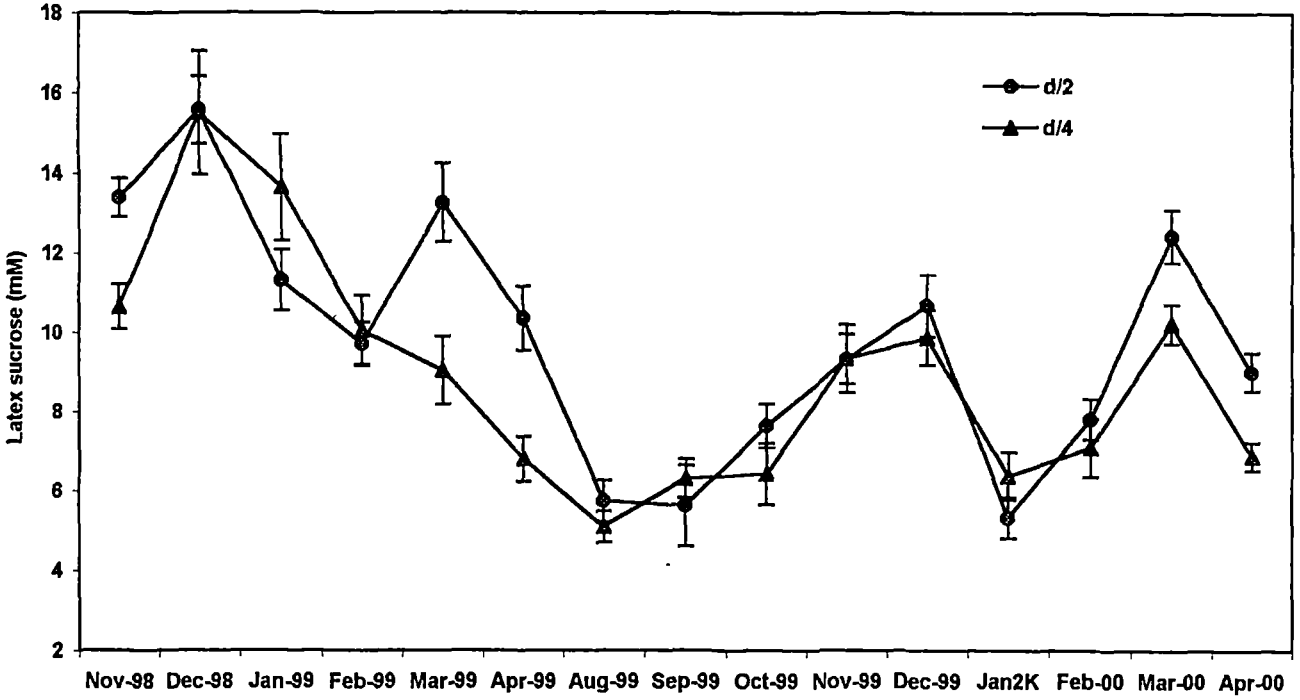


Figure-13 Monthly variations in latex sucrose in newly opened trees of clone RRII 105 under 1/2 S d/2 6d/7 and 1/2 S d/4 6d/7 tapping systems

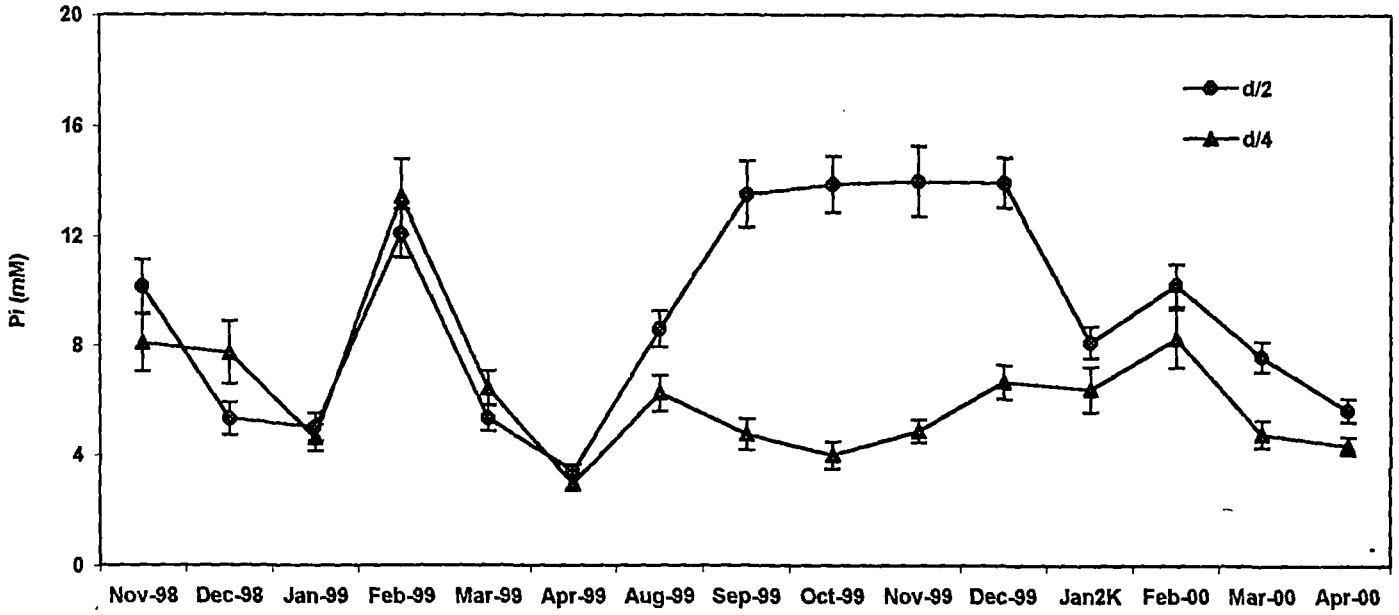


Figure 20 Monthly variations in latex inorganic phosphorus in newly opened trees of clone RRII 105 under 1/2 S d/2 6d/7 and 1/2 S d/4 6d/7 tapping systems

3.4.3. Effect of tapping frequency on parameters regulating latex production (Pi,PPi,C-serum PPase, N-acetyl glucosaminidase& Chitinase in B-serum)

The results are presented in Table-11.C- serum Pi, C-serum PPi and N acetyl glucosaminidase showed an increase in in d/2 tapped trees when compared to d/4 tapped trees. No significant variation was observed in pyrophosphatase in C-serum and chitinase in B-serum between two treatments.

Table-11.Effect of tapping frequency on Pi, PPi, PPase in C-serum, N acetyl glucosaminidase in B-serum, Chitinase in B-serum

Parameters	High frequency 1/2 S d/2 6d/7	Low frequency 1/2 S d/4 6d/7	Significance
c-serum Pi (mg/ml)	0.47±0.022	0.369±0.016	*
c-serum PPi (mg/ml)	0.796±0.038	0.594±0.022	*
Pyrophosphatase (C-Serum)(µmole phos./min/mg protein)	3.2±.15	2.96±1.25	ns
Nacetylglucosaminidase (B-serum)(mg p.nitrophenol/min/mgprotein)	0.42±0.021	0.24 ±0.012	*
Chitinase(B-serum) (units/min/mg protein)	0.0018±0.00026	0.0020±.00018	ns

*Significant at 0.05 level

3.4.4. Effect of tapping frequency on pH and its regulating enzyme

Latex pH and B-serum pH did not show any significant difference between treatments. C- pH showed an increase in d/2-tapped trees. ATPase activity in lutoids did not show any significant significant difference between hugh and low frequency tapped trees.

Table- 12. Effect of tapping frequency on pH and related enzymes in clone RRII 105

Parameters	High frequency 1/2 S d/2 6d/7	Low frequency 1/2 S d/4 6d/7	Significance
latex-pH	7.15±0.062	7.14±0.025	ns
c-pH	6.885±0.065	6.577±0.072	*
b-pH	5.58±0.0599	5.41±0.0292	ns
ATP ase(bottom) µmole phosphorus/min/mg protein	3.91±.36	3.56±0.43	ns

3.4.5. Effect of tapping frequency on amount of bottom, cream and C-serum.

Weight of bottom fraction was high in d/2 tapped trees when compared to d/4 tapped trees. No significant difference was observed in weight of rubber cream and volume of c-serum between d/2 and d/4 tapped trees.

Table- 13. Effect of tapping frequency on weight of bottom, cream and volume of c- serum in clone RRII 105

Parameters	High frequency 1/2 S d/2 6d/7	Low frequency 1/2 S d/4 6d/7	Significance
Weight of bottom fraction (g/g fresh wt.)	0.177±0.008	0.1463±0.011	*
Weight of rubber cream (g/g fresh wt)	0.409±0.019	0.446±0.019	ns
Vol.of c-serum (ml/g fresh wt.)	0.391±0.0093	0.389±0.009	ns

3.4.6. Effect of tapping frequency on latex lipids related to stability of membrane

The results are presented in Table-14. Latex glycolipids and phospholipids in latex and bottom fraction were significantly higher in high frequency tapped trees when compared to low frequency tapped trees. Glycolipids in cream and bottom and phospholipids in rubber cream showed no significant difference.

Table-14. Effect of tapping frequency on latex lipids in clone RRII 105

Parameters	High frequency	Low frequency	Significance
Glycolipid (latex) (mg/g dry wt.latex)	12.69±0.53	10.08±0.603	*
Glycolipid (bottom) (mg/gdry wt.bottom)	38.73±2.969	35.835±3.145	ns
Glycolipid (cream) (mg/g dry wt.cream)	5.508±0.378	5.74±0.756	ns
Phospholipidslatex (mg/g dry wt.latex)	6.43±0.197	5.01±0.171	*
Phospholipid bottom)(mg/g dry wt.bottom)	bottom20.543±1.98	15.87±1.078	*
Phospholipid (cream)(mg/g dry wt.cream)	2.024±0.24	2.68±0.626	ns

3.5 . Effect of stimulation (stimulation with normal tapping, stimulation with intensive tapping , stimulation with tapping rest) on carbohydrate metabolism and ionic balance in clone RRII 105 under ½S d/4 6d/7 system of tapping.

3.5.1.Total volume

Control and stimulated

Total volume of latex increased on all tapping days after stimulation (fig-21).

Stimulation and intensive tapping

A general increase was observed on all intensive tapping days after stimulation , Maximum volume was observed on second and third intensive tapping (Fig-22)

Stimulation and tapping rest

Total volume of latex was found to be maximum in trees with six day rest after stimulation (Fig-23)

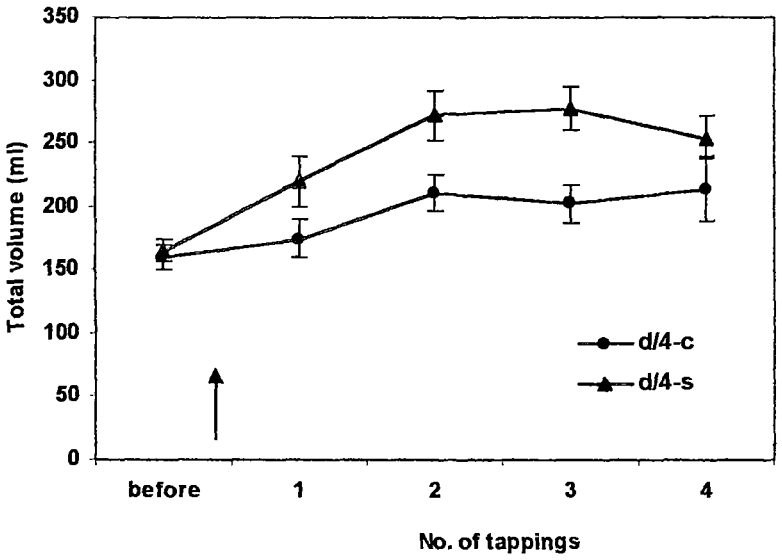


Fig-21. Effect of stimulation on total volume in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ± SE)

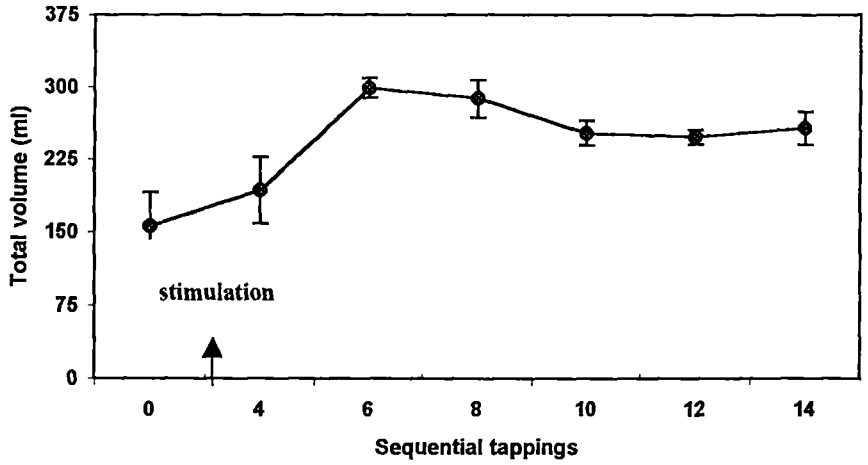


Fig- 22 Effect of sequential tapping after stimulation on latex yield in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ± SE)

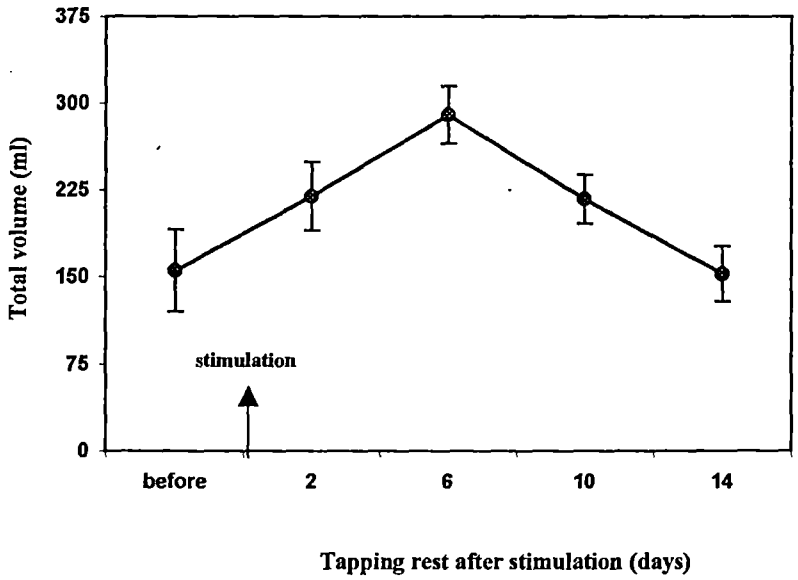


Fig-23 Effect of tapping rest after stimulation on latex yield in clone RRII 105 under ½ S d/4 6d/7 tapping system

3.5.2 Dry rubber content

Control and stimulated

DRC of stimulated trees were always lower than that of control trees. The reduction was observed in the first tapping onwards after stimulation (Fig-24).

Stimulation and intensive tapping

A decrease in DRC was observed after second intensive tapping onwards (Fig-25).

Stimulation and tapping rest

DRC was minimum in trees with six and ten day tapping rest after stimulation and then increased in trees with [illegible] fourteen-day rest it was higher than that of unstimulated trees (Fig-26).

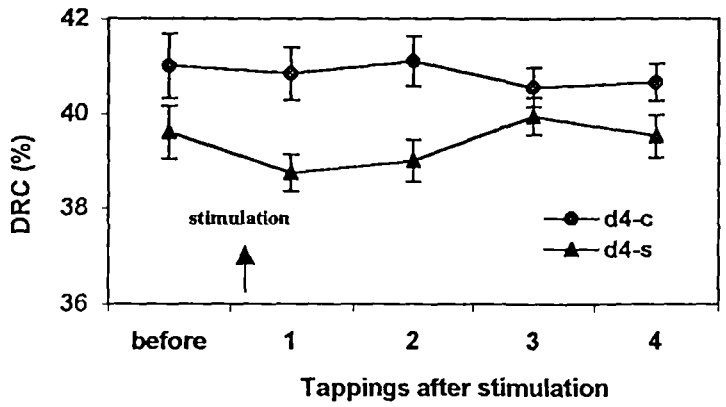


Fig- 24 Effect of stimulation on DRC in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ±SE)
d/4-c: control d/4-s: stimulated

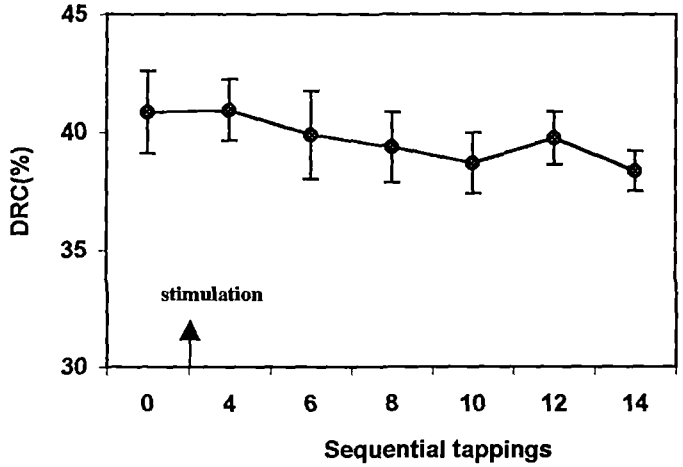


Fig- 25 Effect of intensive tapping after stimulation on DRC in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ± SE).

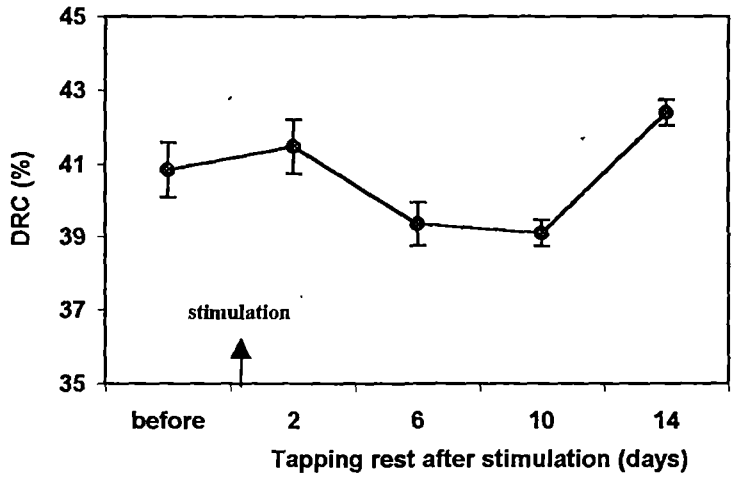


Fig- 26 Effect of tapping rest after stimulation on DRC in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ± SE)

3.5.3. Latex sucrose

Control and stimulated
Maximum sucrose content in latex was observed on third tapping after stimulation. No change in sucrose was observed up to second tapping (Fig-27)

Stimulation and intensive tapping
An increase in latex sucrose was observed up to fourth intensive tapping and then decreases in subsequent tappings but higher than control (Fig-28)

Stimulation and tapping rest
Six day rested trees have maximum latex sucrose and then decreases. Sucrose content in trees was very low (Fig-29)
(14 day rest)

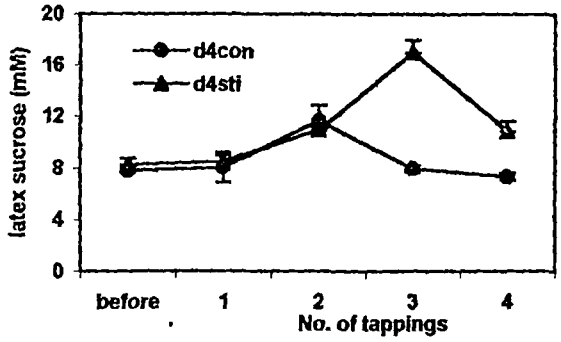


Fig-27 Effect of stimulation on latex sucrose in clone RRII 105 under 1/2 S d/4 6d/7 tapping system (mean of six trees ± SE)

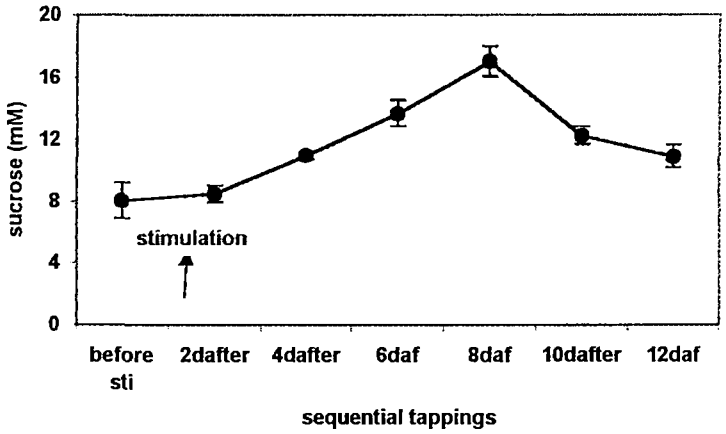


Fig-28. Effect of intensive tapping after stimulation on latex sucrose in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ±SE)

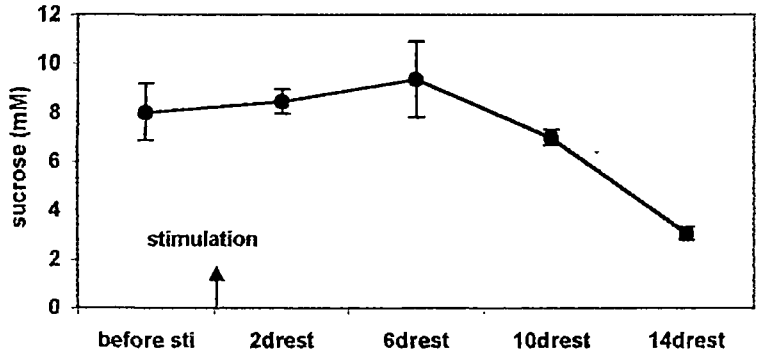


Fig-29. Effect of tapping rest after stimulation on latex sucrose in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

3.5.4. C-SUCROSE

Control and stimulated

Maximum increase in sucrose in C- serum was observed on second tapping after stimulation and then decreases (Fig-30).

Stimulation and intensive tapping

A general increase was observed and maximum sucrose content was on third intensive tapping after stimulation (Fig-31).

Stimulation and tapping rest

An increase was observed in C-serum sucrose up to six day rested trees and then decreases in 10 day rested trees after stimulation (Fig-32)

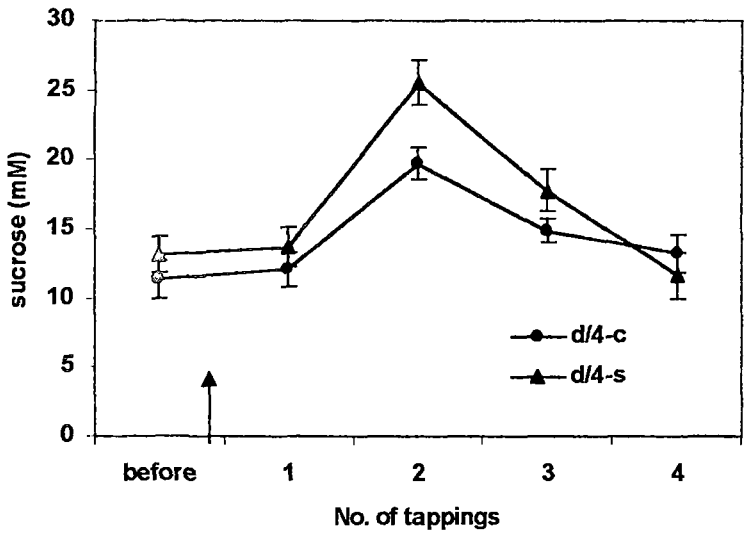


Fig- 30. Effect of stimulation on sucrose (C-serum) in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ± SE).
d/4-c: control d/4-s: stimulated

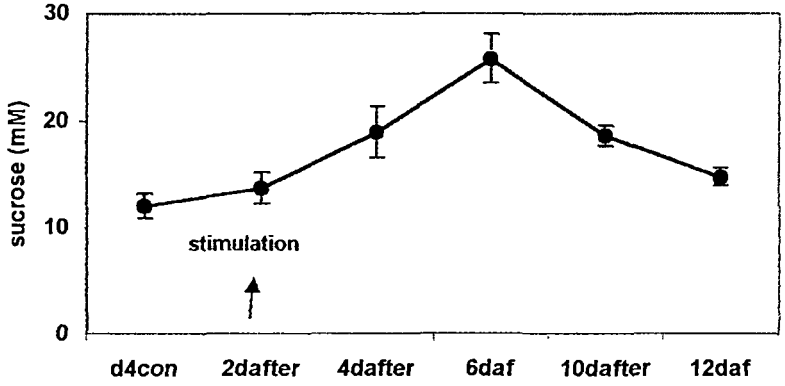


Fig-31 Effect of intensive tapping after stimulation on sucrose (C-serum) in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ±SE)

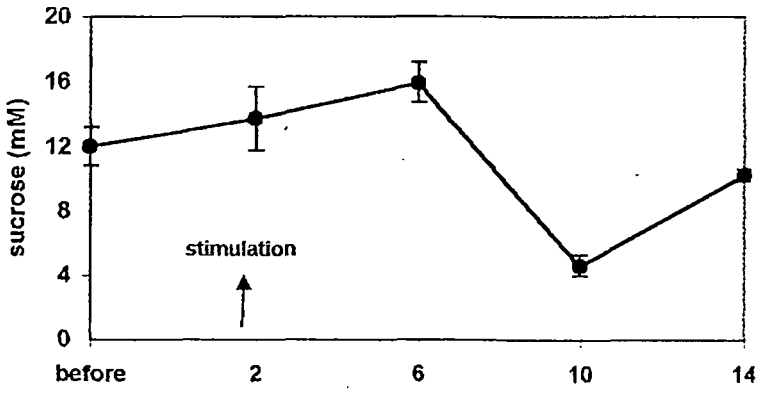


Fig-32 Effect of tapping rest after stimulation on sucrose (C-serum) in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

3.5.5. Invertase activity in C-serum

Control and stimulated

No difference was observed in invertase activity between control and stimulated trees upto 2nd tapping after stimulation , then stimulated trees showed an increase on 3rd tapping after stimulation(Fig-33).

Stimulation and intensive tapping

The activity of invertase showed a decrease up to fourth intensive tapping after stimulation and then increases (Fig-34)

Stimulation and tapping rest

Activity of invertase in 2 and 6 day rested trees showed a decreaseand then an increase was observed in 10 and 14 day rested trees (Fig-35)

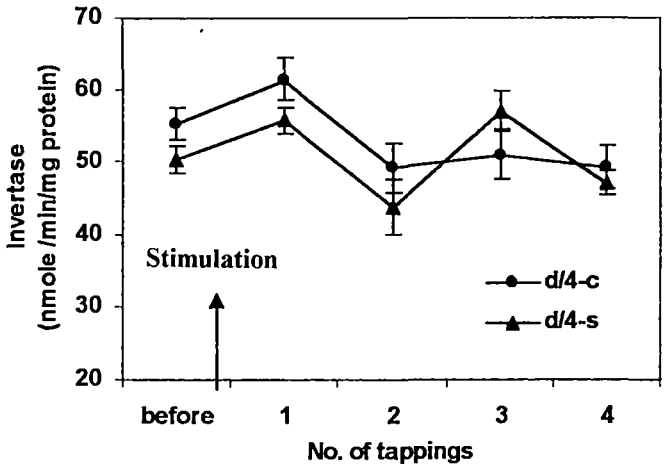


Fig-33 Effect of stimulation on invertase activity of C-serum of clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ±SE) d/4-c: control d/4-s: stimulated

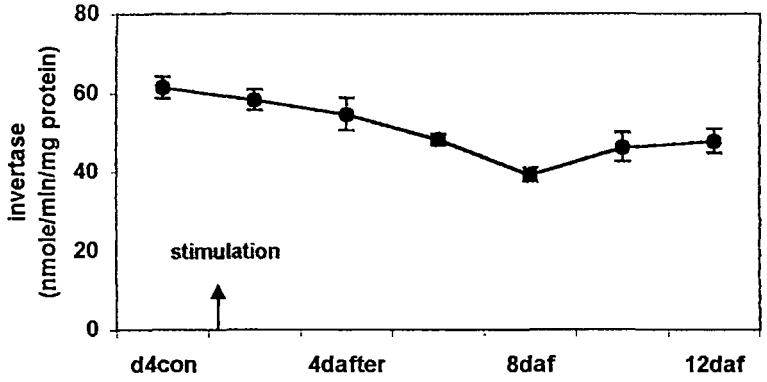


Fig- 34 Effect of intensive tapping after stimulation on C-serum invertase in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ±SE)

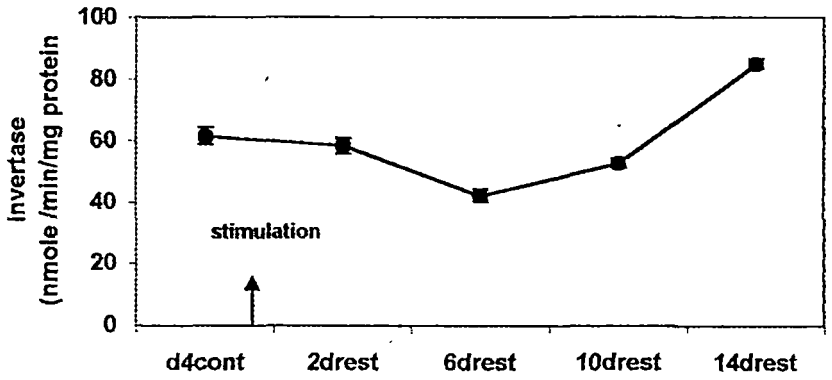


Fig-35 Effect of tapping rest after stimulation on invertase C-serum in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ±SE)

3.5.6. Sucrose synthase in C-serum

Control and stimulated

No difference was observed in the activity up to first tapping after stimulation . Then an increase was noticed in successive tappings (fig-36).

Stimulation and intensive tapping

Sucrose synthase activity showed an increase up to fourth intensive tapping (Fig-37)

Stimulation and tapping rest

Sucrose synthase activity decreases in two day rested trees and six day rested trees and then no variation was observed in 10 and 14 day rested trees.

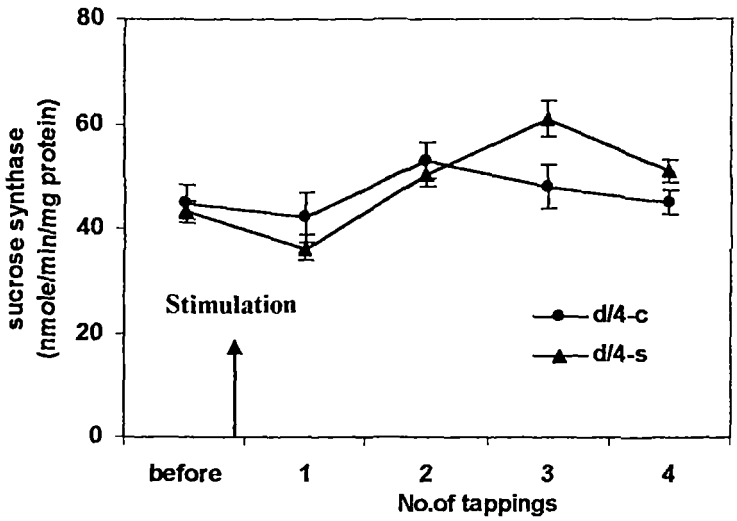


Fig-36. Effect of stimulation on sucrose synthase activity in clone RRII 105 under ½ S d/2 6d/7 tapping system (mean of six trees ± SE)

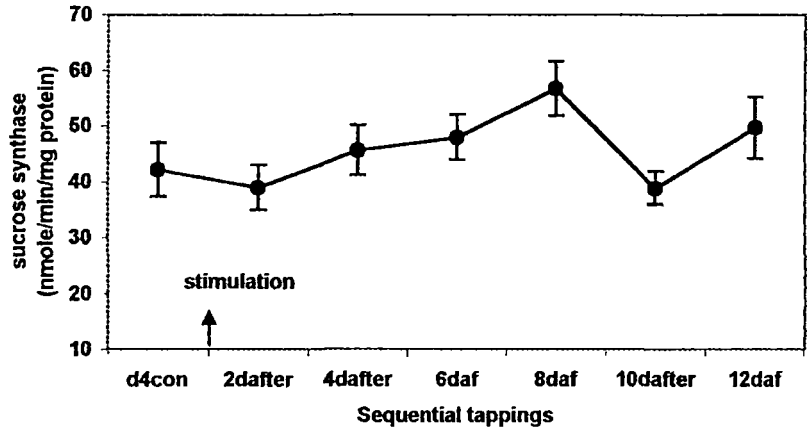


Fig- 37 Effect of intensive tapping after stimulation on C-serum sucrose synthase in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ±SE)

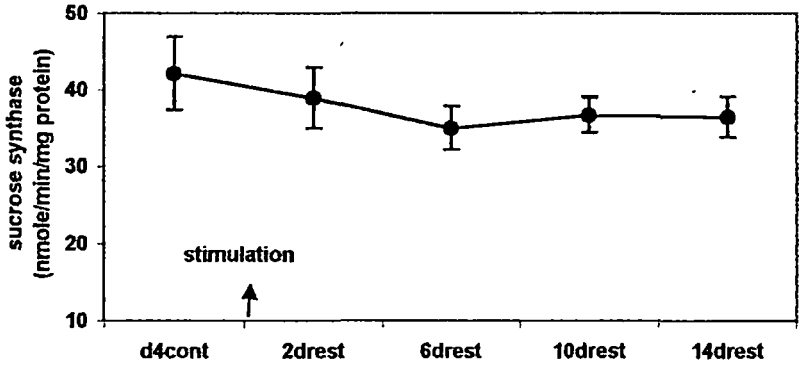


Fig- 38 Effect of tapping rest after stimulation on C-serum sucrose synthase in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ±SE)

3.5.7 LATEX THIOLS

Control and stimulated

Latex thiol shows an overall increase in stimulated trees when compared to control trees on all tapping days (Fig-39).

Stimulation and intensive tapping

There was a general increase in latex thiol in trees with intensive tapping after stimulation. Maximum increase was on third intensive tapping.(fig-40)

Stimulation and tapping rest

An over all increase in latex thiol was observed in trees with different periods of rest and stimulation.(fig- 41)

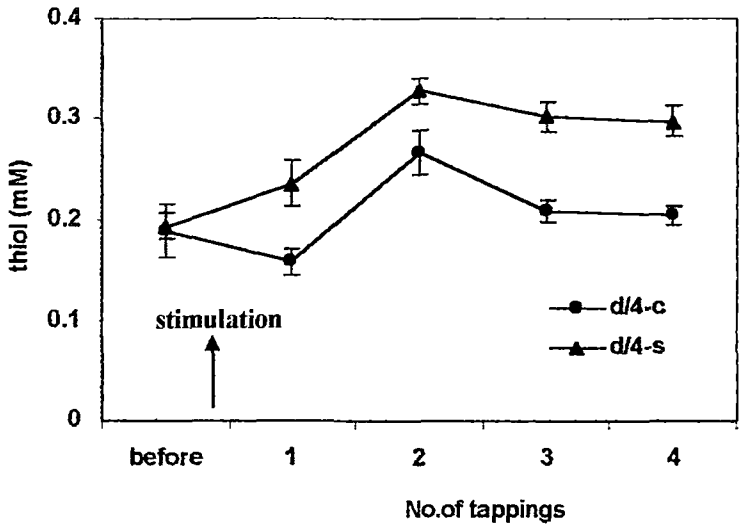


Fig-39. Effect of stimulation on latex thiols in clone RRII 105 under ½ S d/2 6d/7 tapping system. (mean of six trees ± SE

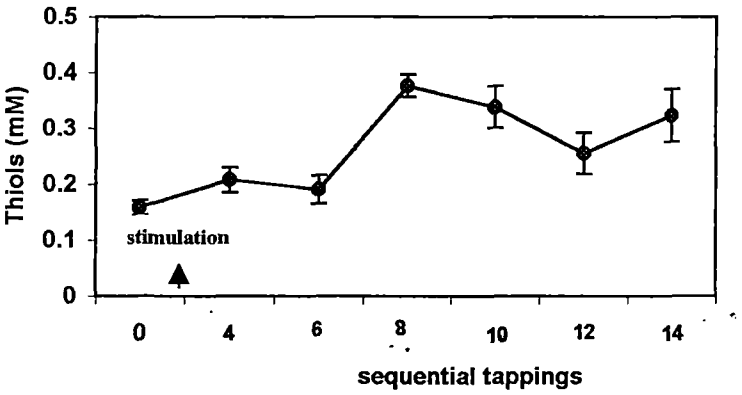


Fig-40 Effect of intensive tapping after stimulation on latex thiols in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ±SE)

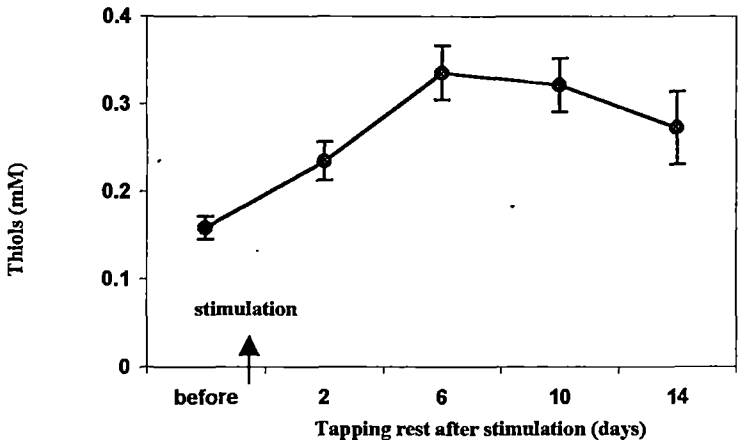


Fig. 41 Effect of tapping rest after stimulation on latex thiols in clone RRII under ½ S d/4 6d/7 tapping system (mean of six trees ± SE)

3.5.8. Pi in Latex

Control and stimulated

Latex Pi showed an increase after first tapping in stimulated trees when compared to control trees (Fig-42).

Stimulation and intensive tapping

A general increase was observed after stimulation on all tapping days (Fig-43).

Stimulation and tapping rest

Maximum Pi was observed on 6 and 10 day rested trees. On 14 day rest a sudden decline was observed (Fig-44).

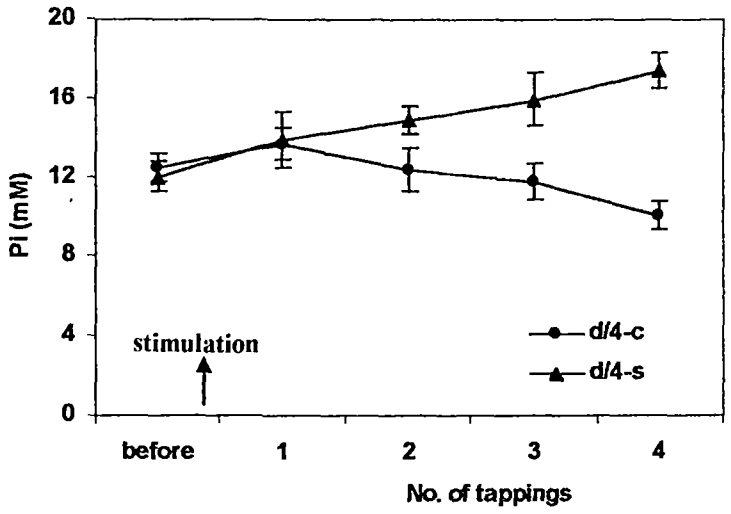


Fig-42. Effect of stimulation on Pi (latex) in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ±SE) d/4-c: control d/4-s: stimulated

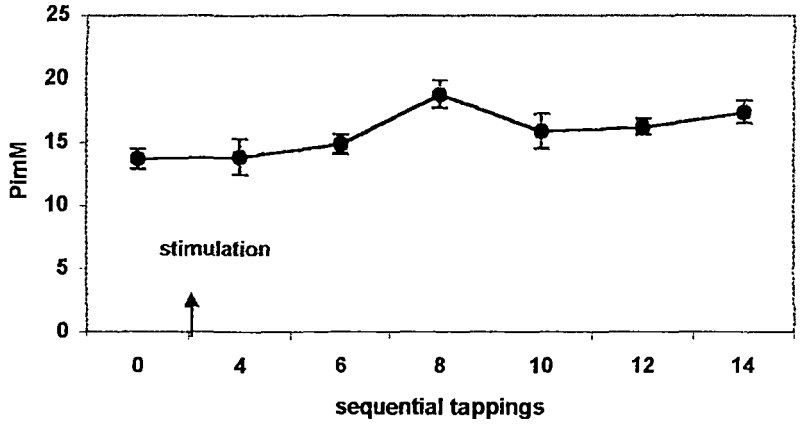


Fig-43. Effect of intensive tapping on latex Pi in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

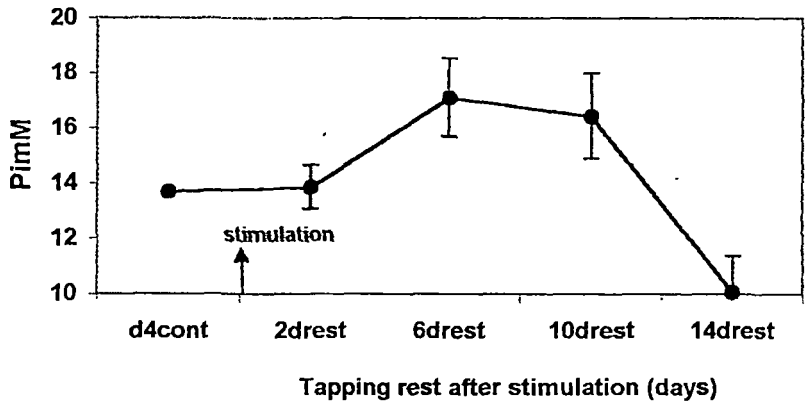


Fig-44. Effect of tapping rest after stimulation on latex Pi in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

3.5.9. Inorganic phosphorus in C-serum

Control and stimulated

No variation was observed in the content of inorganic phosphorus in C-serum in stimulated trees when compared to control trees (Fig-45).

Stimulation and intensive tapping

A general increase in phosphorus content of C-serum was observed on all intensive tapping days after stimulation. Maximum increase was on 4th and fifth intensive tapping (Fig-46).

Stimulation and tapping rest

Up to six day rest, no variation was observed in the content of inorganic phosphorus and then a sudden increase was observed in trees with 10 and 14 day rest (Fig-47).

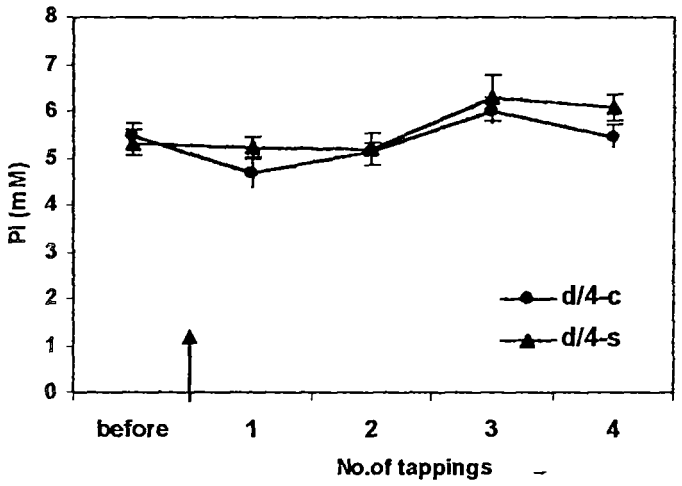


Fig- 45.Effect of stimulation on C- serum Pi in clone RRII 105 under ½ Sd/4 6d/7 tapping system (mean of six trees ±SE). d/4-c: control d/4-s: stimulated

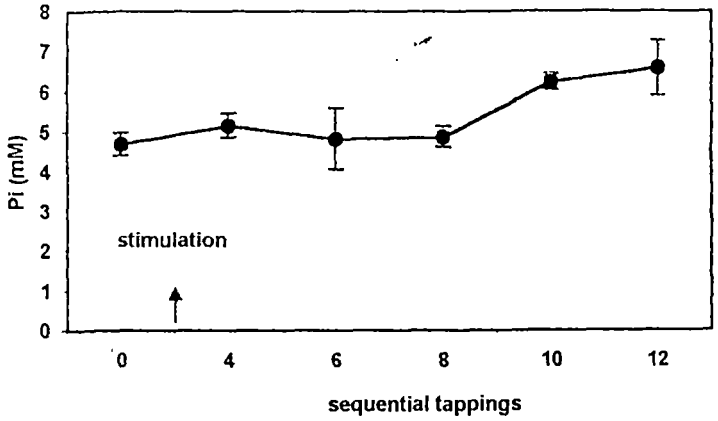


Fig-46. Effect of intensive tapping after stimulation on Pi (C-serum) in clone RRII 105 under 1/2 S d/4 6d/7 tapping system (mean of six trees ± SE)

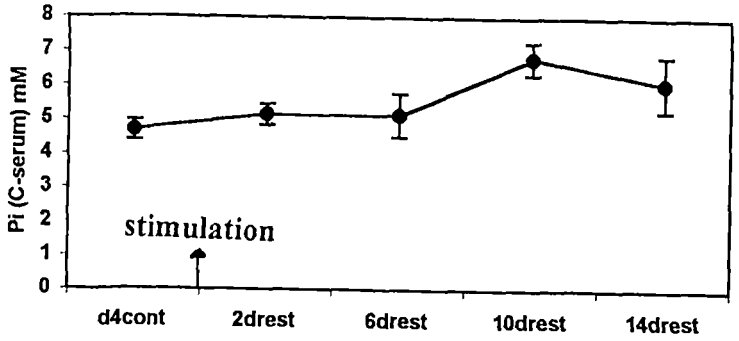


Fig-47 Effect of tapping rest after stimulation on c-serum Pi in clone RRII 105 under 1/2 S d/4 6d/7 tapping system (mean of six trees ±SE)

3.5.10. Pi in B-serum

Control and stimulated
A general increase in Pi of B- serum was observed in stimulated trees when compared to control trees (Fig-48)

Stimulation and intensive tapping
Pi of B-serum showed an increase in first intensive tapping after stimulation and then a sudden decrease was observed in second intensive tapping (Fig-49).

Stimulation and tapping rest
Trees with 2 day rest showed an increase in Pi of B-serum. Then a gradual decrease was observed in 6,10 &14 day rest (Fig-50)

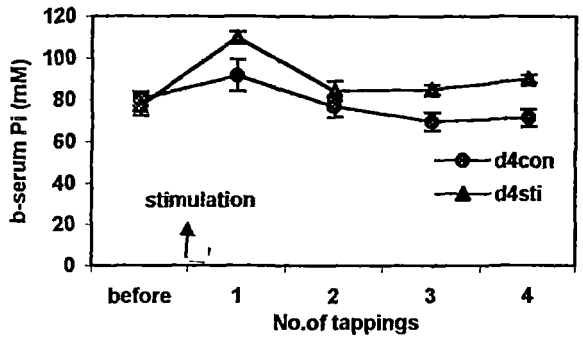


Fig- 48. Effect of stimulation on B-serum Pi in clone RRII 105 under 1/2 S d/4 6d/7 tapping system (mean of six trees ±SE)

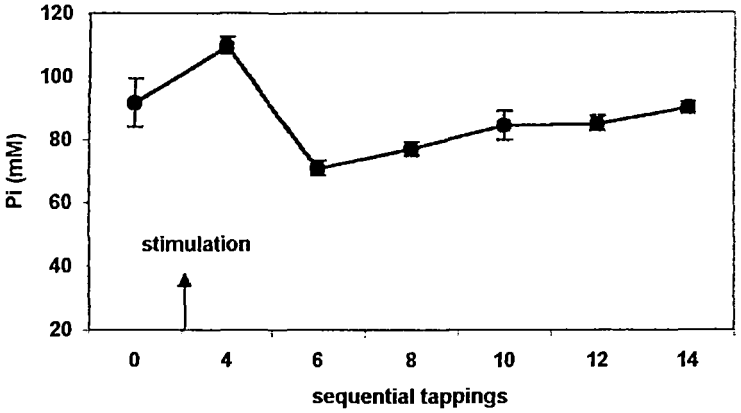


Fig-49. Effect of intensive tapping after stimulation on b-serum Pi in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

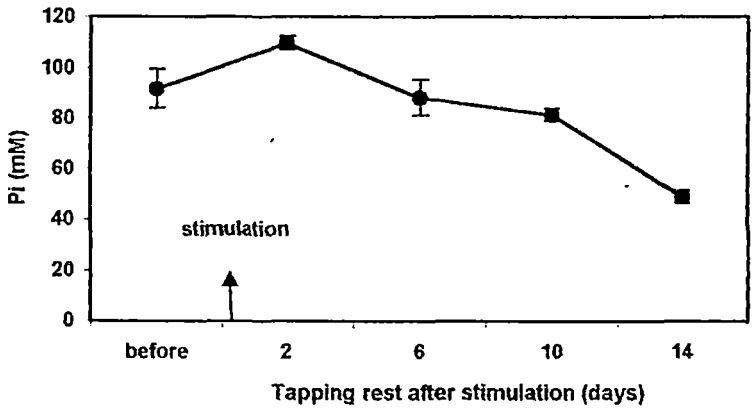


Fig- 50. Effect of tapping rest after stimulation on b-serum Pi in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

3.5.11. Pyrophosphatase (C-serum)

Control and stimulated

Pyrophosphatase activity in C-serum increased on all tapping days after stimulation when compared to control (Fig-51).

Stimulation and intensive tapping

No variation in pyrophosphatase activity was observed in C-serum after stimulation and intensive tapping (Fig-52).

Stimulation and tapping rest

No variation in pyrophosphatase activity was observed in C-serum after stimulation and tapping rest (Fig-53).

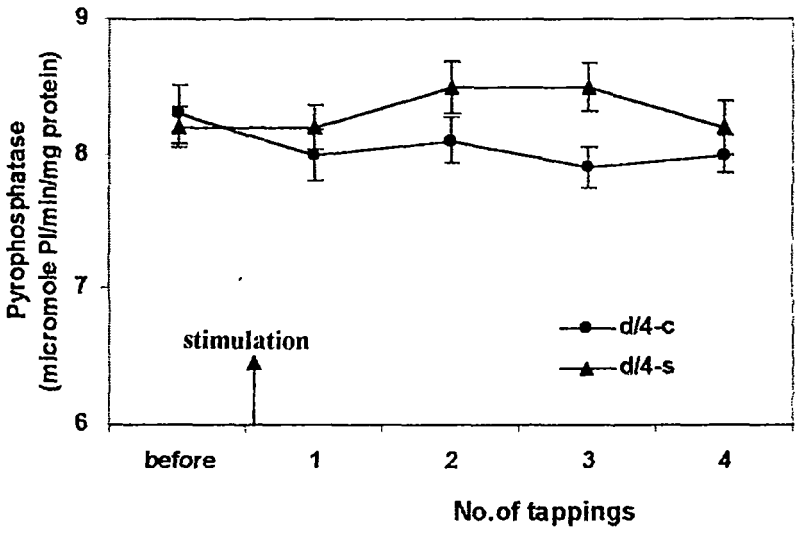


Fig-51. Effect of stimulation on pyrophosphatase (C-serum) in clone RRII 105 under ½ Sd/4 6d/7 tapping system (mean of six trees ±SE) d/4-c: control d/4-s-stimulated

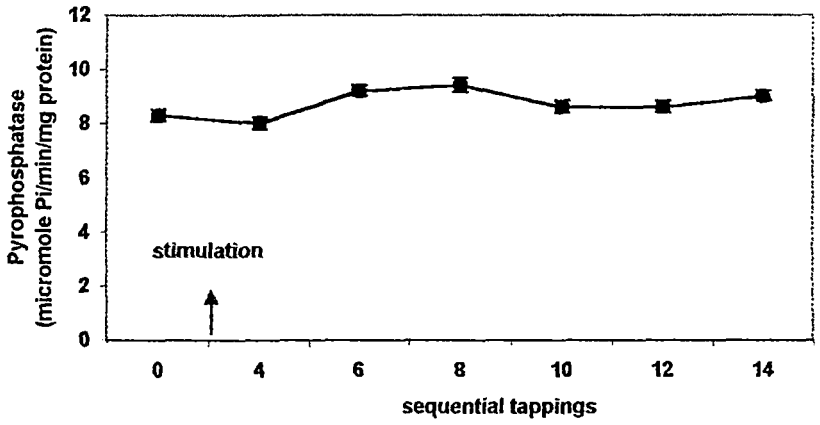


Fig-52. Effect of intensive tapping after stimulation on pyrophosphatase activity (C-serum) in clone RRII 105 under 1/2 S d/4 6d/7 tapping system (mean of six trees ± SE)

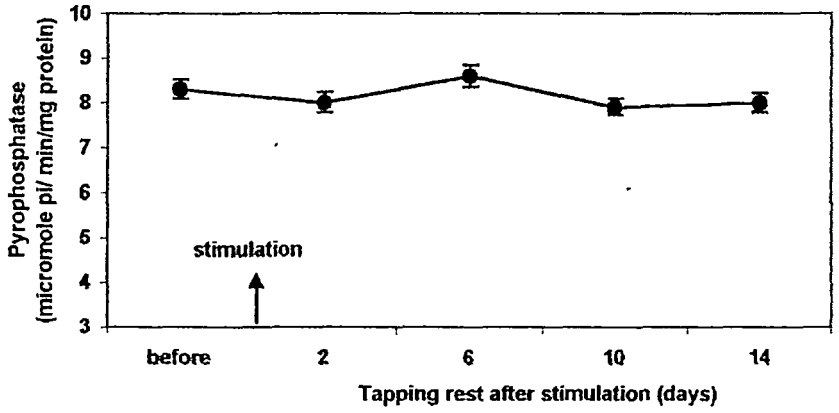


Fig-53. Effect of tapping rest after stimulation on pyrophosphatase activity (C-serum) in clone RRII 105 under 1/2 S d/4 6d/7 tapping system (mean of six trees ± SE)

3.5.12. Latex pH

Control and stimulated

Latex pH after stimulation showed an increase up to third tapping and then decreases (Fig-54).

Stimulation and intensive tapping

Latex pH showed a general increase on all tapping days after stimulation (Fig-55).

Stimulation and tapping rest

No consistent variation was observed in the pH of latex in trees with different periods of tapping rest (Fig-56).

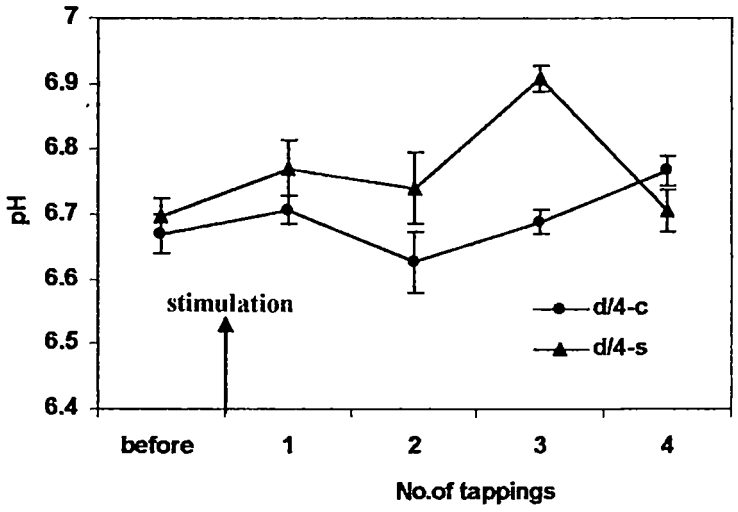


Fig-54. Effect of stimulation on latex pH in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ±SE).
d/4-c: control d/4-s: stimulated

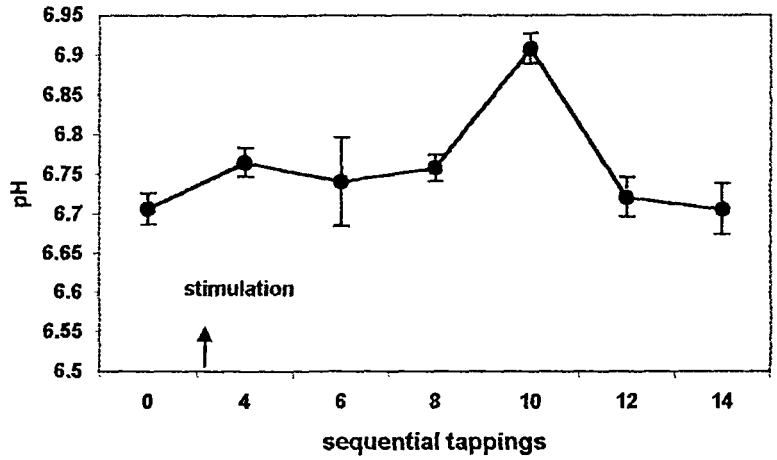


Fig-55. Effect of intensive tapping on latex pH in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

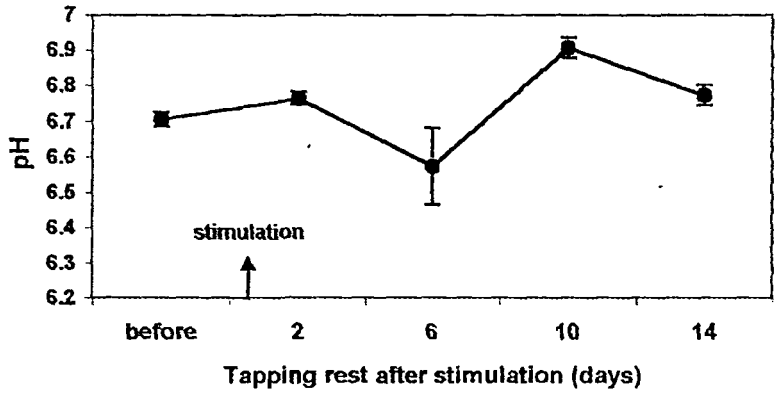


Fig-56 Effect of tapping rest after stimulation on latex pH in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

3.5.13. C-SERUM PH

Control and stimulated

Stimulated trees showed an increase in pH of C-serum on all tapping days when compared to control trees (Fig-57).

Stimulation and intensive tapping

No consistent variation in pH of C-serum was observed was observed in trees with stimulation and intensive tapping.(Fig.58).

Stimulation and tapping rest

No consistant variation in pH of C-serum was observed in trees after tapping rest (Fig-59)

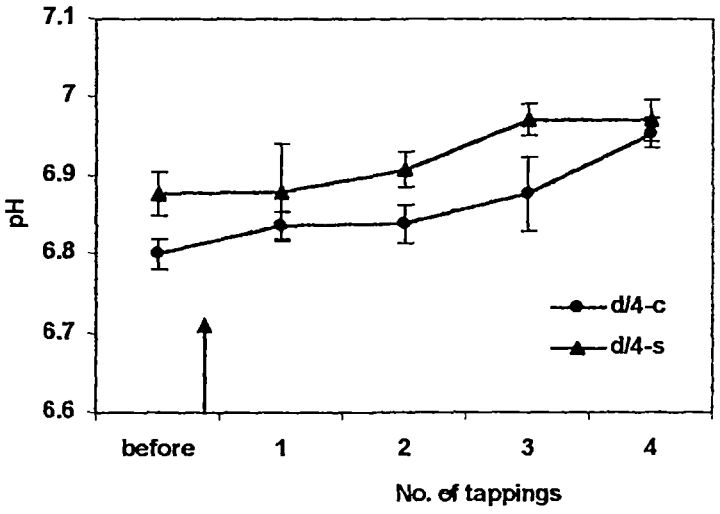


Fig- 57. Effect of stimulation on pH (C-serum) in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ±SE). d/4-c: control d/4-s: stimulated

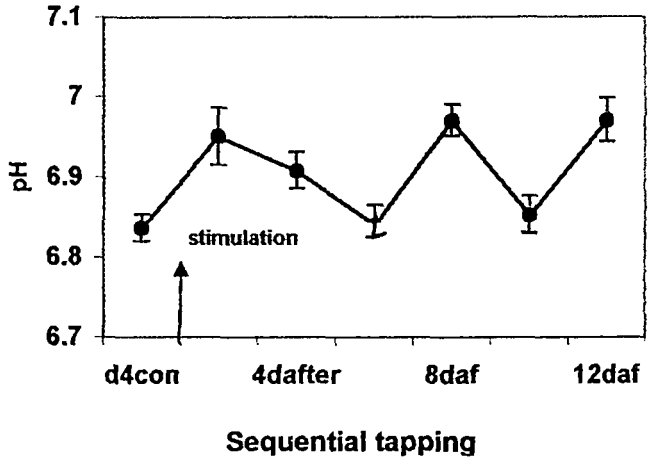


Fig- 58 Effect of intensive tapping after stimulation on pH (C-serum in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ±SE)

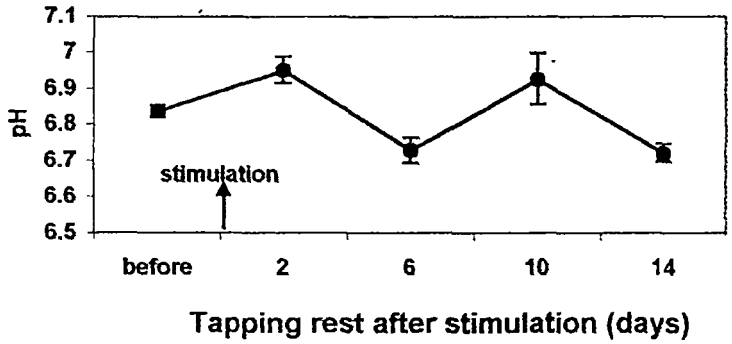


Fig-59 Effect of tapping rest after stimulation on pH (C-serum) in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

3.5. 14. pH of B-serum

Control and stimulated

A general increase in pH of B- serum was observed in stimulated trees up to third tapping. Then a decrease in pH was observed (Fig-60).

Stimulation and intensive tapping

Maximum B-serum pH was observed on second intensive tapping after stimulation and then decreases (Fig-61).

Stimulation and tapping rest

pH of B-serum showed a decrease in trees with 2 day rest and an increase was observed in 6 , 10, and 14 day rest trees. (Fig-62).

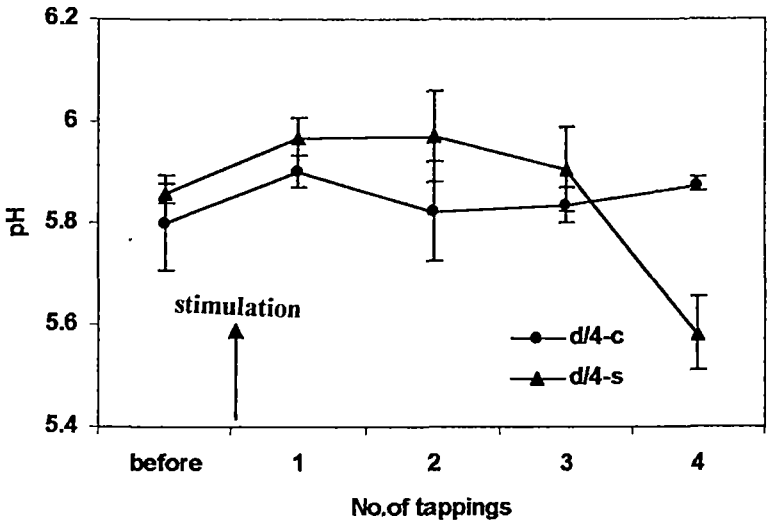


Fig- 60. Effect of stimulation on pH of B-serum in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ±SE)
d/4-c: control d/4-s: stimulated

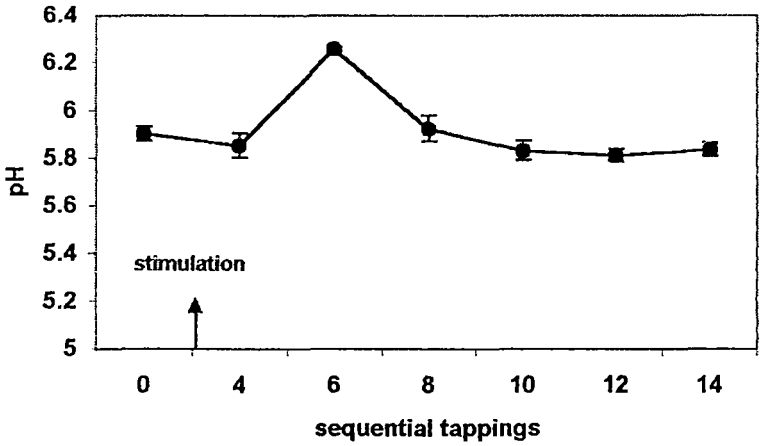


Fig- 61. Effect of intensive tapping on b-serum pH in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

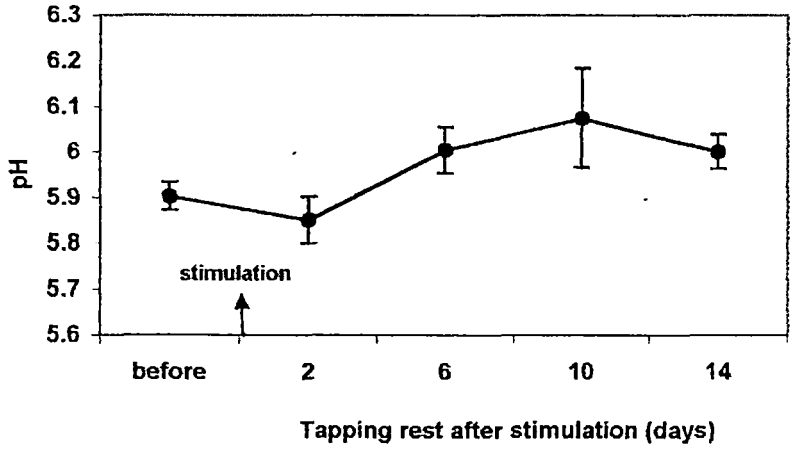


Fig- 62. Effect of tapping rest after stimulation on b-serum pH in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees± SE)

3.5.15. ATP ase activity of lutoids

Control and stimulated

ATPase activity in lutoids increased on all tapping days after stimulation (Fig-63).

Stimulation and intensive tapping

Maximum ATPase activity was observed on third intensive tapping. Then a decreased in the activity was observed on subsequent tappings (Fig-64).

Stimulation and tapping rest

A general increase in ATPase activity was observed in trees with different periods of tapping rest after stimulation. Maximum activity was observed on sixth day rested trees (Fig-65)

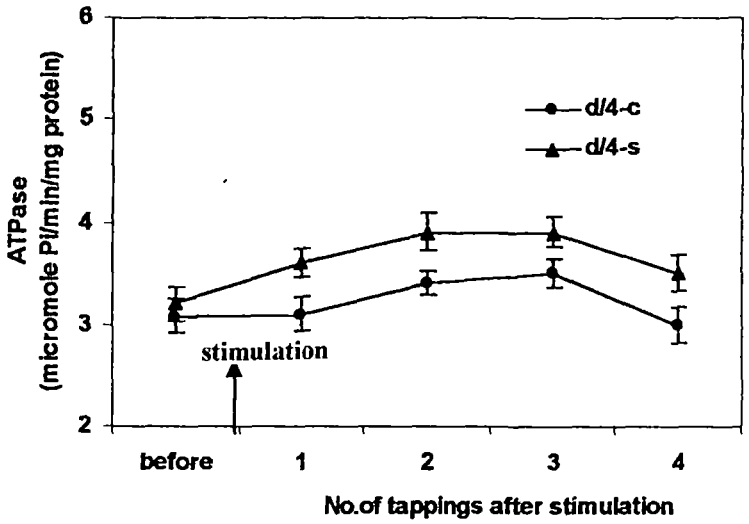


Fig-63.Effect of stimulation on ATPase activity of lutoids of clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ±SE)
d/4-c: control d/4-s: stimulated

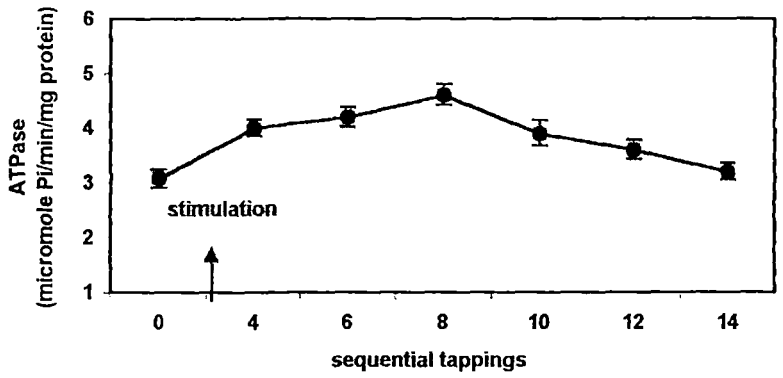


Fig-64. Effect of intensive tapping after stimulation on ATPase activity in clone RRII 105 under 1/2 S d/4 6d/7 tapping system (mean of six trees ± SE)

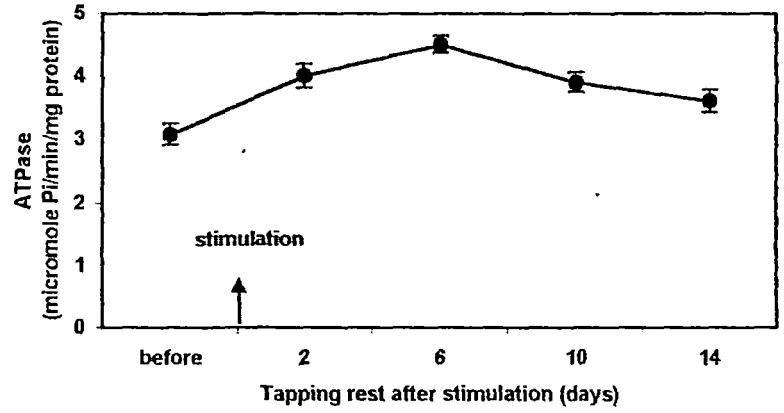


Fig-65 Effect of tapping rest after stimulation on ATPase activity in clone RRII 105 under 1/2 S d/4 6d/7 tapping system (mean of six trees ± SE)

3.5.16. Bursting Index

Control and stimulated

A decreased BI was observed in stimulated trees after the first tapping when compared to control trees (Fig-66).

Stimulation and intensive tapping

The bursting index showed a decrease up to third intensive tapping and then increases (Fig-67).

Stimulation and tapping rest

The bursting index showed a decrease in trees with 2 day and 6 day rested trees (minimum) and showed an increase for 10 day and 14 day rested trees. (Fig-68)

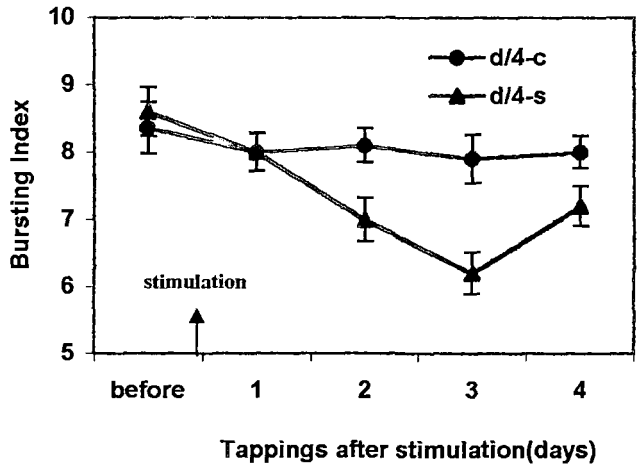


Fig- 66. Effect of stimulation on bursting index in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ± SE)
d/4-c: control d/4-s: stimulated

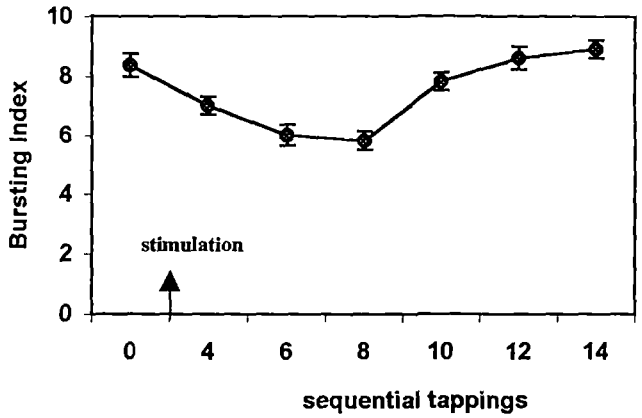


Fig- 67 Effect of intensive tapping after stimulation on bursting index in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ±SE)

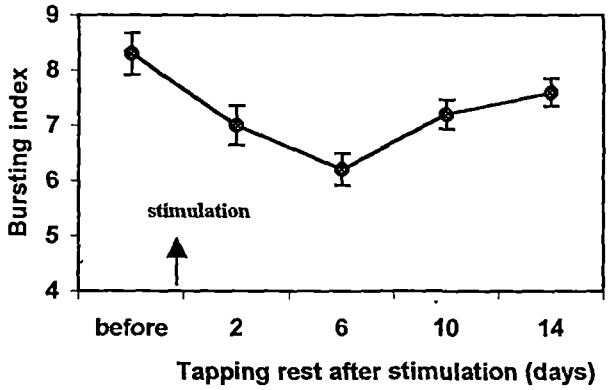


Fig-68 Effect of tapping rest after stimulation on bursting index in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ±SE)

3.5.17. Quantity of bottom fraction in latex

Control and stimulated

A high bottom fraction was observed on all tapping days in stimulated trees when compared to control trees (Fig-69).

Stimulation and intensive tapping

A general increase in bottom fraction was observed on all intensive tapping days after stimulation. Maximum increase was on fourth intensive tapping (Fig-70).

Stimulation and tapping rest

An increase in bottom fraction was observed in trees with 2 day rest and 6 day rest(maximum). Trees with 10 & 14 day rest showed a decrease.(Fig-71)

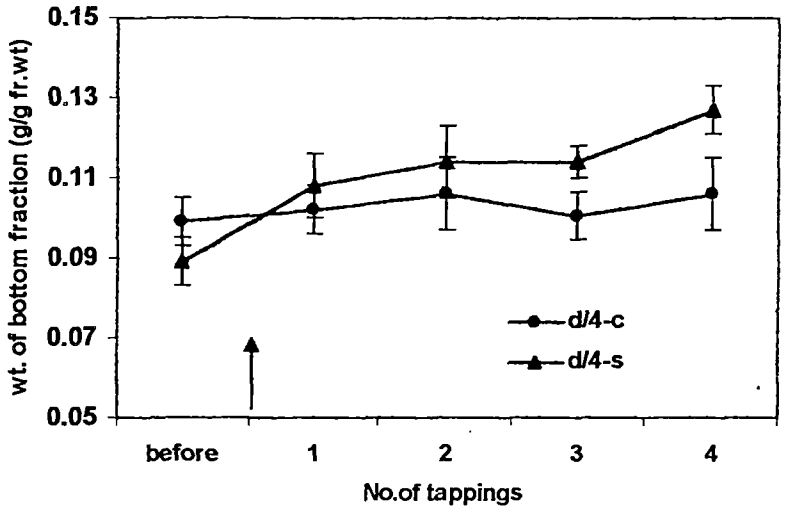


Fig- 69. Effect of stimulation on bottom fraction in latex in clone RRII 105 under ½ Sd/4 6d/7 tapping system (mean of six trees ±SE) d/4-c: control d/4-s-stimulated

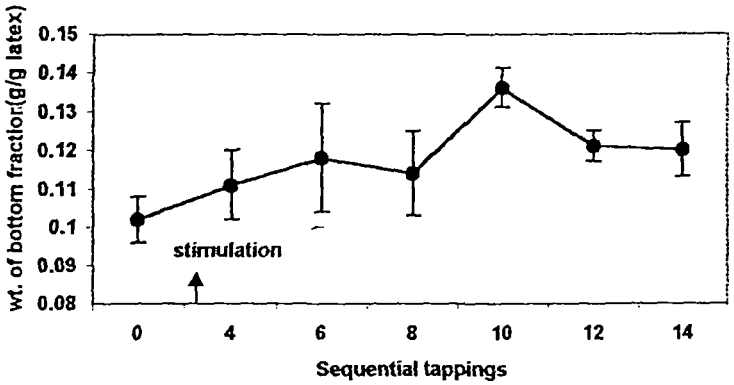


Fig-70. Effect of intensive tapping after stimulation on weight of bottom fraction in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

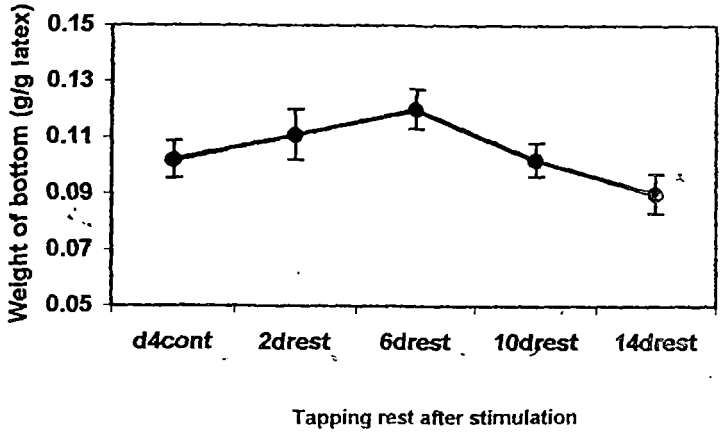


Fig- 71. Effect of tapping rest after stimulation on weight of bottom fraction in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

3.5.18. Volume of C- serum in latex

Control and stimulated

Volume of C-serum was less than that of control trees on all tapping days (Fig-72).

Stimulation and intensive tapping

Volume of C-serum decreases on all intensive tapping days after stimulation (Fig-73).

Stimulation and tapping rest

Volume of C- serum was decreased in trees with different periods of tapping rest after stimulation (Fig-74).

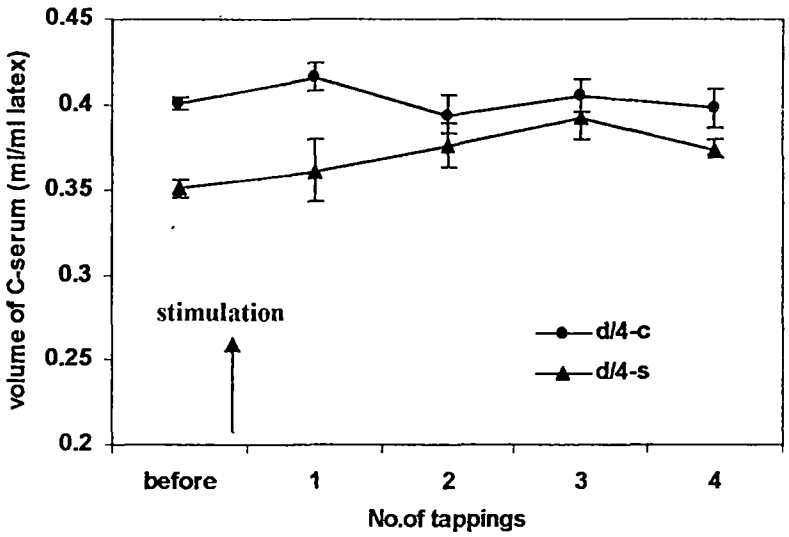


Fig- 72. Effect of stimulation on volume of C- serum in clone RRII 105 under ½ S d/4 6d/7 tapping system (mean of six trees ±SE)
d/4-c: control d/4-s: stimulated

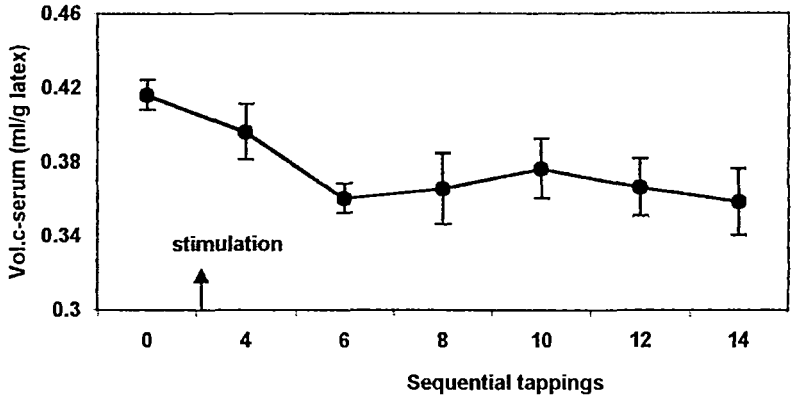


Fig-73. Effect of intensivetapping after stimulation on volume of C- serum in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

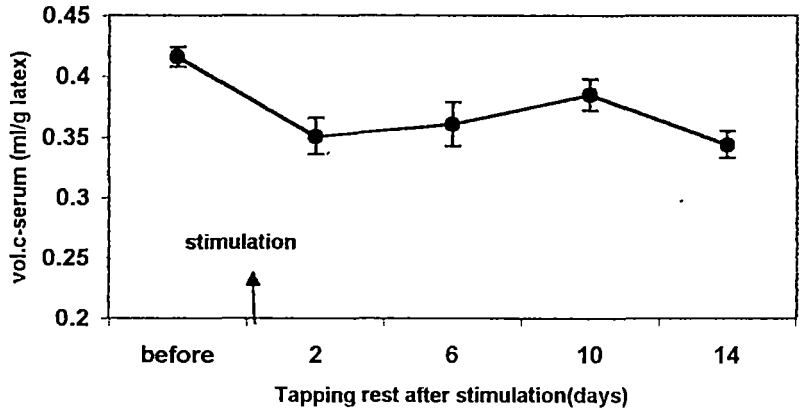


Fig-74. Effect of tapping rest after stimulation on volume of C- serum in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

3.5.19. Weight of cream in latex

Control and stimulated

No variation was observed in the weight of cream in stimulated trees when compared to control trees (Fig-75).

Stimulation and intensive tapping

A general decrease in the weight of cream up to fifth intensive tapping and then increases (Fig-76).

Stimulation and tapping rest

Weight of cream in latex was increased up to six day rest and then no consistent variation was observed in trees with 10&14 day rest (Fig-77).

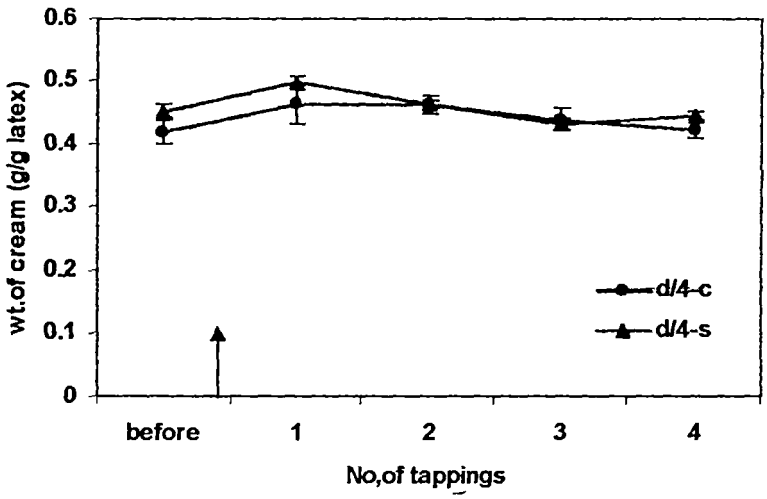


Fig-75. Effect of stimulation on wt. of cream in clone RRII 105under ½ S d/4 6d/7 tapping system (mean of six trees ±SE). d/4-c: control d/4-s: stimulated

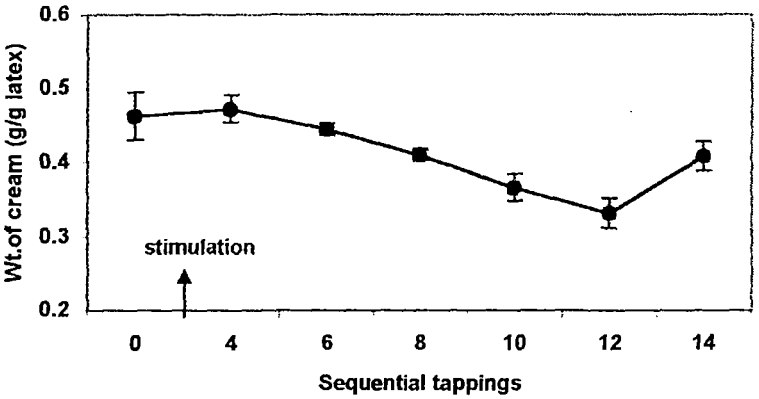


Fig- 76. Effect of intensive tapping after stimulation on wt.of cream in clone RRII *105* under 1/2 Sd/4 6d/7 tapping system (mean of six trees ±SE)

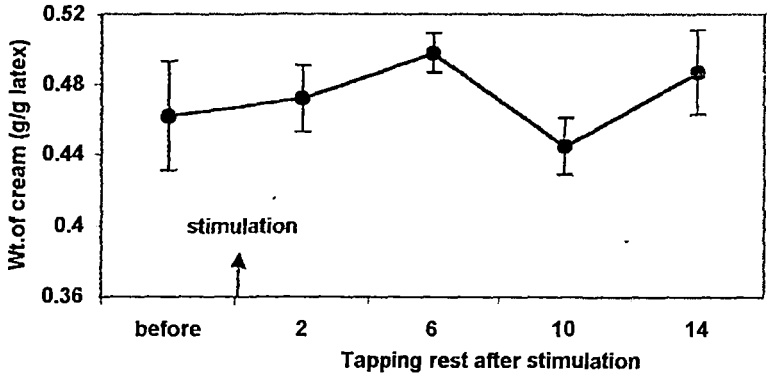


Fig-77. Effect of tapping rest after stimulation on wt. of cream in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ± SE)

3.5.20. N- acetyl glucosaminidase in B- serum

Control and stimulated

A decrease in activity of N-acetyl glucosaminidase was observed in stimulated trees on all tapping days (Fig-78)

Stimulation and intensive tapping

Activity decreases up to fourth intensive tapping after stimulation and then increases (Fig- 79)

Stimulation and tapping rest

The activity showed no variation up to six day rest trees and then decreases on tenth day and increases on 14 day rested trees (Fig-80)

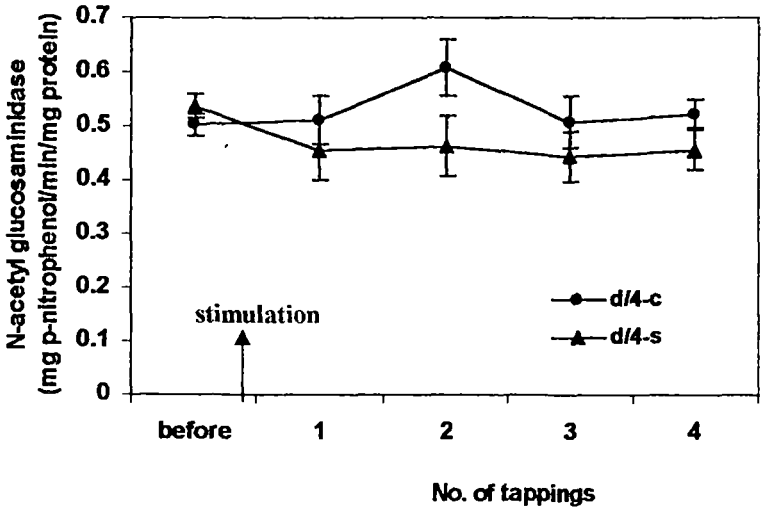


Fig 78. Effect of stimulation on N–acetyl glucosaminidase in B-serum in clone RRII 105 under 1/2S d/4 6d/7 system of tapping (mean of six trees ±SE).d/4-c:control d/4-s: stimulated

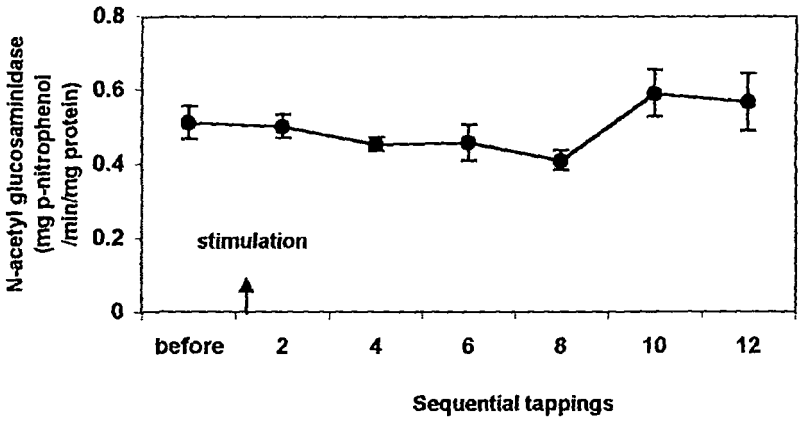


Fig-79 Effect of intensive tapping after stimulation on b-serum N- acetyl glucosaminidase sucrose synthase in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ±SE)

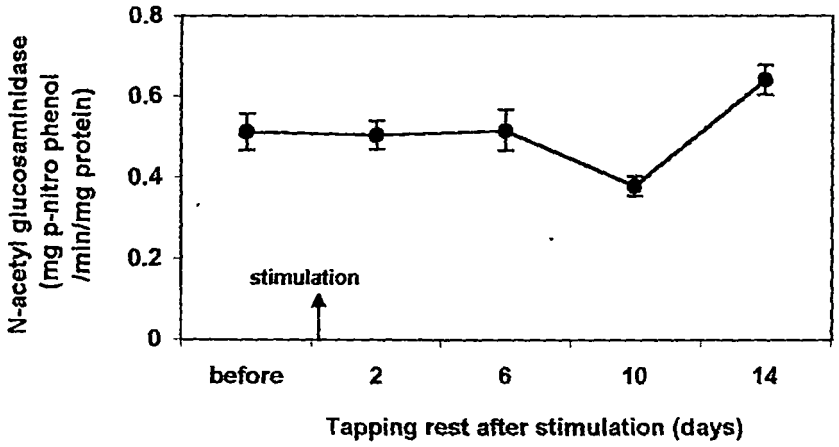


Fig-80 Effect of tapping rest after stimulation on b-serum N- acetyl glucosaminidase sucrose synthase in clone RRII 105 under 1/2 Sd/4 6d/7 tapping system (mean of six trees ±SE)

3.5.21. Glycolipids in latex

Control and stimulated

No change in glucolipids was observed between control and stimulated trees (Fig-81).

Stimulation and intensive tapping

A maximum increase was observed on third intensive tapping after stimulation and then decreases in subsequent tappings (Fig-82).

Stimulation and tapping rest

A general decrease in glycolipids were observed from 6day rested trees after stimulation . Glycolipids in latex was found to be very low in trees with 10 days and 14 days rest. (Fig-83)

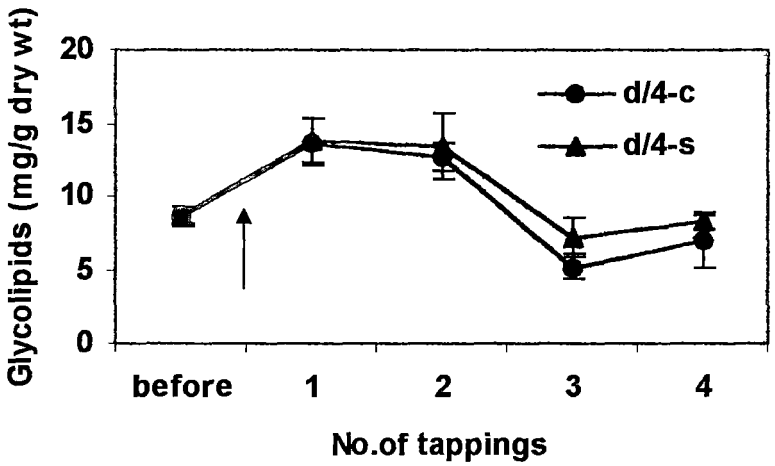


Fig- 81. Effect of stimulation on glycolipids in latex in clone RRII 105 under 1/2S d/2 6d/7 system of tapping. Mean of six trees.d/4-c:control d/4-s stimulated

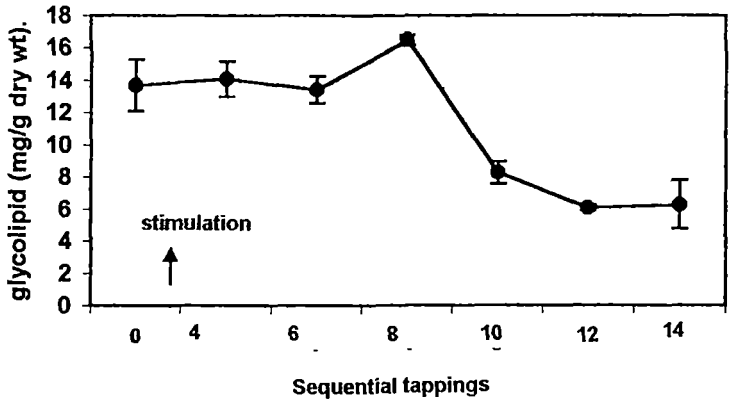


Fig-82. Effect of intensive tapping after stimulation on latex glycolipids in clone RRII 105 under 1/2 Sd/46d/7 tapping system (mean of six trees ±SE)

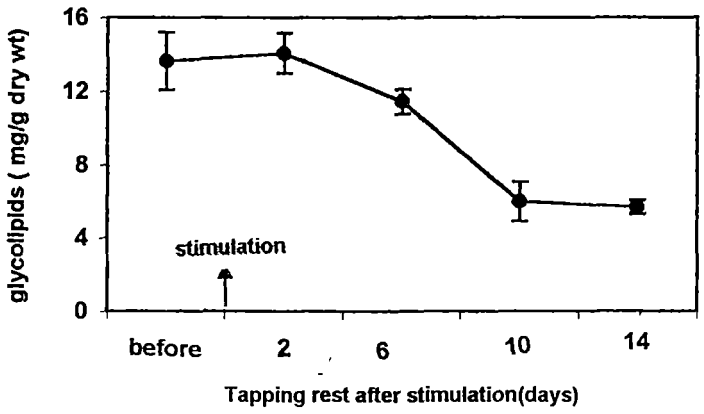


Fig-83. Effect of tapping rest after stimulation on latex glycolipids in clone RRII 105 under 1/2 S d/4 6d/7 tapping system (mean of six trees ±SE)

3.5. 22. Phospholipids

Control and stimulated

Phospholipid content in control and stimulated trees did not show any variation (Fig-84)

Stimulation and intensive tapping

Maximum phospholipids was observed on third intensive tapping and then decreases on subsequent tappings (Fig-85)

Stimulation and tapping rest

Phospholipid content latex showed a gradual decrease in 2,6,10 and 14 day rested trees and a minimum phospholipids was observed in trees with 10 and 14 days rest.(Fig-86)

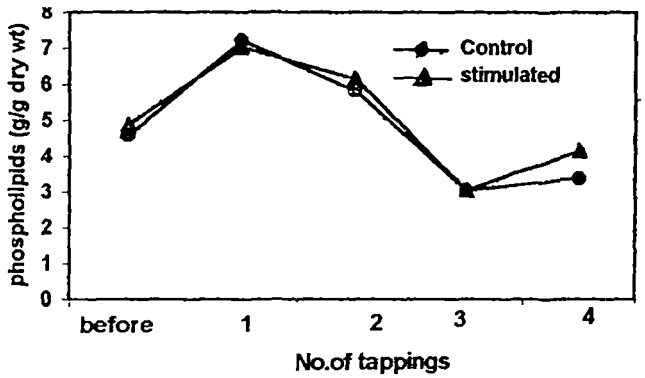


Fig-84. Effect of stimulation on phospholipids in clone RRII 105 under 1/2 S d/4 6d/7 tapping system (mean of six trees ±SE)

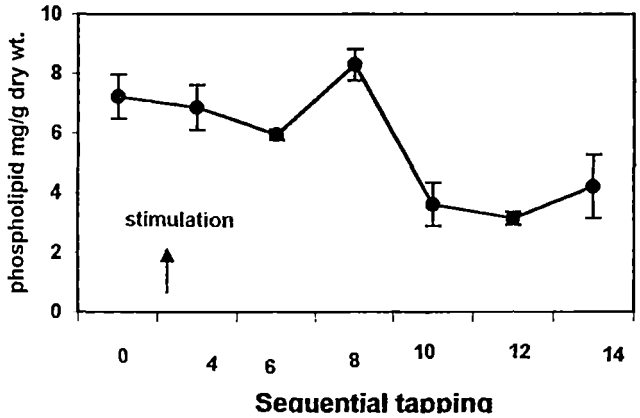


Fig-85 Effect of intensive tapping after stimulation on phosphospholipids in clone RRII 105 in clone RRII 105 under 1/2S d/4 6d/7 tapping system

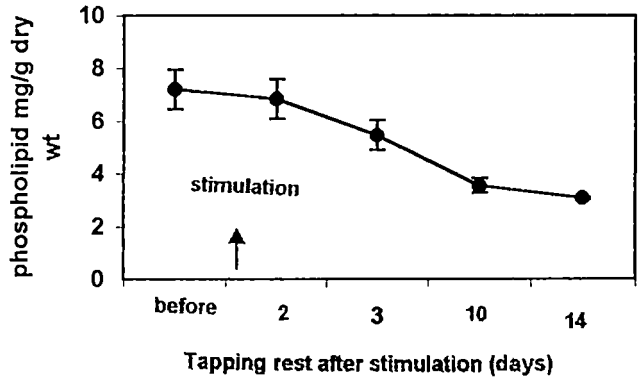


Fig- 86. Effect of tapping rest after stimulation on phospholipids in clone RRII 105 under 1/2 S d/4 6d/7tapping system (mean of six trees ± SE)

3.6. Biochemical parameters related to carbohydrate metabolism in trees affected by TPD

It was found that two trees under 1/2S d/2 6d/7 system of tapping became partially dry (35%)after two years of opening. Basic parameters related to carbohydrate metabolism were done in the latex of these trees. Mean of ten healthy trees was taken as control. When the trees became fully dry bark samples were used for biochemical analysis.

The results are presented in this chapter. Total volume prior to the appearance of partial dryness showed no change. After that the volume decreases in both trees when compared to healthy trees. (Fig. 87) . Just after the onset of TPD both trees showed a decrease in sucrose in C-serum and latex. (Fig. 88,89). Then a sudden increase was observed after six months. Inorganic phosphorus in latex decreases after the development of TPD indicate the low metabolic rate of laticiferous system. (Fig.90)

Bark analysis showed a high sucrose synthase activity in both TPD affected trees(Fig.94). Inorganic phosphorus in the bark of TPD tree-1 was lower and tree-2 was higher than that of control (Fig. 91). Sucrose and invertase activity of tree-1 was higher and tree-2 was lower than that of control.(Fig. 92,93).

Tree-1 showed a higher sucrose synthase activity, invertase activity and sucrose in the bark when compared to tree-2..Pi of tree-2 was lower than tree-2.

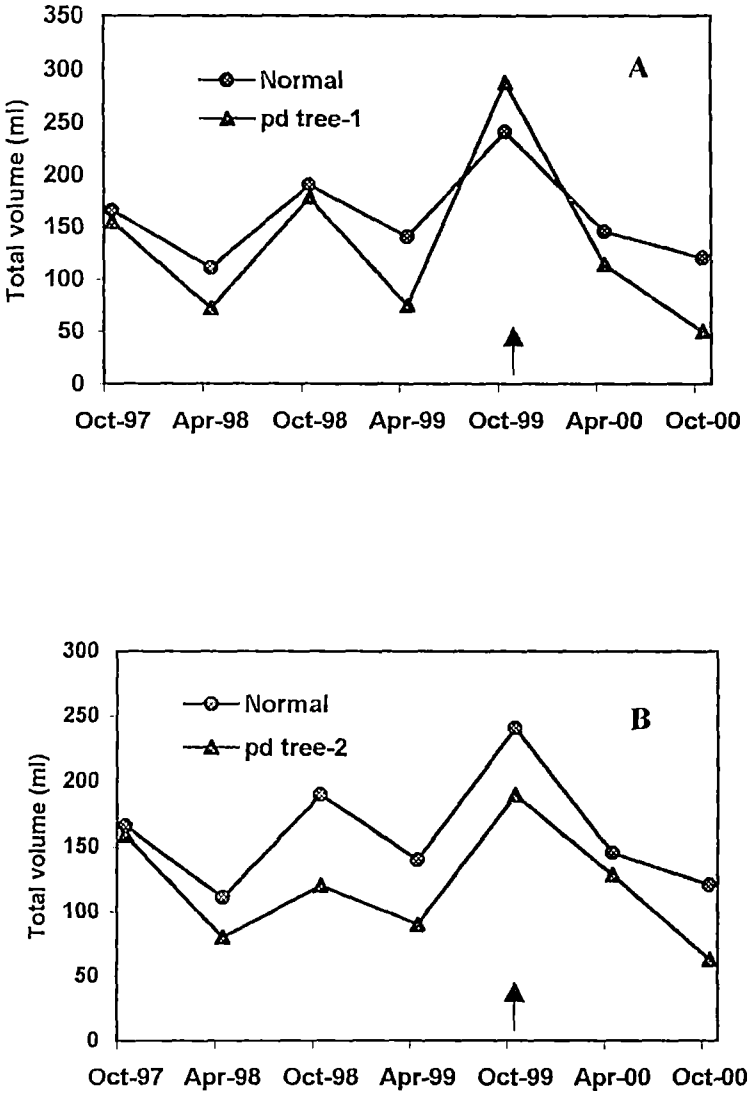


Fig- 37 Latex yield prior to the onset of partial dryness and after the incidence. Normal- mean values of ten healthy trees. Arrow shows the appearance of partial dryness. A- partial dry (pd) tree-1 and B-pd tree-2

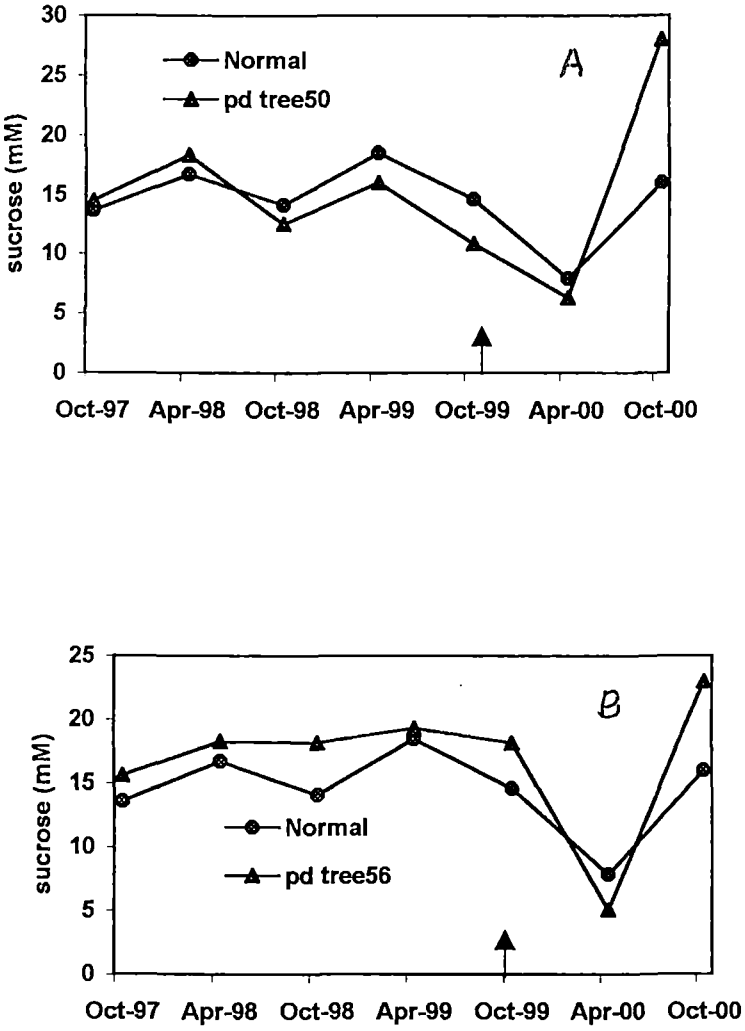
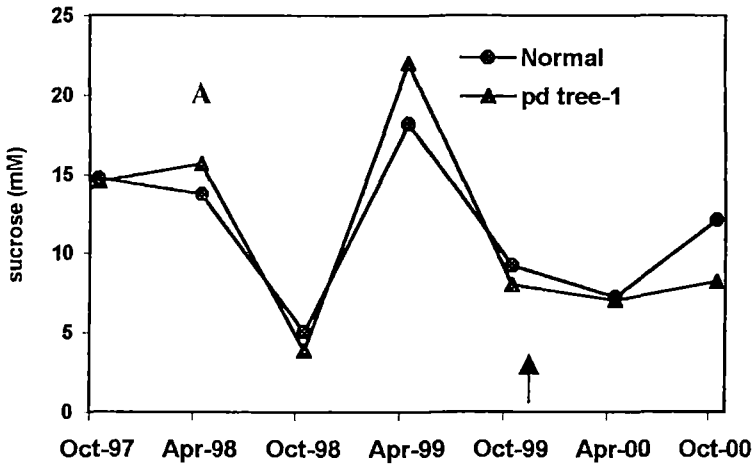


Fig-88 Sucrose content in C-serum prior to the onset of partial dryness and after the incidence. Normal- mean values of ten healthy trees. Arrow shows the appearance of partial dryness. A- partial dry (pd) tree-1 and B-pd tree-2



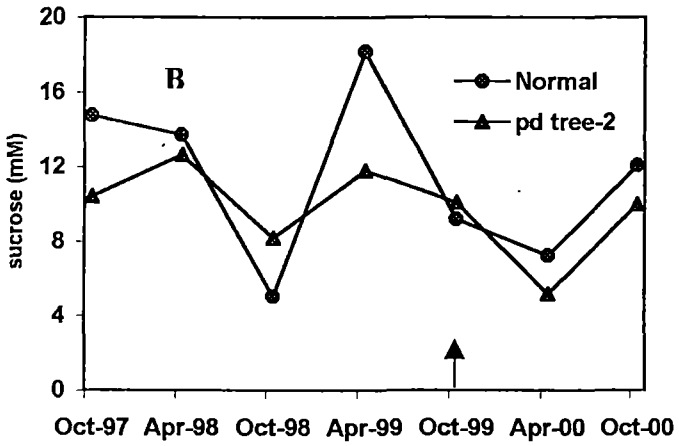
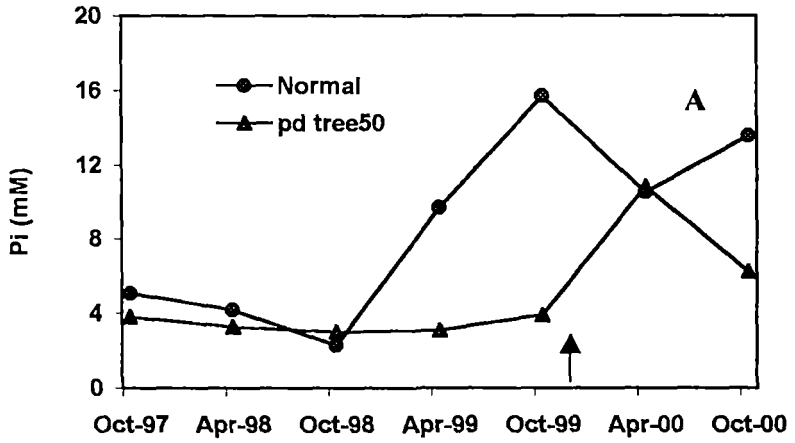


Fig-89 Sucrose content in latex prior to the onset of partial dryness and after the incidence. Normal- mean values of ten healthy trees. Arrow shows the appearance of partial dryness. A- partial dry (pd) tree-1 and B-pd tree-2



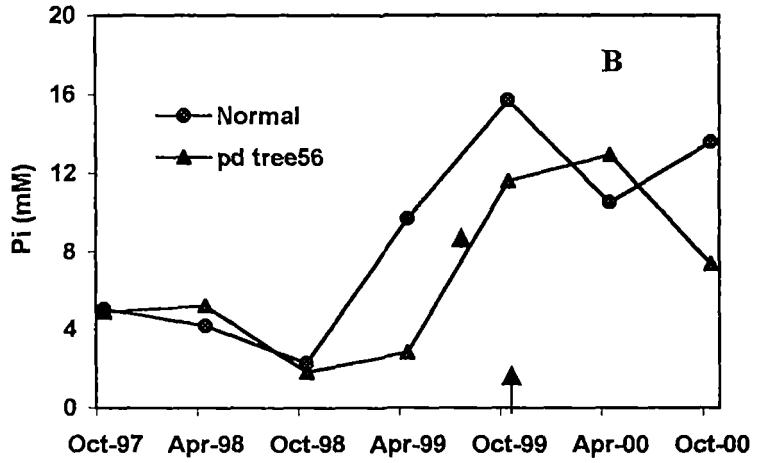
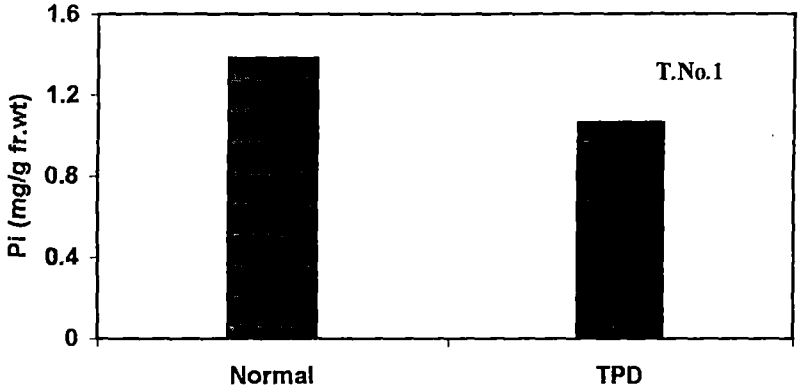


Fig- 90 Phosphorus content in latex prior to the onset of partial dryness and after the incidence. Normal- mean values of ten healthy trees. Arrow shows the appearance of partial dryness. A- partial dry (pd) tree-1 and B-pd tree-2



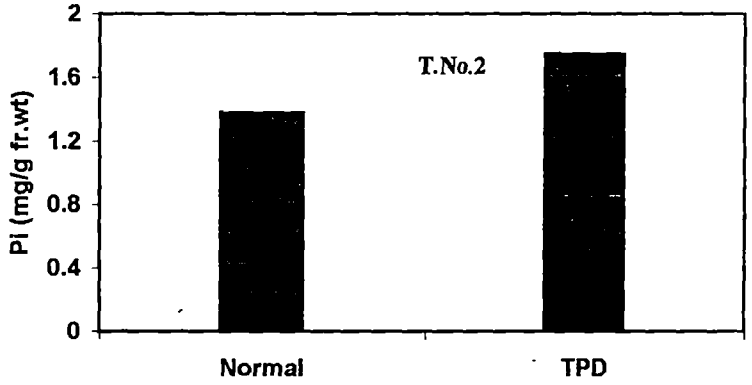
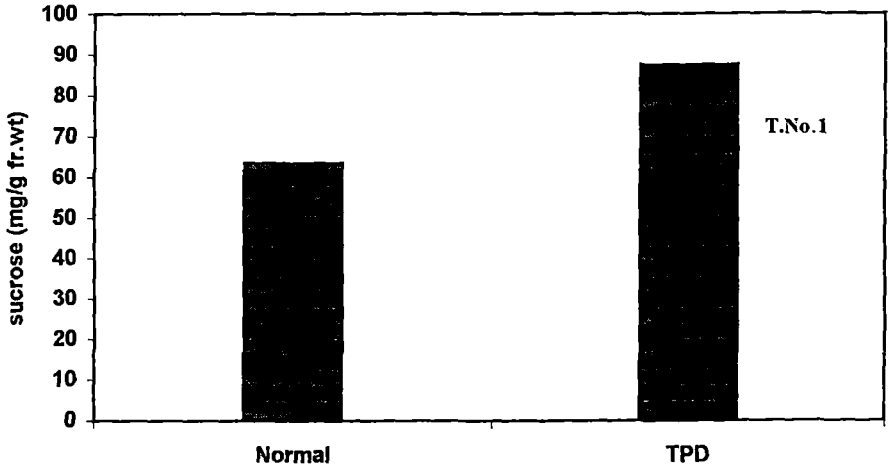


Fig- 9| Phosphorus content in the bark of normal and TPD trees (1&2)



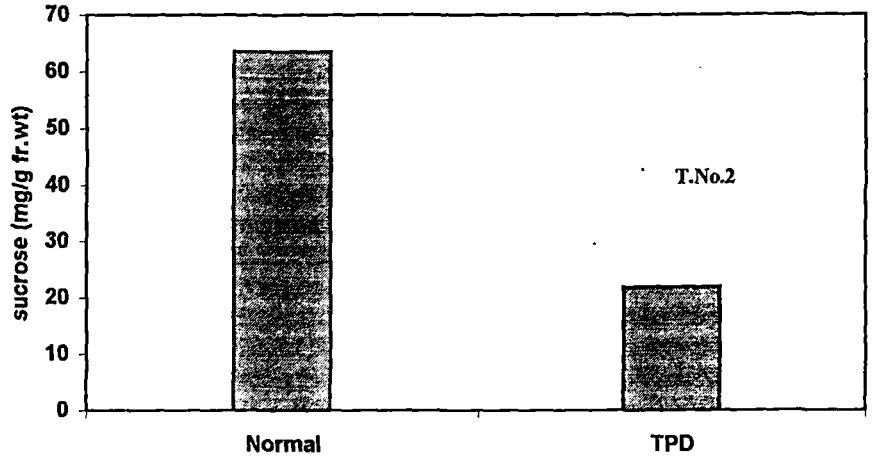


Fig- 12Sucrose content in the bark of normal and TPD trees (1&2)

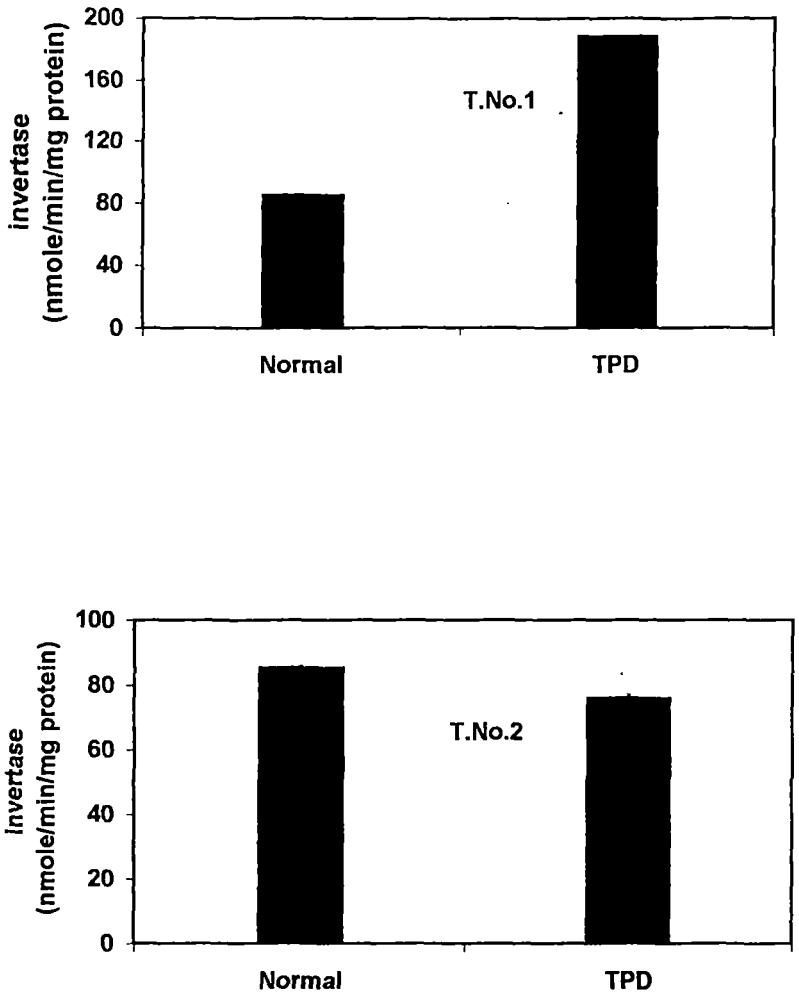
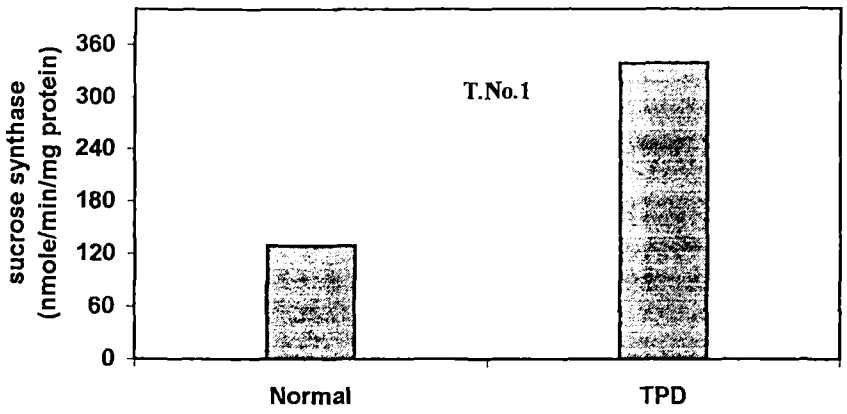


Fig-93 Invertase activity in the bark of normal and TPD trees (1&2)



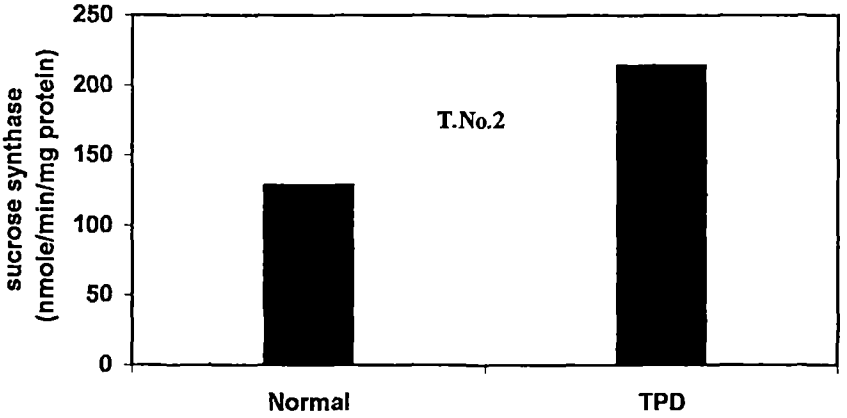


Fig-94 Sucrose synthase activity in the bark of normal and TPD trees (1&2)

CHAPTER 4

DISCUSSION

4.1. Seasonal variations in yield and other physiological parameters related to yield.

A reduction in dry rubber yield and dry rubber content was observed during summer. (Table-1, Figure 1&2). An increase in sucrose during summer (Table1, Figure3) showed a low metabolic utilization of sucrose and low productivity. Sugar content of latex is the result of loading to laticiferous tissue and its use at cell level. A highly significant positive correlation between yield and sucrose content of latex was established by Jacob *et al*, (1986); Eschbach *et al*, (1984); Lacrotte (1991); Auzac & Pujarniscle (1961) and Tupy (1973).

Latex Pi increased during peak season (Table-1 & Figure-5) indicates a high-energy metabolism in latex. This result is in confirmation with the reports of Jacob *et al*, (1989); Prevot *et al.*, (1986) and Thomas *et al.*, (2000). Relative slow functioning of the laticiferous system was indicated by low Pi in latex. A positive correlation between Pi in C-serum and yield (figure-7) was an indication of active metabolism leading to high biosynthesis.

A high thiol content in latex was observed (Table-1, figure-4) during stress period. During summer season more toxic oxygen is produced and more thiol is needed to detoxify these active oxygen species. Sucrose was also high during this period. Sucrose is both the source of energy and isoprene synthesis. Most of the energy is utilized for thiol formation (Dey *et al*, 1995; Chrestin, 1985) during this period where the latex production was minimum.

The bursting index of lutoids showed an increase during stress when compared to peak season (Table-3). A high bursting index during summer indicates low lutoid stability and more degradation of membranes. An inverse relationship between yield and BI was also observed

(Figure-6). This result is in conformation with Ribaillier, (1968); Jacob *et al.*, (1986) and Dey *et al.*, (1995).

Latex and C-serum pH showed a decrease during stress period (Table-3). There was no significant difference in B-serum pH. (Table-3). Latex and C-serum pH became more alkaline during peak season indicates an increase in catabolism of sugars and latex regeneration in laticiferous cells by activating several pH dependent enzymes (Jacob (1970) and Lacrotte *et al.*, (1985). Tupy (1969) also reported the same observation. Alkalinisation of the medium activated glycolysis (Jacob (1970); Tupy (1969) and Tupy, (1973) particularly through invertase (Jacob 1970; Primot, 1976). During summer season pH was low and it is most unfavorable for an active metabolism. The low C-serum pH observed during summer season (Table-3) reflects high porosity of lutoid particles, which may lead to an acidification of the cytoplasm and reduced isoprene synthesis (Coupe (1977); Jacob (1970); Jacob *et al.*, (1983,1977) and Tupy, (1969). Regulation of pH (H^+ concentration in B-serum and cytosol) was very important in rubber biosynthesis. A significantly high ATPase in lutoid, during peak season indicates an activation of proton pumping resulted in greater pH of the cytosol. This alkaline pH was essential for high production. ATPase was involved in pH regulation by regulating H^+ ion concentration between two compartments.

An increase in C-serum PPi and a decrease in C-serum pyrophosphatase were observed during summer (Table-4). Isoprene anabolism releases PPi each time that a molecule of IPP attached to polymer chain and lengthens it (Lynen, 1969). Production of PPi is very rapid and the enzymatic activities capable of hydrolyzing it must be efficient to avoid accumulation of PPi so that the deleterious effect of PPi can be reduced (Jacob *et al*, 1988a). During summer season C-serum pyrophosphatase was low (Table-4) and PPi was accumulated. A negative correlation between yield and C- serum PPi was observed (Figure- 8).

Increased phospholipids in bottom fraction (Table-5) indicate a high stability o lutoid membrane. There exist a positive correlation between phospholipids of bottom fraction anc yield (Figure- 9). During stress period more toxic oxygen is produced and may cause cellula decompartmentalisation following the peroxidative degradation of membrane phospholipid (Chrestin, 1985 and Jacob *et al.*, 1984). Usha Nair *et al*, (1993) reported a greater stability o lutoid was associated with higher content of phospholipids in the lutoid membrane. Sherief and Sethuraj, (1978) observed a negative correlation between phospholipids in bottom fraction and bursting index. Premakumari *et al.*, (1980) reported a decrease in phospholipids content and decreased yield during summer.

Phospholipid content of bottom fraction was an indicator of membrane integrity, which resulted in maintaining ionic balance and an increased yield.

An increase in glycocongugates (fucose and hexose) (Table- 5) in B-serum during peak season indicated that they might have some role in lutoid stability.

4.2. Seasonal variation carbohydrates and related enzymes associated with latex production

Yield output of rubber tree varies with season. (Figure-1). Yield fluctuation were found to be related to the moisture availability in the soil. Highest temperature was found to retard latex flow and reduce yield. During the peak yielding period of November there is adequate mean temperature (26-30^{0}C), soil moisture and sunshine. During March climatic conditions are not congenial for good productivity. This may be due to combined effect of high temperature and soil moisture stress in addition to the effects of annual defoliation and refoliation. Yield depression associated with defoliation, refoliation and summer is of particular importance. Yield was minimum during summer and maximum during peak season (September-November) (Table-1). Depressive effect of defoliation and leafing on production was observed by Evers, *et al* (1960),

Van de Sype (1985). Trees use in priority its organic and mineral reserves for the reconstitution of its leaves and for the fruit growth at the expense of latex producing function. Seasonal yield trend depends on the leaf canopy and climatic conditions. When defoliation- refoliation period was considered, yield was minimum during defoliation (Table-6). Tupy observed a rise in yield during defoliation and a fall in production during leafing. Leaf fall and leafing, which orient the metabolism of the tree essentially towards the replacement of the photosynthetic system, probably hinders the other synthetic activities. Shorter sunshine duration results in low photosynthetic efficiency. Seasonal variation in the level of latex sucrose is related to the variation in sunshine duration and a rise in sucrose level occurs during the process of refoliation. In this experiment latex sucrose during refoliation was lower than that during defoliation. This result was in contradiction with the result of Tupy (1973,1969). Low sucrose during refoliation indicates less loading of sucrose to laticifers. Latex sucrose during summer was more than that during peak season (Table-6). There is a significant relationship between rubber tree yield and latex sucrose content (Lacrotte, 1991). Low sucrose in latex indicates high metabolic conversion of sucrose to rubber leading to high yield. Trend of C-serum sucrose was similar to latex sucrose. Invertase activity was low during defoliation when compared to refoliation period (Table-6). Low yield during defoliation was due to the low activity of invertase. Maximum invertase activity was observed during peak yielding period followed by refoliation, defoliation and summer (Table-6). Yeang *et al.*, (1984) observed a very low invertase activity during wintering month. A positive correlation between yield and C-serum invertase was observed. Rate of carbohydrate metabolism in latex is determined by the invertase catalysed hydrolysis of sucrose. Sucrose synthase during summer was maximum (Table-6). During summer, thiol, an activator of sucrose synthase was

high. This results in an increase of sucrose. High sucrose may indicate less utilization of this through glycolysis. So yield during summer was minimum.

C-serum sucrose was higher during summer than that during peak season (Table- 6). This indicates the accumulation of sucrose in C-serum during summer period where the yield was minimum. No significant difference was observed between defoliation and refoliation period. (Table-6)

4.3.Clonal variations in parameters associated with latex production

Rubber content tends to be lower in clones with higher yield (Table-7) because more latex is lost every time when it is tapped. High sucrose content in latex of low yielding clones (Table-7) indicate good loading capacity of laticiferous cells followed by active metabolism or less utilization of sucrose. Low sucrose level in the latex of high yielders was an indication of enhanced metabolic utilization of sucrose. This result is in confirmation with Tupy & Primot (1976). Tupy (1973) observed clonal difference in latex pH and sucrose level.

Invertase has an important role in controlling the overall metabolic activity by controlling the utilization of sucrose. High pH of C-serum for high yielding clones (Table-8) is an indication of alkalanisation of C-serum of these clones. A high alkalinity is favourable for the activity of invertase whose pH optimum was on alkaline side. High activity of C-serum invertase was observed in high yielding clones when compared with low yielding clones (Table-7). So the sucrose conversion to rubber takes place more quickly in high yielding clones (Conduru Neto *et al.*, 1984; Tupy & Primot 1982). Yeang *et al.*, (1984) reported inter and intra clonal differences in total invertase activity among trees in the same field and system of tapping.

High yielders have high latex Pi (Table-9) indicating the high metabolic rate of these clones. High yielding clones have high Pi in C-serum so the metabolic conversion of sucrose to polyisoprene takes place at a faster rate in these clones leading to high yield. High values of lutoidic ATPase (Table- 8) showed a high influx of protons into the lutoid serum resulting in more alkalanisation of C-serum and hence more yield. ATPases have a role in regulating the hydrogen ion concentration in C-serum and B-serum. High activity of lutoid ATPase increases pumping of more H^+ to lutoid resulted in an increased alkalinity of cytosol and rubber biosynthesis. C-serum PPase was high in high yielding clones (Table-9). High PPase indicate greater production of Pi in latex, which in turn was an indicator of high metabolic conversion.

High activity of N-.acetyl glucosaminidase in the B-serum delays plugging in high yielders (Table-9). This enzyme delays coagulation by blocking the binding site on Hevein or by breaking the glycosidic linkage between N- acetyl glucosamine and 22 KD protein on rubber particle (Gidrol *et al.*, (1994).

4.4. Parameters related to carbohydrate metabolism and ionic balance under d/2 and d/4 system.

Yield was higher in d/2 tapped trees when compared with d/4 tapped trees (Table-10). High tapping frequency of d/2 has significantly higher C-serum invertase than low tapping frequency of d/4 (Table-10). High level of invertase in d/2-tapped trees indicates more conversion of sucrose to rubber. There was no significant difference in latex sucrose or sucrose in C-serum between high and low frequency tapping system (Table-10). Koshy (1997) reported that sucrose availability in latex vessel decreases with an increased intensity of exploitation. High frequency tapping system showed a low sucrose synthase activity in C-serum when compared to low frequency tapping system (Table-10). C-serum in high frequency tapped trees was more alkaline

when compared to low frequency tapped trees. (Table-12). As the C-serum become more alkaline, activity of invertase increases thereby accelerates the catabolism of sucrose and more latex production Do Kim Thanh (1996) observed that pH was not affected by tapping treatments.

High tapping frequency of d/2 produced significantly higher bottom fraction than low tapping frequency of d/4. (Table-13) Bottom fraction (lutoids) has an important role in latex coagulation (Pakianathan *et al.,* 1966; Pujarniscle 1968; Southern. and Edwin1968 and Chrestin (1984). The presence of high percentage of undamaged lutoids in the latex is a reflection of greater latex stability. This result is in confirmation with Koshy (1997).

Trees tapped on high frequency (d/2) have significantly higher latex glycolipids (Table-14). High tapping frequency produced significantly higher bottom phospholipids and latex phospholipids (Table-14). Amount of phospholipids is an indication of integrity of membrane. High amount of bottom phospholipids is a reflection of greater lutoid stability (Sheriff, and Sethuraj 1978). Usha Nair *et al.,* (1995) also reported this. High tapping frequency of d/2 produced significantly higher C-serum inorganic phosphorus than lower tapping frequency d/4 (Table-11). Inorganic phosphorus reflects the energy metabolism in laticiferous system. Jacob.*et al.,* (1986). High content of Pi in d/2 tapped trees reflects the active metabolism in laticiferous cells of these trees resulted in high latex production.

N- acetyl glucosaminidase was high in d/2-tapped trees indicating delayed coagulation. High activity of N-acetyl glucosaminidase and higher bottom fraction in d/2-tapped trees may indicate greater integrity of membrane of lutoid. ATPase activity was not significantly higher in d/2-tapped trees but pH of C-serum was significantly higher in d/2-tapped trees.

4.5. Effect of stimulation on different biochemical parameters related to carbohydrate metabolism and pH regulation in clone RRII 105 under d/4 system of tapping.

Control and stimulated trees

Stimulated trees have higher volume than control trees on all tapping days (Figure-21). Treatment of bark with ethrel efficiently stimulates the production of latex. (d' Auzac and Ribaillier (1969) and Tupy and Primot (1982).

Stimulated trees have higher latex sucrose than control after 3rd tapping (Figure-27). Unacorn silpi *et al* (2001) reported translocation of sucrose to sink intensified by stimulation. Stimulation increases the effect of sink (Jacob, 1984). Lacrotte *et al.,* (1985) reported supply of sugar is an active metabolism It can be intensified by stimulation which induces a sink effect at tapping panel (Lacrotte *et al.,* 1984; Tupy (1973) and Jacob (1983). Treatment of ethrel may be a way of removing the limiting aspects of sugar supply. Eschbach *et al* (1984) and Lacrotte (1991) reported an increase in yield arises from the activation of sucrose metabolism that affects both flow and regeneration..

There was an increase in invertase activity in the latex collected on 3rd tapping after stimulation (Figure- 33). Tupy (1970) and Conduru Neto *et al.*, (1984) reported that ethylene induces activation of invertase activity and the response of stimulation on invertase activity was somewhat delayed in some clones.

C-serum sucrose synthase showed an increase in 3rd d/4 tapping in stimulated trees (Figure- 36). pH of latex and C-serum was high in stimulated latex up to 3rd tapping (Figure-.54, 57). Tupy (1980,1988,1976,1982) claimed that the ethephon stimulation cause an increase in carbohydrate catabolism resulting from the fall off in synthetic activity of sucrose synthase. Latex

pH of stimulated latex was more than that of control on 1st, 2nd and 3rd tapping. But on 4th tappin stimulated latex has less pH (Figure- 54). Tupy (1964&1973) reported that stimulation increase the pH of latex, which in turn increases the catabolism of sugars and high yield.

Coupe *et al.*, (1977) and Brozozowska *et al.*, (1979) reported a high C-serum pH i stimulated trees when compared to control. Treatment of ethylene induces alkalinisation of C serum. B-serum pH decreases in stimulated latex after second tapping , when compared to contro (Figure-60).

Concentration of Pi in latex of stimulated trees was higher than that of control on al tapping days (Figure- 42). Pi is an indication of metabolic rate, glucidic catabolism, more synthesis of rubber. Pi increases on stimulation. Eschbach *et al.*, (1984), D'Auzac and Pujarniscle (1959) reported that stimulation activates laticifer metabolism and also increasing Pi content

There was a slight change in C-serum Pi after stimulation when compared with control (Figure- 45). Pyrophosphate produced during rubber biosynthesis was deleterious to the system. So it should be removed from the system by the rapid hydrolysis by C-serum pyrophosphatase enzyme. Stimulation increases the activity of pyrophosphatase (Figure- 51).

Thiol in the latex of stimulated trees was higher than that of control on all tapping days (Figure-39). Thiol is involved in protection of lutoid membrane by trapping harmful molecules. Do Kim Thanh (1996) reported that stimulation did not significantly affect the thiol content.

Stimulation resulted in an increase in weight of bottom fraction indicating greater stability of latex in stimulated trees (Figure-69). Stimulation effect on latex flow is mediated through delayed plugging in latex vessels Boatman (1966). The delayed plugging would mean less damaged lutoids with fewer plug formation. Thus stimulation provides facility for greater stability

on lutoid particles. The reduced extend of damaged bottom fraction resulted in better yield performance. The result is in confirmation with Do Kim Thanh (1996); Koshy (1997) and Pakianathan *et al,* (1966). Higher bottom fraction was an indication of greater integrity of lutoid. So compartmentalization of ions in lutoids was more leading to faster glycolysis and latex production. N-acetyl glucosaminidase activity was less in stimulated trees when compared to control on all tapping days (Figure- 78).

Stimulation activates lutoid ATPase (Figure-.63). Chrestin (1984); Gidrol,(1984) and Gidrol *et al.,* (1984) reported that by activating lutoid ATPases after treatment with ethrel stimulates an influx of proton to lutoids. Ethephon probably act on ATPase proton pump, which simultaneously causes a rise in latex pH, and pumping of sucrose from neighboring phloem to cytosol by sucrose proton symport. Unakorn Silpi (2001) reported an activation of sucrose-H^+ symport. Alkalinisation of cytosol by ATPase resulted in an increased rate of glycolysis and high yield.

Glycolipid content of latex was higher for stimulated trees on all tapping days when compared to control trees (Figure-81). But phospholipids did not show any variation between stimulated and control trees(Figure- 84) .

Effect of intensive tapping after stimulation on different biochemical parameters in clone RRII 105 under ½ Sd/4 6d/7 tapping system.

Total volume was found to be maximum on 2nd and 3rd intensive tapping (Figure-. 22) followed by a decrease in subsequent tappings. Sucrose in latex (Figure-28) showed an increase up to 4th intensive tapping and then showed a decrease. Sucrose in C-serum showed an increase up to 3rd intensive tapping (Figure-31). This may be due to the activation of sucrose-proton symport by

stimulation (Unakorn Silpi, 2001). The result was in contradiction with Koshy (1997). Intensive tapping with stimulation enhanced loading of sucrose in laticiferous vessel. Combined action of intensive tapping and stimulation caused more drain of latex constituents like sucrose. Stimulation enhanced translocation of sugar into laticiferous vessel. A slight decrease after 4th intensive tapping may be due to the reduced effect of stimulation.

A general decrease in invertase of C-serum up to 4th intensive tapping (Figure-34). First decrease in activity may be due to dilution effect. Sucrose synthase of C-serum increases up to 4th intensive tapping (Figure-.37) and invertase activity decreases. A general increase in latex and C-serum pH was observed after intensive tapping (Figure.55,58) At a high pH, activity of sucrose synthase was towards the synthetic side .this may give an additional explanation to the increased sucrose (latex) up to 4th tapping.

A general increase observed in Pi in latex and C-serum (Figure 43, 46) indicates the activation of metabolic rate, catabolism of sugars and an increase in yield. These results were in confirmation with Ribaillier *et al.* (1971) and in contradiction with Koshy (1997).

Increase in weight of bottom fraction after intensive tapping with stimulation (Figure-.70) resulted in more yield. Supporting results were obtained by Koshy (1997) and Do Kim Thanh (1996). Bursting index showed a decrease up to 3rd intensive tapping (Figure-67) indicates increased stability of lutoid after stimulation. Increase of bottom fraction was also an indication of lutoid stability. Stability of lutoid was responsible for the compartmentalization of different solutes and ions responsible for coagulation of latex in lutoids and good latex production.

N-acetyl glucosaminidase activity in B-serum showed a general decrease up to 4th intensive tapping after stimulation (Figure-79). This may be due to the increased release of enzyme into C-serum after stimulation. This can delay coagulation and increase yield.

ATPase activity increases up to 3rd intensive tapping and stimulation (Figure-64). This results in the increased pumping of H^+ into lutoids, resulted in C-serum alkalinity and an increased yield. Gidrol *et al,* (1988) supports this information.

Increase in phospholipids and glycolipids in latex in 3rd intensive tapping (Figure-82,85) indicates the increased stability of membrane which in turn resulted in high yield.

Tapping rest with stimulation

When d/4 tapped trees were given different periods of tapping rest with stimulation, maximum latex volume was observed in trees with 6 days rest (Figure-23). DRC was minimum for that trees (Figure-26). This may be due to the dilution effect of stimulation.

Sucrose in latex and C-serum was maximum (Figure-29,32) and invertase and sucrose synthase activity was minimum (Figure-35,38) in trees with 6 days rest. Latex thiol which is an activator of both invertase and sucrose synthase activates these enzymes. Activity of thiols was maximum in trees with 6 days rest. Higher sucrose indicates that the rate of utilization of sucrose was less than the rate of translocation .The rate of loading of sucrose by stimulation was found to be maximum in trees with 6 days rest.

Maximum Pi in latex observed in trees with 6 days and 10 days rest after stimulation (Figure-44) indicates the high metabolic activity in laticiferous vessel. Sudden drop after 10 days rest may indicate more drain of inorganic phosphorous leading to low yield.

Maximum weight of bottom fraction was observed in latex of trees with 6 days rest after stimulation (Figure-71). This indicates that synthesis of bottom fraction (lutoids) take place upto 6 days rest. After that stability of lutoid decreases results in degradation of bottom fraction. Minimum bursting index in trees with 6 days rest (Figure-68) indicates the stability of lutoid on

that day. Koshy (1997) reported maximum weight of bottom fraction was in trees with 1-day rest and minimum on 7 days rest. N- acetyl glucosaminidase activity was found to decrease on 10 days rest and an increase on 14 days rest(Figure 80).

Maximum ATPase activity was found in trees with 6 days rest (Figure-65)where yield was maximum showing the influence of this enzyme on latex production. Glycolipids showed a decline in trees after 2 days rest (Figure-83). Phospholipids showed a slight decrease in 2 days rest (Figure-86) and then it decreases as that of glycolipids.

4.6. Changes in biochemical parameters related to carbohydrate metabolism in TPD affected trees

Yield of rubber prior to the appearance of partial dryness showed no change. After that the volume decreases in both trees when compared to healthy trees (Figure-87). Commere *et al.*, (1989) reported 15-20 % decrease in yield in TPD trees.

After the onset of TPD syndrome (35% dry) both trees showed a decrease in sucrose in C-serum and latex (Figure-88, 89). Then a sudden increase was observed after 6 months. Thomas *et al.*, (1998) and Krishnakumar *et al* .,(1999) reported high sugar content in latex of TPD affected trees. Higher sucrose may indicate less conversion of sucrose to rubber. Pi decrease after the development of TPD indicates very low metabolic rate (Figure-90).

Bark analysis showed a high sucrose synthase activity in both TPD affected trees. Pi in bark of TPD tree No. 1 was lower and tree No. 2 was higher than that of control. Sucrose and invertase activity of tree No.1 was higher than that of control. Sucrose and invertase activity of tree No. 2 was lower than that of control. Low Pi in tree No.1 may reduce the metabolic activity even though sucrose and invertase were high.

Conclusions

The present study shows the importance of ATP ase in pH regulation. During the high yielding period, activity of ATPase was high irrespective of the condition, season, clones or stimulation. The study emphasizes the importance of invertase ,a key enzyme involved in rubber biosynthesis .An inverse relation between invertase and sucrose was obtained in all cases of experiment.

Study shows that quantity of lutoid in latex increases with latex yield per tapping irrespective of tapping intensity, stimulation and tapping rest. Increased biosynthesis of lutoid fraction under alternate daily tapping shows quick regeneration. Quantity of lutoid in the latex is a good indicator of latex stability compared to other parameters. Decrease in lutoid content is associated with low levels of glycolipids and phospholipids and increased bursting index. Reduced phospholipids and glycolipids under tapping rest can be attributed to low lutoid stability and low yield. Decrease in glycolipids and phospholipid under tapping rest is a first time report. Hence study on regulating the biosynthesis and breakdown of the lipids is very important.

The study also shows the indirect evidence for the increased release of N- acetyl glucosaminidase from lutoids when stimulated. The presence of high level of the enzyme in the B-serum of high yielding clones indicates more stability of lutoids and high yield.

Invertase activity in C-serum, ATPase activity and quantity of lutoids are good indicators of latex production.

Natural Rubber (cis 1,4 polyisoprene) is obtained from the latex of *Hevea brasiliensis.* Latex is the cytoplasm of specialised cells called laticifers. Production of latex in *Hevea* involves complex mechanisms. Sucrose produced by photosynthesis and transported by phloem pathway to the laticiferous tissue is the main precursor of acetate (most simple initial precursor of isopentenyl pyrophosphate) and hence of rubber. The sugar content remains the main factor that limits the yield of rubber and enzymes involved in isoprene synthesis plays a major role in latex production. The optimum latex production is governed by the availability of sucrose, regulation of limiting enzyme activities, pH and ionic composition of the cytosol in which glycolysis and almost all isoprenic synthesis take place. Contents and distribution of ions such as Mg^{2+}, Pi and thiols in the cytosol and lutoid (vacuolysosomes in latex) compartments influence the activity of certain key enzymes which controls the latex production. The transport of the ions are also linked with the functioning of the lutoid membrane ATPase proton pump and pyrophosphatase. The regulation of the cytosolic pH is important because of the predominant impact of these parameter on latex production. With the above background experiments were carried out to study the variations in physiological and biochemical parameters associated with latex production, seasonal variations in carbohydrates and ionic balance(H+ ion concentration) in latex production, variations in carbohydrates during defoliation and refoliation, effect of stimulation on latex production and clonal variations in the parameters associated with latex production.

During stress period a high sucrose content and a low invertase activity was observed. But sucrose synthase activity was higher. A high PPi in latex, inhibits rubber biosynthesis. This accumulation is due to the low activity of c- serum pyro phosphatase during stress season. Low C- serum pH observed during stress is also unfavorable for invertase functioning.A low phospholipids in the bottom fraction indicate damage of lutoid membrane during stress. A high thiol content during stress indicates a certain amount of protection and it also activates the sucrose synthase activity. ATPase activity of lutoids were also lower which leads to lowering of C- serum pH during stress season.

High yielding clones have in general showed an increase in invertase activity, C-serum pH , ATPase activity of lutoids , Pyrophosphatase and N- acetyl glucosaminidase and a decreased sucrose in latex. Low sucrose was an indication of good utilization or less loading into laticifers. Higher activity of ATPase and Pi were also indicators of high latex production. Hevein did not show any consistant pattern between clones.

High tapping frequency of d/2 showed an increase in invertase activity.. Sucrose synthase activity is less in d/2-tapped trees. An increased bottom fraction and a high glycolipids and phospholipids in the bottom fraction were observed in d/2 tapped trees which increases the stability of membrane.

A high sucrose, C- pH, latex Pi, sucrose synthase and weight of bottom fraction was observed in stimulated trees when compared to control trees.Stimulation leads more production of lutoids. Bottom fraction can be a bettr indicator of latex flow. But N- acetyl glucosaminidase in B- serum showed a decrease in stimulated trees. This may be due to release of this enzyme in to the C- serum without the membrane damage resulting in the prevention of destabilization of latex near the cut end. Release of these enzyme in response to stimulation can be an alternate mechanism to stabilize the latex in addition to increased lutoid stability.Increase in ATPase activity and Pi is an indication of high latex production.

An increased ATPase activity, Pi , bottom fraction ,phospholipids, glycolipids were observed in 3rd intensive tapping after stimulation where the yield was maximum. Quantity of lutoid is an indicator of greater lutoid stability.Pi is an indicator of rate of energy metabolism. Increase in ATPase activity cause an alkalinisation of cytosol by activating proton pumping .A high sucrose may indicate actvation of H+ -sucrose symport.

When trees were tapped with different periods of rest after stimulation , maximum increase in yield was observed in 6days rested trees . Sucrose in latex ,lutoid,Pi and ATPase were maximum in 6 days rested trees.Phospholipids and glycolipids showed a decrease in 10 and 14 day rested trees .This indicates the decreased stability in rested trees.

Parameters related to carbohydrate metabolism were done in trees which became dry during the course of the experiments.Tapping panel dryness affected trees have higher

sucrose and lower Pi in their latex. Bark analysis showed an increase in sucrose synthase activity in TPD trees .Pi , Sucrose and invertase showed variations from normal trees.

Quantity of lutoid fraction is an indicator of latex stability compared to other parameters. ATPase can cause an increase in latex yield by activating proton pumping to lutoid. Reduced phospholipids and glycolipids under tapping rest after stimulation can be attributed to low lutoid stability and low yield.

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