ESTIMATION OF INTRACLONAL VARIATIONS AND ASSOCIATIONS OF CHARACTERS IN RRII 105, A POPULAR CLONE OF *HEVEA BRASILIENSIS* (Willd. ex Adr.de Juss.) Muell. Arg.

THESIS
SUBMITTED TO MAHATMA GANDHI UNIVERSITY, KOTTAYAM
IN PARTIAL FULFILMENT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN THE FACULTY OF SCIENCE

(BOTANY)

By

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JANUARY 2004

DECLARATION

I hereby declare that the thesis entitled **"ESTIMATION OF INTRACLONAL VARIATIONS AND ASSOCIATIONS OF CHARACTERS IN RRII 105, A POPULAR CLONE OF *HEVEA BRASILIENSIS* (Willd .ex Adr. de Juss.) Muell.Arg"** submitted by me for the degree of Doctor of Philosophy in Botany of Mahatma Gandhi University is a bonafide record of the research work done by me at the Rubber Research Institute of India, Kottayam under the guidance of Dr. D. Premakumari, Senior Scientist (Rtd) RRII, Kottayam. I further declare that this thesis has not previously formed the basis for the award of any degree or diploma or other titles to me.

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CERTIFICATE

I certify that this thesis entitled **"ESTIMATION OF INTRACLONAL VARIATIONS AND ASSOCIATIONS OF CHARACTERS IN RRII 105, A POPULAR CLONE OF *HEVEA BRASILIENSIS* (Willd. ex Adr. de Juss.) Muell.Arg."** is an authentic record of the original research work carried out by **Mrs. Mary K.M**. under my supervision and guidance during the period 1999 to 2003. She has passed the qualifying examination conducted by the Mahatma Gandhi University.

I further certify that no part of this work has previously formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar titles of any University or Society to her.

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Acknowledgement.

I have great pleasure to express my gratitude to my supervising teacher, Dr. D.Premakumari,senior scientist (Rtd) Botany Division, RRII, for the excellent and stimulating guidance throughout the course of the study and also for the preparation of the thesis in the present form.

I owe my sincere thanks to Dr. N.M.Mathew, Director of Rubber Research Institute of India (RRII) and Dr. Krishnakumar A.K., Rubber Production Commissioner of RRII for the excellent facilities provided for this endeavour.

I do sincerely acknowledge the encouragement and co-operation rendered by Dr. Kuruvilla Jacob, Deputy Director, Pathology division. I would also like to thank Dr Shaji Philip, Scientist, Pathology division for the kind co-operation and sincere help. I express my heart felt thanks to Dr. Thulaseedharan, Deputy Director, Biotechnology for the valuable help rendered. I am thankful to Dr.Venkatachalam, Scientist, Biotechnology for the timely help. I am grateful to Dr Saraswathyamma C.K, Deputy Director, Botany Division for providing the laboratory facilities. I am deeply indebted to Sri.Ramesh B.Nair, Assistant Director, (Statistics) who has helped me a lot in the statistical analysis, deserves special mention and the staff of the division for their assistance.

Special thanks are due to Dr.Vinod Thomas, Scientist, for the co-operation and help in the preparation of photomicrographs. I am indebted to Dr.Meenakumari T., Junior Scientist, for the remarkable insight into the subject given to me through several discussions I had with her. I do acknowledge the help of all my friends in the Botany division, especially that of Ms. Sobhana Sanker, Senior Scientific Assistant, for the co-operation and timely help during my work.

I am deeply indebted to Smt. Accamma Korah, Senior Librarian, for the meticulous scrutiny of references and sincere help rendered. I do acknowledge the services of the staff of the Library, especially Mrs. Mercy Jose, Documentation Officer. The services of Mr Kurian Thomas, Junior Publication Officer, is also acknowledged. The sincere services of the staff of the nursery(C.N.Karikkattoor) are acknowledged. Mr. Thampy Andrews and Mr. Kunjunny K deserve special mention for their valuable help.

I do acknowledge the help of my respected teachers, Dr.Joseph P.Varghese, H.O.D. of Botany Department, C.M.S.College, Kottayam and Dr.Raju, E C, H.O.D.of Botany Department, Baselius College, Kottayam.

I affectionately thank my brother, Professor Varghese K.M., founder Principal of St. Mary's College, Manarcaud, for permitting me to carry out the part-time research work, while working as a lecturer in the College.I do thank the succeeding Principals Dr.John Kuriakose and Mr.Bobby A. for allowing me to continue the work. Special thanks are due to Manager, Rev. Fr.Thomas Mattathil for the permission and support extended.

It's my pleasant duty to express my gratitude to my colleagues, friends, and relatives especially Dr.George M.Cherian, Mr.Biju Chemmanam, Mr.Govindraj and Praveen Chandran for their encouragement and support.

The immense help and encouragement rendered by my beloved husband Mr.Babu John Jacob and my loving children Paul Baven and Anish Baven, who appreciated my involvement in the study with patience and affection are greatly acknowledged. I remember my beloved parents who rest in peace.

Above all, I thank God for making my PhD programme a success in my fifties without UGC (FIP) facilities.

Mary K.M

Dedicated To My Most Beloved Parents

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INTRODUCTION

1. INTRODUCTION

Domestication of *Hevea brasiliensis* in the Far East is the most spectacular event in rubber industry. Production of natural rubber, the most versatile raw material, on plantation basis, is only 100 years old. In this short span, a novel plantation enterprise covering an area of over 5,69,000 hectares, was created by research and development, to meet the industrial demand.

Natural rubber is a constituent of latex of certain plants, including *Hevea.* In the plant kingdom laticifers are present in over 12500 species belonging to about 900 genera of which about 1000 species of 76 families contain rubber (Polhamus, 1962; Esau, 1965 and Metcalfe, 1966, 67).

Of all the latex yielding genera, *Hevea,* a member of *Euphorbiaceae*, is the most important one and natural rubber as an industrial raw material is recovered mostly from a single species, *Hevea brasiliensis* which is also known as Para rubber tree.

Productivity enhancement, reduced cost of production and varietal adaptability to agroclimatic situations and biotic constraints are the needs in the field of agriculture to cope with the globalised economy and so also are the thrust areas of research. Rubber is not an exception. The priority areas to be explored are:

1. Development of improved varieties.
2. Proper utilization of the available information for the effectiveness of clonal characterization in a time saving and money saving manner.
3. Agroclimatic zoning of clones.
4. Improvement in nursery practices to enhance the productivity of nurseries.
5. Use of proper stock and selection of properly formed budded stumps for planting in polybags /field to improve the productivity of available clones.
6. Developing propagation techniques to reduce the immaturity periods and collecting feed back information on its impact in the field.
7 Managing TPD in the field rather than further going to the ultra minute details of TPD affected trees.

Yield of *Hevea* is a multi-factorial trait, which can vary due to varietal difference, age, location, and climatic changes and even due to stock- scion interaction. Now in India, RRII 105 is an accepted clone for yield and for tolerance to some diseases, in spite of TPD as a major defect. Some introduced clones like PB 260 also perform well in India. A few RRII clones now are in the pipeline, which show encouraging yield and growth in the small-scale trial and for the initial performance in the large-scale trial. Efforts for generating data on the agro-climatic responses of such clones and for their characterization and cataloging are in good progress We might be able to get some exotic clones like IRCA clones with good growth and a few more PB clones like PB 314 and PB 255 as promising for growth and yield which could be utilized for multi-clone planting. The clones thus available are the achievements of tremendous progress in breeding and selection programmes of the various rubber growing countries. According to the situations research priorities have to be shifted.

To achieve the maximum productivity from the high yielding clones and for the progress towards the theoretical maximum of the productivity of such clones, research priority has to be fixed aiming at the progress of economic feasibility of cultivation practices. Better management practices without additional burden to the planters should be the next motto. Once a high yielding and agro-climatically suited clone is developed, the most important productivity improvement area is TPD management.

Both generative and vegetative methods of propagation are possible in *Hevea.* During the early stages of rubber plantation industry, rubber cultivation was practised by planting seedling stumps produced by generative method. But the yield was poor. Researchers observed that major portion of the yield in a plantation is contributed by a small number of plants, which pointed to the need of seed selection. Tremendous progress was achieved in this field resulting in the development of high yielding primary clones such as Tjir 1 and GT 1(Dijkman, 1951) and monoclonal seedling progenies of selected clones were used as planting materials for preparing seedling stumps.

Development and perfection of bud grafting technique in rubber marked the most important milestone in the crop improvement programmes of the plant, which facilitated perpetuation of scion genotype by vegetative propagation. This event marked the start of a speedy progress in the field of propagation and clone development. The technique was refined in different areas to develop much improved types of planting materials such as brown budded stumps, green budded stumps young budded stumps and poly-bag plants. Poly-bag plants of improved clones, prepared from bud-grafted plants are widely accepted planting materials now.

In the beginning of application of bud grafting in *Hevea*, some observations had been made on the influence of stock on the growth of budded plants and some reports on such influence of stock on the growth of grafted bud are available. Based on this, the early practice was using monoclonal seeds of selected clones. In India open pollinated seeds of the clone Tjir 1 were popularly using for raising stock seedlings. Later, due to high demand for the stock seedlings, when rubber cultivation was promoted into a very extensive area in the traditional and non-traditional areas in India, it was necessary to use readily available assorted seeds for this purpose and that became a general practice. Nobody bothered to assess the impacts, if any, of this practice on the productivity of the nursery or on the performance of field plants.

Now some questions are raised on the validity of assorted seeds as source of stock seedlings in terms of germination percentage, seedling growth, budding success, stock- scion compatibility and subsequent impacts on establishment success, uniformity of growth, productivity and TPD incidence in the plantations. The basis of such thinking is some field experiences of lack of uniformity of plants and high incidence of TPD in plantations and the observations of some recent studies showing considerable amount of variations among the individuals of the same clone. Some observations on TPD affected plants indicate the possibility of genetic contribution of root stock material for TPD occurrence (Shobhana *et al.*, 1999). The findings of some studies to estimate the magnitude of intra-clonal variations of some major economic traits (Dijkman, 1951; Buttery, 1961; Chandrasekhar *et al*, 1993 and Thomas *et al.*, 2000) and some physiological traits (Sethuraj, 1977), even at the enzyme level (Shobhana *et al.*, 2000), highlights the need of generating more data on intraclonal variations of important characters of *Hevea* plants and also highlights the need of identifying the causal factors controlling such variations. The present study was planned for partial fulfilment of this objective. This study aims at generating data

(1) on the magnitude of intraclonal variations of the seed characters such as volume, density and germination percentage of the monoclonal seeds of three clones readily available in the traditional rubber area of South India, in comparison to the assorted seeds available in the seed source of the traditional rubber tract of India,

(2) to estimate the magnitude and nature of intra-clonal variations of some important growth characters of the resultant stock plants in terms of buddable seedlings produced,

(3) to assess the variability of budding success due to stock,

(4) to identify the types of stock-scion incompatibility occurring in rubber

(5) to estimate the nature of such incompatibility as to genetic or due to other factors

(6) to assess the variations for incompatibility, among grafted plants , due to the root stock sources

(7) to assess the variability on the biochemical activities of the scion part of bud-grafted plants due to stock variations

(8) to identify the nature of stock effect on the agronomic characters of the scion part, in terms of the early trend of growth, yield and important leaf diseases with a view to examine the importance of stock selection and nursery selection of budded stumps in *Hevea* to enhance the growth and productivity.

(9) to estimate the intraclonal variations of growth characters, major yield components, summer variations in yield components and the bark characters of mature budded trees, under tapping stress, which were raised on assorted seeds as root stock source.

(10) to estimate the associations of characters of the mature trees to study the stock effect on the characters. Finally

(11) to observe the positional variations of bark growth of the tapping trees and also to discuss the theoretical possibilities of graft incompatibility to contribute for TPD.

RRII 105, the most popular clone in India at present was selected as the right clone to be used as scion material for this study.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Propagation

Management of perennial crops involves judicious selection of most suitable planting materials based on scientific evidence. Low yield and high variability necessitate crop improvement programmes in any crop. There are two basic types of plant propagations - generative and vegetative. Preserving the genetic identity during mass multiplication was achieved through vegetative propagation. Propagation of some species may be easier, more rapid and more economical by vegetative than by generative methods. The concept of clone does not mean that all individual members are necessarily identical in all characteristics. The actual phenotype results from the interaction of its genes with the environment in which it was growing.

Bud grafting

Grafting is uniting two or more pieces of living plant tissues by means of regeneration so that they grow as a single plant, and is a horticultural technique that dates to antiquity (Andrews and Marquez, 1993). Many horticultural applications had been derived from grafting, including exploitation of desired characteristics of rootstock and/or scion into a single plant, increasing precocity of bearing by eliminating the juvenile period, hastening the growth of seedlings in breeding programmes, obtaining special growth forms, rapid cultivar change on existing root systems, repairing trees with bark or root damage, and virus indexing and the study of viral diseases (Hartmann *et al*; 1990). Budding is often termed as "bud grafting" since the physiological processes involved are the same as in grafting.

Technique

Bud grafting is a process that involves choice of stock and scion species, creation of a graft union by physical manipulation, healing of the union and acclimatization of the compound plant. It is limited to plants, which developed the secondary plant body; the principal tissue concerned is cambium, which have the ability to make new cells. The sequence of structural events in grafts of herbaceous plants had been reviewed by Andrews and Marquez, (1993). During grafting the two structures, stock and scion were prepared in such a way that the vascular cambium of each was placed close to or in contact with each other and held together until the two structures grew together. By grafting, new combinations unite and grow together. Tissues which form the union is called *callus,* a bridge of living tissues between the scion and the stock and thus facilitate the passage of water, hormones and essential raw materials from stock to scion, manufactured food and hormones in the scion which pass from the scion to stock. The sequence of structural events occurred in compatible grafts, in various plant species had been studied by researchers (Stoddard and Mc Cully, 1979,1980; Moore and Walker, 1981a; Mc Cully 1983; Moore, 1984; Gebhardt and Goldbach; 1988). First the ruptured cells at the graft interface collapse and form a necrotic layer, which disappears during subsequent events. Then living cells from both stock and scion extend into the necrotic zone. A callus bridge of interdigitating parenchyma cells forms by cell division, rupturing and invading the necrotic layer. During these events the strength of the graft increases due to physical cohesion between the partners. This strengthening occurred as dictyosome- mediated secretion of cell wall precursors aided in cohesion. Now new vascular cambium is differentiated from parenchyma cells. Secondary xylem and phloem are produced by this cambium, providing vascular connection between stock and scion. The bridge between the graft partners differentiates primarily from cortex and pith tissues. Pericycle and cambium are involved to a lesser extent (Stoddard and Mc Cully, 1979).

Most of the fruit and nut species are propagated by budding or grafting on root stock seedlings. Rootstock breeding research of avocado received high priority at the University of California (Reuther, 1961) and large-scale rootstock experimental system was established (Halma, 1954). Blueberry cultivars were propagated by budding (Hartmann and Kester, 1972). Cherry nursery trees were generally propagated by T-budding the desired cultivars on seedling rootstock. The T-bud method of vegetative propagation was very successful with citrus (Hartmann and Kester, 1972). The mere presence of graft or bud-union tends to stimulate earlier heavier bearing. Good quality fruits of excellent size were produced on grape fruit stock. Tree vigor, height and yield were influenced by rootstock in mango cultivars (Reddy *et al.*, 1989; Kurian *et al.*, 1996). In an imperfect graft union, there was probably a partial blocking effect on the movement of nutrients, which resulted in increased fruitfulness (Hartmann and Kester, 1972).

A satisfactory method of establishing grape varieties on resistant rootstocks is by field budding. Mangoes were commonly propagated by veneer grafting or by chip budding (Hartmann and Kester, 1972), Olives were propagated in a number of ways such as by budding or grafting on seedling or clonal rootstocks, by hard or semi hard wood cuttings or suckers from old trees. Plums are always propagated by T-budding.

Advantages of Vegetative Propagation

Vegetative propagation is used for cultivar change, repair or invigoration of older established trees. Small trees were created by the use of dwarfing root stock (Brrientos – Priego *et al.*, 1992). The use of graftage overcomes the difficulty in root formation in many fruit trees, in which propagation through cuttings was impractical.

Grafting was an important research tool in the study of secondary metabolites (Heuser-1983), plant growth regulators (Sachs, 1981; Jones, 1986), translocation (Beeson, 1986) water relations (Schmid *et al.,* 1988), anatomical development (Gebhart and Gold bach, 1988), cell relations (Basic and Clark, 1988), senescence, stress physiology (Feucht, 1988) and molecular genetics (Ochatt, 1989). Grafting had also been used as means to study the transmission of signals affecting vernalization and photo period (Suge, 1992), as well as transmission of virus into indicator plants and to eliminate viruses. Grafting was restricted to dicots since their vascular bundle arrangement and presence of continuous cambium offered greater degree of potential grafting success (Hartmann and Kester, 1972).

The interactions of stock and scion affected growth and productivity (Beak bane and Roger, 1956, Rom and Carlson, 1987). Studies on different species had yielded enormous amount of information on the anatomical and physiological events that occurred during the formation of compatible graft union.

Rootstock

Considerable variability existed in the tolerance of various rootstocks to adverse soil conditions and hence choice of rootstock is very important. Avocado orchards were not uniform either in tree vigor or in productivity; the explanation for such a phenomenon was the rootstock variability resulted from seed heterozygosity. Certain American grape varieties obtained great yield when they were grafted on vigorous rootstocks in comparison with own rooted plant (Vaile; 1938). Grape varieties were definitely influenced by the type of rootstock in which they were grafted (Harmon 1949). Cases in which rootstocks impart disease resistance to the scion variety were common. One example in this case was the infection of stone fruit in California by bacterial canker. In a number of instances apricots and plums, when grafted on myrobalan plum roots, became much more seriously infected than when they were worked on the peach roots. Pear decline disease, which occurred in America and California, killed hundreds of pear trees. In early studies it was believed that trouble was related to the rootstock used (Batjer and Schneider, 1960).

According to Hartmann and Kester (1972), clonal rootstocks were desirable not only to produce uniformity but, also to preserve special characteristics and specific influence on scion cultivars such as disease resistance, growth, soil stress factors etc. Stronger rootstocks can more easily withstand complex stresses. Vigorous, strongly growing rootstocks in some cases resulted in larger and more vigorous plant, which produced greater crops over a long period of time. Dwarfing rootstocks may be more fruitful if closely planted.

The salinity problem of Avocado is partly due to sodium translocation and could be solved by using resistant rootstock (Ayers *et al;* 1951). Kadman and Ben-Ya'acov, (1976) observed that sodium was accumulated in the root system and little accumulates in the leaves. In Avocado dwarfness could be achieved by growing dwarf cultivars. For large –sized cultivars, dwarfing rootstock would be the best means of size- control (Bergh, 1976; Brokaw, 1982). Rootstock effect on tree size and vigour is strongly related to tree productivity. The "tree efficiency" in many horticultural races was influenced by rootstocks (Gregoriou, 1992).

Inter specific hybrids possessing excellent tolerance to diseases had been developed for root stock use in many crops, like *Pepper*, *Capsicum* and other Solanaceous vegetables. In egg plants the rootstocks played an important role in disease resistance (Katao and Lou, 1989; Kim, 1999). Root stock selection in *Solanum* species was usually done based on the population of soil borne pathogens in the relevant production area. The influence of rootstock as a source of drought tolerance in tomato had been reported by Bhatt *et al.*, (2002). The study indicated the existence of considerable interaction of scion and stock in cultivation strategies of tomato under water stress condition.

Biochemical studies

Plant improvement programme in any perennial crop needs reliable biochemical studies. Isozymes provide useful evidence in the study of variation within crop cultivars in terms of intensity as well as presence or absence of bands (William and Mujeeb Kazi ., 1992). A study in which growth rate of scion and rootstock were measured close to the graft union, in incompatible pear quince combinations, failed to show that the incompatibility was due to difference in growth rates or time of cambial activity. Incompatibility may be attributed to physiological and biochemical differences between stock and scion (Gur, 1957). Various laboratory methods had been developed for evaluating stock – scion compatibility in nursery trees without growing trees to maturity (Evans and Hilton, 1957). One bio-chemical method was based upon pear quince incompatibility due to the presence of glucoside (Samish, 1962).

Enzyme polymorphism was used as genetic markers in Avocado (Torres *et al.*, 1978), datepalm (Torres and Tisserat, 1980), *Camellia Japonica* (Wendel and parks, 1983), sugarbeet (Vangeyt and Smed, 1984), apple cultivars (Weeden and Lamb, 1985). Bower and Nel (1982) found that rootstock played a role in the final expression of bio-chemical activity in the scion. The affinity between the scion and different rootstocks could be predicted by the relative electrophoretic mobilities of the total proteins in *Vitis vinifera (*Masa, 1989). Increased peroxidase activity and accumulation of lignin were considered as symptoms of incompatibility. Peroxidases appeared as large numbers of isozymes and participated in numerous physiological activities. Peroxidase activity also was observed in compatible grafts only for a short duration (Deloire and Hebant1982). High lignification had been observed in incompatible host pathogen combinations and absent in compatible combinations, Santamour (1988b) reported potential application of electrophoresis as a means of predicting incompatibility before grafting. Santamour (1988a) in studies of intraspecific grafts of red oak, found abundant variation in cambial isoperoxidase banding patterns. Isoelectric focusing of peroxidase and acid phosphate isozymes were used for characterizing *Diascoria* food yams by Twyford *et al.*, (1990).

Incompatibility

The inability of the parts of two different plants, when grafted together to produce a successful union, and of the resulting single plant to develop satisfactorily was termed as incompatibility. It appeared as genetically controlled by multiple genes with additive effects (Copes 1970,1978). Moore and Walker (1981a; b; 1983) and Moore (1983) described and compared the anatomical and physiological events occurred in compatible autografts and incompatible heterografts. Salesses and Al kai (1985) reported that incompatibility acted as a genetic dominant, which needed at least two genes for expression. Lee (1989, 1994) and Ko (1999) studied graft incompatibility in cucurbits and concluded that it could be changed depending on the grafting methods and growing environments.

Propagation of many ornamental conifers were affected by stock-scion incompatibility. Since more than 50% of grafted trees showed incompatibility responses, tree growers in New Zealand were forced to change (Sweet and Thulin, 1973) from grafting to seedling orchards of Monterey pine. Researchers considered graft incompatibility as the most serious limitation to grafting success (Garner, 1979).

Incompatibility symptoms were diverse, depending upon species, internal symptoms usually preceded the external ones. The observations disproved the concept that the vascular cambium was the only source of callus for bridging. While using the standard grafting or budding methods, there were numerous possible variables, which may affect the success of the operation (Roberts, 1927). If the union was healed properly, growth will proceed in normal manner. Attention to proper polarity is very important to make the graft union permanently successful. Sometimes the grafting technique is so poor that only very little portion of the cambium of stock and scion is brought together. It was still recommended that the cambium of stock and scion be well aligned, so that new vascular elements were formed longitudinally providing greater mechanical strength (Crafts, 1934; Esau, 1965, Kollmann *et al.*, 1985). A comprehensive review had been written by Lachaud (1975). Grafting errors like uneven cuts, delayed waxing, use of desiccated scion etc resulted in grafting failure, which caused incompatibility. .

Phloem tissues were more severely affected than xylem tissues in incompatible combinations, Symptoms included phloem and cortex degeneration, (Breen, 1974) atypical axillary xylem parenchyma (Copes, 1980); lack of axillary parenchyma in the *phloem and necrosis of cortex cells, increased peroxidase activity in both partners and* deposition of lignin and polyphenols at the graft union, impeding formation of vascular connections and translocations (Deloire and Hebant, 1982). Due to these problems in vascular conductivity higher concentrations of starch, free sugars and sugar alcohols had been found in the scion and higher concentrations of inorganic nutrients (N, P, K, Ca, Mg), were found in the root stock (Breen, 1975; Salesses and Al Kai, 1985). Changes in nutrient levels accompanied or followed changes in carbohydrate levels, but never preceded them (Breen and Muraoka, 1975). Findings by Breen and Muroaka (1975) explained the starch distribution as a consequence of vascular discontinuity, refuted the early and long accepted hypothesis that considered differential starch distribution as a cause of graft incompatibility (Rogers and Beakbane, 1957; Garner, 1979).

Incompatibility symptoms included excessive suberization and bark thickening due to over production of sieve cells, and under production of tracheides, excessive tannin accumulation, indicated by abnormally dark stained bark, and a wavy pattern of annual growth rings (Copes, 1980). Late bud break (Nelson, 1968), abnormal leaf morphology and premature leaf abscission (Copes, 1980), reduced vegetative growth, shoot dieback, ill health, and premature death (Hartmann and Kester., 1972), and discolouration in scion leaves were the external symptoms observed. Due to vascular discontinuity the scion leaves showed discoloration (Breen, 1975).

Swelling often develops above the union with vascular discontinuities. Over growth at the graft union was considered as a symptom of incompatibility (Bhattacharya and Dutta, 1952; Garner, 1979; Westwood, 1988). In trees, a clean breakage at the graft union was considered as the most reliable diagnosis of incompatibility (Garner, 1979; Eleftheriou, 1985). It implied that very few or no functional vascular connections and interlocking fibres between scion and rootstock confer significant mechanical strength and lead to full graft development.

Types of incompatibility

Herrero (1956) classified graft incompatibility into four categories; failed bud growth, virus caused graft failures, mechanical obstruction at the union, and abnormal union structure usually associated with disproportional starch accumulation. Mosse (1962) divided incompatibility into translocated and localized type. Localized incompatibility occurred at the graft interface and required actual tissue contact between the two components, both incompatibilities can occur in the same graft combination as in pear and quince graft union. Translocated incompatibility is due to the phloem degeneration of which a brown line or necrotic area in the bark was developed, which resulted in the blocking of movements of carbohydrates at the graft union. Virus induced incompatibility cases were included in translocated incompatibility. One component of the combination may carry a virus and may be symptom- less, but the other component may be susceptible to it.

Delayed incompatibility had been used to describe graft combinations that failed after a period of successful growth (Argles, 1937; Randhawa and Bajwa, 1958). Delayed incompatibility had been identified as disease such as pear decline, which occurred in Italy, California and North America and killed hundreds of pear trees (Shalla Chiarappa, 1961; Hibino *et al.*, 1971). It was found that trouble was related to the rootstock used. Further researches showed that this was associated with a virus, the scion variety were tolerant of the virus, but the rootstock was susceptible showing a phloem degeneration just below the union, for the decline condition to appear, the concurrent presence of virus and a susceptible rootstock was necessary (Jensen *et al;* 1964).

Physiologically based "delayed" incompatibility was possible if the production of incompatibility toxin took place as graft partners age, such as the developmental transition from juvenile to mature tissues (Andrews and Marquez, 1993). More recent studies revealed the fact that stress and senescence were considered as triggers of delayed incompatibility response (Feucht, 1988;Treutter and Feucht, 1988). Prominent differences in the development of interspecific plasmodesmata between stock and scion suggested that cell recognition and functional co-ordination might be involved in graft formation (Kollmann and Glockmann, 1985; Kollmann *et al.*, 1985)

Prediction of incompatibility

Prediction of incompatible graft combination is a very important area of study for preventing economic loss due to graft incompatibility. The incompatibility could be partially controlled in cases where the mechanism depends on the presence of toxins, by selecting rootstocks or scions that produced lower levels of these metabolites.

Isozymes separated by electrophoresis was one of the earliest methods used for the prediction of graft incompatibility (Copes, 1978). Electrophoretic techniques for detection of incompatibility have advantages over anatomical observations, since testing could be done before actual grafting and graft union need not be destroyed. The differences occurred in the isozymes patterns predict the anatomical or external symptoms of delayed incompatibility. Seedlings, with similar peroxidase composition, were compatible and vascular continuity was restored. However, if composition differed, incompatibility was observed following grafting.

Rubber

Hevea brasiliensis (willd, ex Adr. de Juss.) Muell. Arg. (Family Euphorbiaceae) is a quick growing perennial tree. Pollination is entomophilous and fruits, a regma, mature within five months. Seeds weigh 4 to 6 grams and, possess characteristic mottling (Polhamus, 1962), which is helpful for clone identification. Each seed has a dorsal and ventral side and had frontal and lateral depressions.

Commercial rubber is recovered from the latex extracted from the bark of the trunk. A comprehensive description of the structure of mature bark was made by Bryce and Campbell (1917). In the bark, below the protective tissue or cork, there were two distinguishable zones: an inner zone consisting of soft tissues, termed soft bast and an outer zone made up of hard and thick walled cells, the major component being sclerified cells or stone cells. Most of the functional latex vessels are present in the soft bast region. Towards the outer portion of the hard bast, the latex vessels, sieve tubes, etc become discontinuous and non-functional due to age and senescence.

The latex vessels are oriented in an anti-clockwise direction at an angle of inclination of two to seven degrees, they are produced in discrete rows and the vessels belonging to the same row are interconnected tangentially. The laticifers appear as straight tubes in radial, longitudinal sections where as the structure resembles an expanded meshwork in tangential longitudinal sections while in cross section it is circular shape.

Tapping and bark renewal

Rubber tree is commercially exploited for latex by a systematic regular excision of bark of the trunk. The economic period of *Hevea* tree is 20 –23 years from the commencement of tapping by which both virgin and renewed bark are exploited.

During every tapping, a thin slice of bark 1.0-1.5 mm in thickness is shaved off to cut open the latex vessels, the cambium is not injured in this process. Moreover, a layer of soft bast is also left uncut during tapping which gives protection to the cambium. The protective tissue removed on tapping is replaced by the formation and activity of a new phellogen below the cut surface (Bobilioff, 1923; Panikkar, 1974), and the vascular cambium continues its activity.

Importance of the extension of drainage area mediated through dilution reaction after tapping was well elucidated (Sethuraj, 1977). Physiological parameters such as initial flow rate (Volume of latex obtained per minute for the first five minutes after tapping) and plugging index received much attention.

The occurrence of intraxylary phloem in *Hevea* was identified by Premakumari *et al*, (1985 b). Clones vary significantly for the quantity of intraxylary phloem and occurrence of high quantity of internal phloem was found to be beneficial to reduce girth retardation on tapping (Premakumari and Panikkar, 1988).

Propagation

Seedlings:

Hevea is propagated both generatively and vegetatively. Vegetative method is by budding. Rubber seeds are collected during the rainfall season. Due to considerable tree-to-tree variations recorded in yield among the seedling populations, raised during early periods of rubber cultivation attempts were made to propagate *Hevea* vegetatively (Dijkman, 1951). These seeds retain viability only for a short period (Edgar,1958). Fresh and heavy seeds show early germination (Saraswathyamma and Nair, 1976). Seedlings raised from assorted seeds were being used as the main source of stock seedlings for bud grafting.

Budding

Vegetative propagation in *Hevea* to produce clonal materials began with introduction of brown budding (Dijkman, 1951). Young seedlings were raised in seedling nurseries to be used as rootstocks for budding and desired clones were budded on them. Patch budding was adopted in *Hevea* (Marattukalam and Saraswathiamma, 1992). According to the age and colour of scion shoot used for bud collection, budding is termed as green budding or brown budding.

Green budding

Green budding was an advanced method of vegetative propagation developed in Indonesia in 1960 by H.R Hurov. Both stock plant and bud wood used for green budding are comparatively young compared to brown budding. Seedlings, which were two to eight month old, were used as stock. Buds are collected from six to eight weeks old budwood plants. These buds are green in colour and hence the name green budding. Green budwood is obtained from budwood plants grown in nurseries. Budding can be carried out at anytime of the year. However, too dry or very wet weather is unsuitable (Desilva, 1957). Green budding gives good success during the first half of summer months. Days with heavy rainfall are not suitable for budding (Marattukalam and Premakumari, 1982).

Polybag plants

Green budded stumps are planted in the polybags of appropriate size and are transplanted in the field without disturbing the shoot and root systems These plants are transplanted to the field at two to three whorl stage or at four to six whorl stage according to the size of the bag used. Polybag plants are advanced planting material now under use for rubber cultivation. The maintenance of these plants in the early stages will be easier in the nursery than in the field. The immaturity period in the field can be reduced by planting these advanced planting materials (Sivanadyan *et al.*, 1976). Uniform growth, less casualty, early establishment, less weed growth and cost reduction were also the advantages of polybag plants.

Stock-Scion Interaction

The stock-scion interaction is an area in rubber research, now gaining much attention due to the fact that rootstock influence varies with the variety of scion used and stock can influence the growth and yield of scion at varying degrees (Singh, 1980). Stocks raised from monoclonal seeds of PB5/51,are found to influence favourably the growth and yield of several scion clones, while some other stocks like RRIM 600 affect the growth performance of the scion negatively. The high yield potential of a clone could be exploited to the maximum by using the most compatible stock material. Early attempts to study the influence of stock on scion and vice versa were employed using twin plants raised from split clonal seedlings (Dijkman, 1951). The results showed a high correlation indicating the influence of stock on the productivity of the scion. Growth of *Hevea* budding was observed with particular reference to the vigour of various clones by Templeton (1960): Seneviratne *et al.*, (1966) observed that the growth vigour of the stock influenced the scion growth in rubber. They observed a positive correlation between girth and height of the plants. Ng *et al.*, (1981) reported the influence of six rootstocks on growth and yield of six clones of *Hevea*. The study indicated that rootstock influenced the growth and yield of scion significantly. Dijkman, (1951) and Buttery (1961) reported that rootstock influence on yield was independent of its influence on growth. Sagy and Omokhafe (1996) evaluated the variation in clonal combinations of rootstocks and scion among five clones of *Hevea*. They observed significant variation in rootstock effects and general combining ability. In none of these works any incompatible symptoms have been identified to be utilized for nursery selection of compatible grafts.

Yeet *et al.*, (1977) studied protein and enzyme variation in some cultivars of *Hevea*. The polyclonal rootstocks used for the evaluation of stock influence on *Hevea* by Yeang *et al.*, (1996) showed a clear evidence of rootstock effect both in the stock as well as in the scion portions.

Krishnakumar *et al.*, (1992) observed polymorphic isozyme expressions caused by stock – scion interaction among the plants of the same clone raised on seedlings of assorted seeds. Variability in isozyme expressions in different *Hevea* clones, raised using assorted seedlings as rootstock was observed by Sobhana *et al.*, (2000). Though the work mentioned above had established the effect of stock on scion, there was no work to quantify the variability occurring among the available assorted seeds and monoclonal seeds or within the groups of rootstock seed sources. The nature of variability and the type of incompatibility occurring to influence the growth and yield or other agronomic characters of the grafted plants are yet to be studied.

Constraints in Rubber Cultivation:

Jayasekare *et al.*, (1977) used regression analysis to study the genotype environment interactions in some *Hevea clones* and reported that the clones can be categorized into different adaptability groups based on their significant linear components. The summer drop pattern of various clones and some biochemical and physiological factors influencing the seasonal effect of yield variations have been reported. Diseases are also constraints, which affect yield of *Hevea.* In India two major leaf diseases are the abnormal leaf fall disease (during monsoon) caused by *Phytophthora* sps and powdery mildew (during January – March) caused by *Oidium Heveae* had received much attention (John *et al.,* 2001). Disease susceptibility is clone specific and effective selection parameters for disease resistance are lacking.

Tapping Panel Dryness (TPD)

The productivity of a plantation was seriously affected due to panel dryness of trees and this constraint is more affected in the case of high yielding clones (Premakumari *et al.*, 1991) and in association with increased tapping intensity (Sethuraj, 1988; Vijayakumar *et al.*, 1991). In advance or in association with the dryness, various external and internal symptoms occurred. The external symptoms were expressed differently. It could be partial or complete drying of the tapping cut leading to cessation of latex flow (Sanderson and Sutcliffe, 1921); in some cases the dryness reached up to the cambium (de Fay, 1981). Cracking and flaking of the bark

(Rands, 1921) are external symptoms. In some cases prolonged flow of latex before the expression of other external symptoms were common. It was also reported that the trees which were initially slow growing were more readily suffering from the problem and after the onset of the disease vegetative growth accelerates and the trunk becomes larger than usual (Jobbe-Duval, 1986).

There were different arguments on the causal factors as tapping intensity, especially frequency of tapping (Bealing and Chua; 1972; Beauchamp and Fridovich, 1971; Chua, 1965; 1967). It is now widely known that stress due to mechanical causes, tapping, chemicals, or pathological infections cause internal ethylene formation (Yang and Prat, 1978) and it was accepted that endogenous and exogenous ethylene were involved in natural stimulation of *Hevea* (Abeles, 1973; Lieberman, 1979). One theory postulate nutritional stress (Chua, 1966) but this theory had been contradicted on the ground that carbohydrate reserve remains plentiful in the trunk of dry trees (Chua, 1967; Krishnakumar *et al.*, 2001). Premakumari (1991) reported that the number of latex vessel rows and the number of intraxylary phloem groups along with the total volume of latex governed 49 % variation in the occurrence of tapping panel dryness.

Krishnakumar *et al.*, (1998) observed higher peroxidative activity in TPD affected plants. Higher activity of peroxidase was negatively correlated with cytokinin in the tissue of TPD affected trees (Krishnakumar *et al.*, 1998). While ethylene tilted the metabolic equilibrium from anabolic to catabolic (Wang *et al.*, 1990) leading to senescence, cytokinins have antiseniscence effects in plants through prevention of free radical production as well as their scavenging activities (Leshem, 1984).

Increased activity of peroxidase in the TPD affected bark may be an indication of increased production of active oxygen species such as superoxide radicals (O_2). Crestin (1985) reported an abnormal rise in the production of toxic oxygen species in TPD affected plants. Production of free radicals and active oxygen species damaging membrane systems, including lutoids and consequent disturbance in lutoid stability and premature *in situ* coagulation of latex on the panel of TPD affected trees was suggested by Crestin (1985). The chances of accumulating such toxic oxygen species in the tissue would lead to oxidative stress to the cellular constituents including mitochondria. Inhibition of the mitochondrial activity could lead to a possible accumulation of carbohydrates as observed by Krishnakumar *et al.*, (1999) and a decreased availability of ATP for the conversion of sucrose into rubber, a process where high energy was consumed (Jacob and Prevot. 1992). However the production of toxic substances in trees confronting stress situation and the possible accumulation of such substances in the panel of some trees leading to TPD is a possible theory.

Genetic control of TPD was suggested by various researchers. Mydin *et al.*, (1999) reported that tapping panel dryness was confirmed to be a distinct clonal characteristic with high heritability and low genetic advance. Non-additive gene action in the inheritance of TPD had also been suggested. Sobhana *et al.*, (1999) had reported that the greater the genetic distance between rootstock and scion, the greater the possibility of the scion showing symptoms of TPD, which was randomly distributed in the field.

Genetic parameters and associations

Co-efficient of variation

In any comparative study, estimation of variations of quantitative characters is an essential part. The comparison is meaningful when the standard variations represented by different units are converted into a unit less measurement. Co-efficient of variation which is the standard deviation from the mean expressed as percentage of the mean value, thus provides such a measurement for comparing the extent of variation between different characters measured in different scales. Genotypic coefficient of variation is the relative magnitude of variability contributed by genetic factors and helps in the comparison of genetic variability present in a population for different characters. In the case of quantitative characters, which are involved by a large number of minor genes with cumulative but small individual effects, it becomes impossible to measure the contribution of each and every gene to the total variance directly. The external expression of genetic values as modified by the environment is measurable as phenotypic values. The available variability in a population could be partitioned into heritable and non-heritable components. The heritable component is genotypic co-efficient of variation (GCV) and the non-heritable component is phenotype co-efficient of variation or PCV. In the case of grafted plants, the genetic make up of the scion part of all-individuals of a clone is the same. Hence any amount of genotypic co-efficient of variation pertaining to a character is contributed by the stock part.

Heritability

The term 'heritability' was first introduced and defined by Fisher (1918) as the ratio of the fixable genetic variance to the total genetic variance. Lush (1937) defined heritability in the 'broad sense' as the proportion of total genotypic variance to the total phenotypic variance and in the narrow sense as the ratio of additive genetic variance to the total variance. Robinson *et al.*, (1949) defined it as "the additive genetic variance in percent of the total variance.

Clonal variability for yield and associated traits in rubber.

Development of improved varieties depends on the available variability in the existing population. Unidirectional selection for yield, adoption of cylindrical generation wise assortative breeding and wider adoption of clonal population by bud grafting led to further narrowing down of the genetic base (Wycherley, 1969). Still considerable variability has been recorded for both source and sink components in *Hevea*. High genotypic and phenotypic variability of dry rubber yield was observed among *Hevea* clones in various studies (Markose, 1984, Premakumari, 1992a). Vigorous growth of the tree in the juvenile phase will reduce the unyielding time. Thus breeders task is to maximize latex yield in a tree on a sustainable basis. There were various reports on clonal variability for girth and girth increment on tapping (Templeton, 1969; Napithapulu, 1973; Premakumari,*et al*; 1987, Premakumari, 1992;a Wycherley, 1975, 1976; Licy, 1997; Mydin, 1992) of mature tree and also on the girthing rate at immature phase (Licy, *et al.*, 1992; Varghese *et al.*, 1993, 1996).

Latex production and storage is in the laticiferous tissue in the bark. Clonal variability for bark thickness, number of latex vessel rows, density and diameter of latex vessels, laticifer area and phloem ray characters had been extensively studied (Ho *et al.*, 1973; Gomez 1982, Markose 1984; Premakumari *et al.*, 1985a; Premakumari 1992a). Production of an internal core of phloem tissue in *Hevea* was reported by Premakumari *et al.*, (1985b). Significant clonal variations for such phloem points were also noticed. The clonal variability of latex flow characters such as plugging index, initial rate of flow and duration of flow (Sethurag, *et al* 1974; Saraswathyamma and Sethuraj, 1975,) as well as the total volume of latex and dry rubber content had been well established.

Correlation

Correlation explains the degree of relationship between characters and it is measured as correlation coefficient, which defines to what degree two variants are related when they vary together. Correlation was first defined by Galton (1889) and was later elaborated by Fisher (1918) and Wright (1921). Such information on the magnitude and direction of correlation existing between different characters are very usefully applied in selection work in biological sciences. It is also an advantage that selection for some of the corrected characters will results in the improvement of the other characters also.

Associations among yield and yield component traits

In *Hevea* juvenile yield is an indication of mature yield showing significant association Samsuddin *et al.*, 1987.Hence information on correlation between the yield at juvenile and adult stage was useful to strengthen the feasibility of early prediction.

Intra -clonal variations in rubber.

Intraclonal variation and association in rubber have been reviewed by Henon *et al* (1984). In a nursery study yield displayed the greatest variability with a coefficient of variation of 60% and anatomical characters displayed lesser co-efficient of variation ranging from 5% to 20%. Intra- clonal variations in yield and certain component traits had been reported (Dijkman 1951; Buttery, 1961; Chandrasekhar *et al.*, 1997; Thomas *et al.*, 2000a; Premakumari *et al*, 2002). Sobhana (1998) also observed enzyme polymorphism among young plants in a given clone, produced using heterogenous type of rootstocks. Intraclonal variations in isozyme profiles of three-enzyme system such as peroxidase, catalase and esterase were reported by Sobhana, (1998) and Sobhana *et al.*, (2000). RAPD analysis of the bark tissues of both root- stocks and scion tissues of a clone by Sobhana *et al.*, (1999). Thomas, *et al.*, (2000 b) revealed genetic homogeneity among the scion tissues while appreciable variation was expressed among the root stocks confirming the heterogeneity of the root stock. Thomas *et al.*, (2000a) observed intra-clonal variation for yield and certain biochemical components of yield in a popular *Hevea* clone. In most of the above studies regarding quantitative traits, the nature of variability as genotypic or phenotypic has not been examined and hence any major role of rootstock in such variability cannot be assured. Premakumari *et al*, (2002) made a new approach to assess the nature of intra clonal variability of yield and girth in 13 Hevea clones by estimating the 'b' values along with the coefficient of variations since a high value of ' b' indicates high genetic influence in the expression of the respective character. Stock sensitivity of the clones with respect to the two traits have also been advocated Premakumari *et al;(*2002) also reported higher coefficient of variation for yield between trees within clone, in 13 *Hevea* clones compared to the CV values for girth of the respective clones. In this case the 'b' values were higher for girth indicating high genetic influence for the intraclonal variations of girth than that for yield.

Intra-clonal associations in rubber

Henon *et al.*, (1984) has reviewed the work on intra-clonal associations among yield and bark structural characters. Girth and latex vessel rings were suggested as important determinant traits for yield. The scope of identification and utilization of stock sensitivity of clones for the improvement of growth and productivity is also discussed. Chandrashekar *et al.*, (1997) estimated intra-class correlation coefficients to measure the relative magnitude of the variations existing between trees as well as between different tappings of a tree for yield in *Hevea* clones to assess the seasonal consistency of yield. They observed poor consistency of yield for RRII 105 during summer. According to Premakumari *et al.*, (2002), intraclonal association between yield and girth was significant in certain clones only.

The present review is to estimate the intra clonal variability of important agronomic traits of rubber to study the nature of variability to trace out stock effect for collecting information useful to promote stock selection. Finally it helps to identify incompatibility symptoms as parameters to cull out undesirable graft combinations. The purpose of this review is to elucidate the recently proposed mechanisms of graft incompatibility in various species and to suggest potential techniques for predicting incompatible combinations.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study was carried out from 1999-2003. The programme included two sections. One part was a nursery study laid out in the Central Nursery of the Rubber Board, established in Karikkattoor near Ranni (plate, 1.fig:1) .The other part was a study on mature tapping trees which was laid out in the Experiment Station of the Rubber Research Institute of India at Pampady in Kottayam.

A. Nursery Study

Materials

For the nursery study the materials used were

(1) Four types of seeds as stock sources.

a. Assorted seeds, collected from the commercial seed sources at Kanyakumari region of the traditional rubber tract at South India. . This was conventionally used in rubber nurseries for raising stock seedlings.

b. Monoclonal seeds of three popular clones namely RRII 105,RRIM 600 and PB 28/59, readily available in Kanyakumari region, the seed source of South India. The seeds were collected from the central portion of large monoclonal areas.

(2) The seedlings were raised from the four types of stock seeds.

(3) RRII 105, as scion clone. Green buds taken from the appropriate stage of green shoots collected from the green–shoot nursery raised in the central nursery of the Rubber Board, at Karikkattoor, were used for bud grafting.

(4) The bud grafted plants and budded stumps prepared after green budding.

(5) The poly-bag plants raised using the green budded stumps.

(6) The plants raised by transplanting the poly-bag plants at a spacing recommended for raising a bud-wood nursery (plate 1,fig.4) for the purpose of collecting brown bud-wood.

Lay out of the study

During the 1999 seed fall season, 1000 seeds each, of the four types were collected. Seeds of the respective types were mixed well and divided into five lots of 200 seeds per lot. The seed characters were recorded from 40 numbers of randomly selected seeds per lot .The seeds were put for germination, on specially prepared germination beds (plate 1,fig.2) as recommended (Marattukalam and Mercykutty, (2000) and watering was done once in two days using sprinklers.

From the 10th day onwards-germinated seeds were collected from the beds once in two days, the numbers counted and planted in seedling nursery. The seedling nursery and the planting beds were prepared following the conventional method (Potty, 1980) and the recommended spacing for seedling nursery (plate 1,fig. 3) was adopted. Normal cultural operations were followed. The establishment and vigor of the seedlings were observed two months after the transplantation of the first germinated seed. Further observations on growth aspect were done at the age of four months just before budding.

Green budding was carried out on the seedlings which attained a girth of 2.5 cm or above at the collar region. Budwood was collected from green shot nursery (plate 2,fig3). Two rounds of budding were done for utilizing maximum number of buddable seedlings .The green budding technique conventionally used in rubber was followed (Marattukalam and Mercykutty, (2000). Fifteen days after the budding operation the bandage was opened and the initial success of bud take was recorded. For testing the bud take, retention of green color of the scion part inserted on the stock during grafting was checked. Those which retained green colour was recorded as successful grafts on initial observation. Ten days after the first observation, final observations on budding success was made and the numbers of successful grafts were recorded.

The grafted plants remained in the seedling nursery for one month and after that the grafted plants, 50 numbers of successful grafts per plot, were pulled out and each plant properly labeled and observations taken. On the same day of pulling out, after taking the observations, the budded plants were stumped as per the standard procedures recommended (Marattukalam and Mercykutty, (2000) and the budded stumps were prepared as standard methods proposed (Potty, 1980) and planted in poly-bags (plate2, fig·1). A polybag nursery (plate2, fig2) was raised. For polybag planting polythene bags of the size 55x25 cms were used and standard methods were followed for filling the bags. Normal cultural operations and manuring procedures were followed for the maintenance of polybag nursery. Observations on sprouting and growth of the poly-bag plants were recorded.

At two to three whorl stage of growth the plants were transplanted to the field in a spacing recommended for bud-wood nursery (Punnose and Lakshmanan, 2000).

The plants were grown and maintained in the bud-wood nursery for two to three years and detailed studies on various aspects were undertaken.

Design of the experiment

Throughout the experiment, from seed bed to the field planting of polybag plants the same design was adopted with some modifications in plot size according to the availability of materials. A completely randomized design with five replications was followed. The plot size was 200 seeds for nursery studies and 40 seeds for recording seed characters in the laboratory. In seedling nursery 160 germinated seeds were taken as plot size. Slight variations occurred as per the availability of germinated seeds. For the observations on bud grafted plants and for raising poly-bag nursery 50 plants per plot was planted. Finally when the polybag plants were transplanted to the field, the plot size was 30 plants.

Observations.

Seeds and Seedlings.

Each seed was weighed accurately using an electronic balance and seed volume was taken by water displacement method using a measuring cylinder and water. Seed germination was recorded once in two days and the total number of seeds germinated, per plot, and percentage germination were computed.

Plant vigor, was observed at the age of two months. The parameters used are number of leaf stories, plant height and biomass production. The number of leaf stories per plant in each plot was counted and the proportions of plants with single storied, double storied and multistoried leaves were computed. The plant height was measured using a meter scale. For recording the dry weights of root and shoot five plants per replication, selected randomly, were pulled out, washed and dried in an oven and weighed using an electronic balance and the root/shoot ratio was computed.

At the age of four months, the number of plants established per plot was counted and establishment percentage was worked out. Plant height was recorded using a meter scale and collar girth was recorded using a tailor's tape. The plants having a girth of 2.5 centimeters or above at the collar region were selected for bud grafting and the number of seedlings which attained buddable girth, per plot was assessed.

Budding

From among the plants, which attained a girth of 2.5 centimeters or above, those, which had, good peeling quality were selected and green budding (plate3) carried out using RRII 105 as scion. Two weeks after the first round of budding, a second round of budding was carried out and the total number of plants budded, per plot was counted and recorded. The budding success, initial and final, was also recorded as per standard methods, as described earlier.

Bud-grafted seedlings and budded stumps.

Observations on surface morphology of the graft area and positional growth variations, including the graft area, were recorded from the bud-grafted seedlings, before planting in polybags.

Growth

For studying the positional growth variations after bud grafting, girth measurements were taken from five positions of each bud- grafted seedling as detailed below:

Position 1 - Two cm above the upper joint of the bud patch.

Position 2 - At the upper joint of the bud patch.

Position 3 - At the bud point of the bud patch.

Position 4 - At the lower joint of the bud patch.

Position 5 - Two cm below the lower joint of the bud patch.

The measurements of girth were taken using twine and scale.

Surface morphology of the graft area

Morphological observations of the graft area taken were (1) surface texture of the union part as to whether it is rough and corky or smooth and level; (2) completion of the filling growth (plate 4) in between the stock tissue and the scion patch. The number of plants in each category per plot was counted and the proportion as percentage of total plants per plot was calculated.

Polybag Nursery

The observations taken from polybag nursery are

1. Sprouting.
2. Stem growth.
3. Disease.

For taking sprouting observations, the sprouted plants were noted, two weeks after poly-bag planting .The observations repeated for three more times at two weeks interval. Number of plants dried after sprouting was also noted. The percentage of sprouted plants was calculated. Growth aspects of the stem recorded are

1. Girth at scion base.
2. Girth at lower joint of stock and scion.
3. Girth at 2 cm below the lower joint (stock part).
4. Total plant height.

The powdery mildew disease was observed in the disease season. The numbers of affected plants were noted and the percentage was calculated.

Bud wood Nursery

From the plants in bud-wood nursery, various observations were taken on different aspects such as

(1) Growth .

(2) Leaf diseases.

(3) Bark anatomical studies with emphasis to the graft union.

(4) Quantification of intra-xylary phloem in the stem.

(5) Biochemical aspects.

(6) Molecular aspects .

(7) Test tap yield.

Growth

After one year's growth in the bud wood nursery the following growth observations were taken.

1. Plant height.
2. Girth of the stock (Two cm below the bud joint).
3. Girth at the bud joint.
4. Girth of the scion base.
5. Girth of the scion plant at 50 cm height.

After two years growth in the bud-wood nursery the parameters studied were

1. Girth at 10 cm above the bud union.
2. Girth at 50 cm above the bud union.
3. Plant height.
4. Number of buds per meter of bud-wood.
5. Yield on test tapping.

The girth and plant height were recorded using a tailor's tape and a meter scale respectively. The number of buds available from one meter of bud-wood was assessed by counting the numbers from one meter per plant, from above 15 centimeters from the base of the shoot. The plot mean was estimated.

Test tapping was carried out by the incision method proposed by Annamma *et al.*, (1989).

Bark anatomy of the graft union.

Sample collection.

For anatomical study two plants per plot, at random positions were selected. Bark samples were collected from two height positions of the stem. One is the graft area of the plant and the other is a more distal area, at 50 cm above the bud union. Collection from the graft area was done so that the sample covers the stock-scion joint, a portion of the scion bark and a portion of the stock bark. The samples were collected using a bark sampler designed for this purpose. Using the sampler, four linear cuts were made in a square shape with two cm sides and in a depth to reach the surface of the wood and the piece of bark along with the cambium were removed carefully.

Processing of tissue and observations.

The bark thickness was measured using a scale and the samples fixed in formalin acetic acid. Sections were cut from the bark samples in two longitudinal planes; radial (100 μm thickness) and tangential (70 μm thickness) using a Leitz sliding microtome. The sections were stained in freshly prepared Sudan 111 for staining the latex vessels. The sections were put in the stain for 10- 12 hours, washed well with tap water and subsequently with distilled water and stored in dewatered glycerine. For observation, the radial longitudinal sections (RLS) were mounted in glycerine and observed under a student's microscope. The bark anatomical characters were recorded with the help of an eyepiece micrometer and the values were converted as actual measurements. The bark-anatomical characters recorded from the RLS were

1. Total bark thickness at four positions; stock stem, interface, scion base and distal position from the bud-union, at 50 centimeter height.
2. Thickness of soft bast at the four positions.
3. Number of latex vessel rows at the four positions.

Observations were taken from three sections per bark and the mean was computed before converting to the actual measurements. The percentage of hard bast was computed using the total bark thickness and thickness of soft bast.

The continuity of latex vessels and the orientation and deviations in the running direction at the union area were studied. Photomicrographs were taken using a camera – attached Leica Diaplan microscope and stereomicroscope.

The tangential longitudinal sections, stored in glycerin after Sudan staining, were washed and restained with a mixture of Harri's haematoxylin and phenolic bismark brown in a proportion of 2:1 by volume which is an excellent stain for nuclei and cytoplasm. The stains were prepared and staining procedures followed as suggested by Purvis and Collier, (1966). The sections were observed under a research microscope. The nature of axial connections and continuity of sieve tubes and axial parenchyma of stock and scion at the stock- scion interface was observed and photomicrographs were taken.

Intraxylary phloem.

Sample collection.

Stem samples of one-year growth were collected from the same plants selected for bark sample collection. Two stem pieces per plant were collected from 4 cm below the tip of the branches. The samples were fixed in formalin acetic acid.

Processing and observations

Cross sections of the stem were cut in 40 μm thickness and double stained with safranin and fast-green following the procedures suggested by Sass; (1958). The sections were mounted in glycerine, observed under a Student's microscope and the number of intraxylary phloem and primary xylem points present in each section was counted. Three sections per stem were observed.

Yield recording

Yield recording was carried out using the method suggested by Annamma *et al.*, (1989) for test tapping two to three year old rubber plants. The device used for test tapping have two blades, fixed parallel to each other on one side at a distance of 10 cm in between. The blades are fixed in such a way that the incisions made on the plant are at an angle of 25 ^{0} to the horizontal.

By applying this device at a height position, 10 cm (same height on all plants), tapping was done. The latex oozed out from the incisions of each plant was collected separately on pre- dried and weighed blotting paper and dried in an oven. The dry weight was recorded and the dry weight of rubber was computed by reducing the paper weight from the dry weight of rubber with paper. Using the data on tree yield plot mean was calculated.

Leaf Diseases

Observations on powdery mildew were recorded during summer season. In two consecutive years (2002 and 2003), the second and third year after planting. Leaf samples were collected from five trees randomly selected per plot. Five leaves from the top storeys of the branches from both sides of the stem were collected at pendant stage, (Premakumari, 1992). These leaves were graded according to the intensity of infection on 0-5 scales and mean score per plot was calculated and expressed as percentage of disease intensity (Horsfall and Heuberger; 1942). The common formula used is,

$$\text{PDI} = \frac{\text{Sum of all disease ratings} \times 100}{\text{Total No; of ratings} \times \text{Maximum No. of disease grade}}$$

Soon after south - west monsoon, shoot rot observations were made from the bud wood nursery at the second year of planting. Number of shoot rot affected plants per plot was counted and computed as percentage of total plants per plot.

Biochemical study

For biochemical assay young leaves at early pendant stage were collected from two-year-old bud wood plants. For sampling one plant per replication was selected, the total number of plants per treatment being five. These leaves were used for the isozymes study and for the estimation of total protein content. The isozymes studied were peroxidase and esterase.

Estimation of Peroxidase

Sample preparation

Extracted 1 gm of plant tissue in 3ml of 0.1 ml phosphate buffer (pH 7) by grinding using a pre- cooled mortar and pestle.

Preparation of Phosphate buffer

1.	Monobasic sodium phosphate	3.70grams.
2.	Dibasic sodium phosphate	3.58 grams.
3.	Sterilized water	200 ml .

Centrifuged the homogenate at 18,000-x g at 5^0 centigrade for 15 minutes. The supernatant was used as enzyme source within 2-4 hours, which was kept in an icebox. Pipetted out 3 ml buffer solution, 0.05 ml guaicol solution, and 0.1 ml enzyme extract and 0.03 ml hydrogen peroxide solution into a cuvette. (buffer solution was brought to 25^0 centigrade before the assay). A water blank was also used in the assay. Mixed well and placed the cuvette in the spectrophotometer. Waited until the absorbance was increased by 0.05. The time required to increase the absorbance to 0.1 was noted using a stopwatch. The enzyme activity of peroxidase was expressed in terms of rate of increased absorbance per unit time per mg protein or tissue weight.

Total Protein Estimation

Protein content was determined by the method of Lowry's *et al.*, (1951). Enzyme extracts prepared by homogenizing 1 gm leaf tissue in 3 ml of 0.1 m phosphate buffer, which was grinded with a pestle and mortar. Buffered extract was filtered through a corah cloth. Centrifuged and used the supernatant for protein estimation.

Reagents used

(a) 2% Sodium Carbonate in 0.1 N Sodium Hydroxide (Reagent A).

(b) 0.5 % Copper Sulphate in 1 % Potassium Sodium tartarate (Reagent B).

(c) *Alkaline copper solution*: Mixed 50 ml of A and 1 ml of B prior to use (Reagent c).

(d) *Folin- Ciocalteau Reagent* (Reagent D).

Working Standard

Diluted 10 ml of the stock solution to 50 ml with distilled water in a standard flask. One ml of this solution contains 200 micro gram proteins.

Estimation

1. 0.2, 0.4, 0.6, 0.8, and 1 ml of the working standard was pipetted into a series of test tubes.
2. 0.1 ml and 0.2 ml of the sample extract was pipetted into two other test tubes.
3. The volume was made up to 1 ml in all the test tubes. A tube with 1 ml of water served as the blank.
4. 5 ml of Reagent C was added to each tube, mixed well and allowed to stand for 10 minutes.
5. Added 0.5ml of Reagent D mixed well and incubated at room temperature in the darkness for 30 minutes. Blue colour was developed.
6. Readings were taken at 660 nm.
7. Standard graph was drawn to calculate the amount of protein in the sample and expressed as milligram per liter.

Det ection of Esterase

Esterase enzyme activity was detected by Native PAGE gel.

Preparation of Gel

Thoroughly cleaned and dried the glass plates and spacers, then assembled them properly. Held the assembly together with bulldog clips. Clamped in an upright position in order to seal the chamber between the glass plates 2 % agar (melted in a boiling water bath) was applied around the edges of the spacers. Sufficient volume of separating gel mixture was prepared and carefully poured into the chamber between the glass plates and placed the comb in the gel. Layered distilled water on top of the gel and left to set for 30-60 min.

Preparation of separating gel

Stock acrylamide solution	6.6 ml.
Tris – HCL	4.0 ml.
Water	9.0 ml.
Ammonium persulphate solution	1 ml.
TEDMED	10 micro lit.

After polymerization the combs were removed carefully.

Carefully installed the gel after removing the clips, agar etc in the vertical slab of the electrophoresis gel unit. Filled it with electrode buffer and removed any trapped air bubbles at the bottom of the gel. Connected the cathode at the top and turned on the DC- power briefly to check the electrical circuit. Marked the positions of wells on the glass plate with marker pen.

Sample Preparation

Homogenized 3 gm leaf tissue with 5 ml of phosphate buffer grinding with a pre- cooled mortar and pestle, centrifuged for 10 min. Kept the supernatant in low temperature. These 20 samples were cyclomixed with bromophenol. Kept the sample solutions at very low temperature and injected it into the sample well through the electrode buffer. Turned on the current to 10-15 m A for initial 10-15 minutes until the samples travel through the stacking gel. The stacking gel helps concentration of the samples. Then continued the run at 30 m A until the bromophenol blue reaches the bottom of the gel (about 3 hours). After the run is complete, carefully removed the gel from between the plates and incubated the gel in a solution given below at 37 degree centigrade for 20-30 minutes in darkness.

Sodium dihydrogen phosphate	2.8 g.
Disodium hydrogen phosphate	1.1 g.
Fast blue RR salt	0.2g.
Alpha –naphthyl acetate	0.03g.
Water	200 Ml.

Stopped the enzyme reaction by adding a mixture of methanol, water, acetic acid and ethyl alcohol in the ratio 10:10:2:1. The esterase fractioned into bands was seen coloured. The gel was photographed. The number and intensity of bands were counted, graded and computed the mean for each group.

Genetic analysis:

RAPD analysis was done to evaluate the genetic polymorphism among the four groups of seedlings and also for studying the genetic incompatibility and its possible impacts.

Sampling:

Samples of bark were collected from the seedling portion of bud-grafted plants. Samples were taken from four plants each from the four different treatments in terms of stock source variations; of which two each were showing external symptoms of graft incompatibility. Thus the samples included eight numbers each from incompatible and compatible grafts. The samples were kept in icebox immediately after collection and brought to the laboratory.

Preparation of Genomic DNA

Genomic DNA, from bark samples was isolated and purified following the modified CTAB extraction procedure (Doyle and Doyle, 1990). About 1g of fresh bark tissue was ground to a fine powder in liquid nitrogen using a mortar with pestle and homogenized in DNA isolation buffer.[2% CTAB; (hexadecyl triethyl ammonium bromide), 1.4M Nacl, 20nM EDTA (pH 8.0), 100nM Tris-Hcl (pH 8.0), 1% polyvinyl polypyroidone (PVPP), 1% 2-mercaptoethanol]. The homogenate was then incubated in a water bath at 65° C for 30 min ('and the tubes were agitated frequently). The extracts were centrifuged for 15 min (at 8000 rpm) and the supernatant was transferred to fresh centrifuge tubes and emulsified with an equal volume of phenol: chloroform: isoamyle alcohol (25: 24: 1) and spun at 10000 rpm for 10 min. The top aqueous phase was removed carefully to new tubes and incubated at 37° C for 3h after adding 10µl of Rnase A (10 mg/ml). The samples were emulsified with chloroform and spun at 10,000 rpm for 5 min and re-extracted until a clear aqueous phase was obtained. The DNA was precipitated with an equal volume of isopropanol. After 15 minutes of centrifugation at 10,000 rpm the DNA pellet was washed with 70% ethanol and air-dried. Then it was dissolved in about 300ml of TE buffer [10 mM Tris – Hcl, (pH 8.0); 1 mM EDTA (pH 8.0)]. DNA quality was tested by agarose gel 0.8% electrophoresis and stored at 20°C until use for PCR amplification.

DNA amplification by PCR

PCR was carried out in a 20μl reaction mixture containing 10- 15 ng of template DNA, 250 nM of primer, 1.5mM $mgCl_2$, 100μM each of dATP, dGTP, dCTP and dTTP (Amersham-Pharmacia, UK), 0.5 unit of Taq DNA Polymerase enzyme and 1x reaction buffer. In order to avoid evaporation, the reaction mixture was overlaid with approximately 25 μl of mineral oil (Sigma, USA). Amplification was performed in 0.5 ml tubes placed in a 48 –well thermal cycler (Perkin-Elmer DNA Thermal Cycler 480, USA). Tubes containing all the reaction components, except for the DNA template were included as a control for each primer used. The PCR programme included: a 4 min initial denaturation step at 94°C, 1 min denaturing at 94°C, 1.30 min at 38°C for annealing and 2.0 min at 72^0C for extension. Thirty-five application cycles were performed and the last cycle was followed by 7 min at 72°C to ensure that primer extension reactions proceeded to completion. Eighty oligonucleotide random primers, each of 10 nucleotides long (Operan Technologies Inc; Alameda, CA, USA) were tested individually for the amplification of genomic DNA. Eight primers, which produced clear banding, pattern after PCR amplification were selected for further RAPD analysis. In order to confirm whether the amplified products are reliable, amplification with each primer was repeated at least three times.

Gel electrophoreis and photography.

After PCR amplification, loading buffer was added to the amplified products. The RAPD products were separated by electrophoresis using 1.5% agarose gels containing 0.5 μg/ml ethidium bromide in 0.5X TBE buffer (Sambrook et al. 1989). Electrophoresis was performed at 50V power supply for about 4 h until the bromophenol blue dye front had migrated to the bottom of the gel. The molecular standard used was the lamda DNA double digested by EcoRI/Hind111. The gels were visualized and photographed under UV- light. The reproducibility of the amplification products was tested at least thrice for each experiment.

B. Observations on mature trees

Materials:

The experiment was started in 2000 April. One hundred tapping trees of the clone RRII 105 were selected randomly from a plantation in the campus of the Regional Engineering College at Pampady, in Central Kerala. The trees were ten year old and were under tapping for the previous one year, with an intensity of 1/2S d/3. On the start of the experiment the system of tapping was changed to 1/2S d/2. Tapping was continued.

Observations

Total volume of latex:

The total volume of latex (ml) of individual trees was measured on normal tapping days at monthly intervals and annual mean yield per tree was computed.

Dry rubber content:

The dry rubber content (DRC), of individual trees was. recorded. DRC is the quantity of dry rubber contained in the latex expressed as percentage by weight. From the latex collected from a tree, a sample of 20ml was taken and coagulated using 1% formic acid. The coagulum was pressed and dried in an oven and oven dry weight was taken .For each tree DRC was calculated as:

$$\frac{\text{Oven - dry weight of rubber}}{20} \times 100$$

From the data annual mean of DRC per tree was calculated.

Summer variations:

For estimating the summer variations of Latex volume and DRC of individual trees, the mean values of the respective characters over five months, from January to May were computed from the monthly data. The difference between this mean and the annual mean was expressed as percentage of annual mean.

Growth characters:

The growth data in terms of girth and bark thickness at five height positions of the tree trunk were also recorded at six monthly intervals from April 2000 onwards. Girth was recorded using a tailor's tape and bark thickness using a bark gauge, specially prepared for that purpose. The height positions selected for data collection were marked as 0 (Scion base), 1 (60 cm above the bud union), 2 (95 cm above the bud union) 3 (125 cm above the bud union) and 4 (150 cm above the bud union). Bark thickness was recorded separately from the tapping and untapped sides of each tree. Girth and bark thickness at the stock portion (just below the bud union) was also recorded.

Anatomical characters:

For anatomical studies, 30 trees at random positions, from among the experimental trees, were selected. Bark samples were collected from three height positions (60cm, 95cm and 125cm above the bud union) of the tapping side as well as from the untapped side in April 2000. Samples (at a position of four centimeters from the tip) of stem were collected from one year old twigs (two twigs per tree) from three groups of trees; (1) tapping trees (2) untapped trees of the same age, in the same plantation, and (3) from two year old plants of the same clone in a bud wood nursery. The samples of stem were cut from the same position of all twigs, four centimeters below the tip.

The samples were fixed in formalin acetic acid. Longitudinal sections of the bark in tangential (80μm thick) and radial (100μm thick) planes were taken and stained with Sudan III. Cross sections (40 μm thick) were cut from the stem and stained with safranin and fast - green. Sectioning was done using a Leitz sledge microtome. For microscopic observations a projectina / student's microscope with a micrometer attachment were used and measurements were computed and photographs taken with a Leitz Diaplan microscope.

The characters recorded are: (plate-5).

1. Bark thickness.

2. Thickness of the soft bast.
3. Number of latex vessel rows.
4. Density and width of latex vessels.
5. Height and width of phloem rays .
6. The numbers of tanniferous and untanniferous cells per unit area.

The percentage of tanniferous cells, out of total cells, in a unit area of 2.5mm^2 and the percentage of hard bast thickness out of total bark thickness were assessed. The ratio of ray height to the ray width was also computed which is an indicator of the inclination of axially running tissue.

For all the characters mean values of the three positions of individual trees, with respect to each side, were calculated and used for comparison of tapped vs untapped sides.

The anatomical characters recorded from the stem are

1. Number of primary xylem points.
2. Number of intraxylary phloem points (plate- 6).

Tapping panel dryness

The number of TPD affected trees (plate -8) were assessed during 2003 April.

Statistical Analysis

Simple and appropriate statistical tools described in standard books (Gomez and Gomez, 1976) were used for data analysis. CRD analysis was done for estimating the variability among stock sources, for different characters, and for comparing different positions of grafted plants at various stages of growth. For testing the genetic influence on the expression of important nursery characters, the variances were partitioned into genotypic and phenotypic and broad sense heritability estimated. Student's 't' test, for comparison of means, was applied for comparing the incompatible vs. compatible grafts for different characters at different stages of growth in nursery experiment. For comparing the structural characters on tapping and untapped sides of mature trees also 't' test was done using the formula suggested for paired samples. Intra-clonal variations were estimated for seed characters and for the important traits of mature trees. Co-variance analysis was done to estimate the useful associations in both experiments. For the inferences test of statistical significance was also done following the standard procedures.

RESULTS

4. RESULTS

A. NURSERY EXPERIMENT

1. VARIABILITY DUE TO STOCK SOURCES

1.1.Root stock seeds and seedlings.

Table. A. 1.1.1 Comparison of seed types for seed characters and germination percentage

Seed type	Seed volume (cm^3) **	Seed weight (g) **	Germination (%)**
	SE 0.14 CD 0.42	SE 0.04 CD 0.13	SE 2.86 CD 8.57
Assorted	7.47	4.73	72.20
RRII 105	8.58	5.50	80.40
RRIM 600	6.85	4.25	79.20
PB 28/59	7.26	4.58	46.20

** $P < 0.01$

Table. A. 1.1.1 depicts the comparative values of assorted and monoclonal seed types with respect to the three traits. Seed volume recorded a range of 6.85 to 8.58 cm^3 with maximum value for the monoclonal seeds of RRII 105 and minimum value for RRIM 600. The range of values recorded for seed weight and germination percentage were 4.25 to 5.50 and 46.20 % to 80.40% respectively. The stock sources showed significant differences for the three traits. For seed volume and seed weight

RRII 105 was superior to the other two clones and assorted seeds. RRIM 600 recorded the lowest values for both the traits. For germination percentage also RRII 105 recorded the highest value though the difference with assorted seeds was not statistically significant. Germination percentage was the lowest for PB 28/59.

Table. A. 1.1.2. Estimates of variability among the seed sources for seed characters and germination

Characters	GM	MS	VR	GCV	PCV	H^2
Seed Volume	7.54	2.74	28.60**	9.65	10.49	84.66
Seed weight	4.79	1.63	171.71**	11.90	12.07	97.25
Germination %	69.50	1271.80	31.13**	22.58	24.38	85.77

** $P < 0.01$

Variability parameters for seed characters and germination percentage are shown in table. A. 1.1. 2. The variance ratios for all the three characters were highly significant. GCV values showed a range of 9.65 to 22.58, for different characters against the corresponding PCV values of 10.49 to 24.38.The values of genotypic and phenotypic coefficients of variations showed that variation for seed weight, seed volume and germination percentage have high genetic involvement and those characters are highly heritable. For all the three characters the heritability values recorded are very high.

Table. A. 1.1.3. Intraclonal variations (CV values) in seed types for seed weight and seed volume.

Seed type	Seed weight	Seed volume
Assorted	20.42	20.44
RRII 105	10.16	17.18
RRIM 600	11.10	13.49
PB 28/59	12.20	12.32

The coefficients of variations for seed weight and seed volume with respect to the seed types are given in table. A. 1. 1. 3. Seed weight recorded CV values ranging from 10.16 to 20.42 for the different seed types with maximum value for assorted seeds. For seed volume the range of CV among the seed types was 12.32 to 20. 44 with maximum value for assorted seeds. As expected, the assorted seeds recorded higher CV for both the characters when compared to the monoclonal seed types assuring better uniformity of monoclonal seeds.

Table. A. 1.1.4. Comparison of seed types for biomass production (dry wt.) and plant height of young seedlings at the age of one month.

Seed types	Root weight (g)	Shoot weight (g)	Root/shoot ratio	Plant height (cm)
Assorted	17.52	25.60	0.72	23.7
RRII 105	16.76	27.00	0.63	24.98
RRIM 600	17.40	25.88	0.68	25.82
PB 28/59	17.32	25.76	0.71	23.54
SE	0.82	0.96	0.03	0.64

Table. A. 1. 1. 5 Estimates of variability for biomass production and plant height of young seedlings

Characters	GM	MS	VR
Root weight	17.25	0.57	0.17 NS
Shoot Weight	26.06	2.03	0.44 NS
Root/shoot ratio	0.68	0.01	1.54 NS
Plants height	24.51	5.89	2.92 NS

The mean values of root weight shoot weight, root/shoot ratio and plant height recorded for the different seed types are given in table A. 1.1. 4. Table A. 1.1.5 depicts the mean and variances for the same characters. Root weight, shoot weight, root/shoot ratio and plant height were observed at juvenile age, one month after transplantation into the nursery. Root weight showed a range of 16.76 g to 17.52 g Shoot weight and root /shoot ratio recorded a range of 25.60g to 27.00g and a range of 0.63 to 0.72 respectively. Plant height ranged from 23.54 cms to 25.82 cms. For none of the characters, variance ratio was significant. For root weight and root/shoot ratio, assorted seeds recorded better values than the monoclonal seed types.

Comparative vigor, of two-month-old plants in terms of number of leaf flushes, of the stock seedlings raised from assorted seeds and from monoclonal seeds of the three clones - RRII105, RRIM 600 and PB28/59 is shown in Fig 1. For the proportion of single storied leaf all the four types were comparable. Around 25 percent of the plants in all the four groups had only one leaf flush. 50 percent of the plants raised from the seeds of RRII 105 developed two leaf flushes; but growth uniformity was low for this group. PB 28/59 also showed better growth vigor.

Table A. 1.1.6. Comparison of seed sources for establishment and growth of the seedlings

Seed sources	No.of transplantable germinated seeds ** (As% of seeds sawn)	No.of seedlings established ** (As % of transplanted seeds)	Number of seedlings which attained buddable girth after 4 month's growth ** (As % of established seedlings)
	SE 5.86 CD 17.56	SE 8.76 CD 26.27	SE 8.22 CD 24.63
Assorted	145.80 (72.20)	120.40 (82.58)	68.40(58.16)
RRII 105	160.60 (80.40)	122.20 (76.09)	80.40(65.46)
RRIM 600	160.80 (79.20)	126.20 (78.48)	61.80(47.87)
PB 28/59	91.80 (46.20)	58.00.(63.18)	35.40(60.10)

** $P < 0.01$

Actual numbers and percentages (in parenthesis) of transplantable germinated seeds, establishment success in the seedling nursery and number of buddable seedlings obtained per plot are depicted in table A. 1.1.6.Treatment differences were statistically significant at 1percent level. The number of transplantable seedlings generated from 200 seeds/lot showed a range of 91.80 (PB 28/59) to 160.80 (RRIM 600). For the numbers established and attained buddable girth the values ranged from 58.00 (PB 28/59) to 126.20 (RRIM 600) and 35.40 (PB 28/59) to 80.40 (RRII 105) respectively For all the three characters, PB 28/59 was significantly inferior to the other types. The absolute values indicated superiority of RRII 105 and RRIM 600 over the assorted seeds for the percentage of transplantable seeds.

The variability parameters of the characters under study are furnished in table A.1.1.7. The variance ratio observed for all the characters were highly significant. The Coefficients of variation (CV) recorded for the characters showed a range of 9.37 to 29.87 with the lowest value for the number of transplantable seedlings and the highest value for the number of buddable seedlings. For number of seedlings established the CV recorded was 18.36. GCV values showed a range of 23.04 (for transplantable germinated seeds) to 29.39(number of seedlings established). For number of buddable seedlings also good GCV (27.91) was recorded. The PCV values for the three characters are 24.87, 34.65 and 40.88 respectively. Heritability values showed a good range of 46.60(number of buddable seedlings) to 85.81 (number of transplantable germinated seeds).

Table A.1.1.7. Estimates of variability for transplantable germinated seeds, establishment success and buddability in terms of growth.

Characters	General Mean	MS	Variance ratio	CV %	GCV	PCV	H^2 %
No. of transplantable germinated seeds	139.75	5356.05	31. 24**	9.37	23.04	24.87	85.81
No.of seedlings established	106.70	5299.80	13. 80**	18.36	29.39	34.65	71.92
No.of buddable seedlings (in terms of girth)	61. 50	1810.20	5.36**	29.87	27.91	40.88	46.60

** P < 0.01

1. 2. Budding success.

Table. A. 1.2.1. Comparison of seed sources for budding success

Seed sources	Total no. of plants budded *	Budding success (No)	
		Initial **(%)	Final **(%)
	SE 8.08 CD 24.22	SE 7.78 CD 23.31	SE 7.42 CD 22.26
Assorted	64.00	62.80(98.13)	62.40 (97.50)
RRII 105	77.60	77.00(100)	74.60(96.88)
RRIM 600	56.20	55.80(99.29)	52.00(92.86
PB 28/59	34.00	33.60(98.82)	33.00(97.06)

* P< 0.05 ** P < 0.01

The number of seedlings budded and budding success, initial and final, are given in table A. 1.2.1. For all the three characters treatment differences were significant. Total number of plants budded represent the number of plants, which attained buddable girth and had peeling quality. This character showed a range of 34.00(PB 28/59) to 77.60(RRII 105) among the seed sources. Budding success, initial and final, recorded were in the range of 33.60(PB 28/59) to 77.00 (RRII 105) and 33.00(PB 28/59) to 74.60(RRII 105) respectively. For these traits also PB 28/59 was the only inferior type, which was not reflected on the percentage values, which ranged from 98.13 to 100 for initial success and 92.86 to 97.50 for final success. From the data it is also implicated that the inferiority of PB 28/59 is resultant of the low germination percentage.

Table A 1. 2. 2. Estimates of variability for the number of plants budded and budding success.

Characters	General Mean	MS	Variance ratio	CV %	GCV	PCV	H^2 %
Number of plants budded (having peeling property at the time of budding).	57.95	1665.65	5.10*	31.17	28.24	42.07	45.08
Initial bud take (Nos).	57.30	1637.13	5.42**	19.51	28.52	41.64	46.90
Final budding success (Nos).	55.50	1551.53	5.63**	29.91	28.78	41.51	48.09

* $P < 0.05$; ** $P < 0.01$

The estimates of variability for the total number of plants budded and budding success are shown in table A. 1.2.2. For all the three characters the variance ratios were significant. The significance was at higher level for budding success The CV values recorded for number of plants budded, initial success and final success are 31.17, 19.51 and 29.91 respectively.

1.3. Morphological observations of bud union

The two major morphological features observed on the graft area are (1) Whether the joining at the periphery of bud patch and the cut portion of the stock seedling is complete or partial (2) Texture of the surface at the union part, whether it is corky and rough or smooth and level. Quantitative estimation of the different types was made as percentage of total number of grafted plants. In the extreme case of corky and undulating surface, a bulging of the plant base was evident

Quantitative data on the surface features of the graft union are given in table A.1. 3.1. Treatment differences for the percentages of plants with corky rough surface and with smooth level surface were significant at I percent level. For rough surfaced graft area with scar, the four stock sources showed a range of 11.90 percent (PB 28/59) to 34.40 percent (assorted seeds). The plants raised with RRII 105 as stock source produced 15.20 percent of rough surfaced grafts while the group with RRIM 600 as stock source 31.73 percent was of such type. Smooth surfaced graft area was observed for 65.60 percent of the plants raised on seedlings of assorted seeds and 88.10 percent of the plants raised on the seedlings of PB 28/59. For RRII 105 and

RRIM 600 the proportions were 84.80 and 68.27 respectively. The differences due to stock differences were highly significant.

Table A.1.3.1 Comparison of seed sources for the surface morphology of bud union

Seed sources	Surface morphology		
	% of plants with incomplete union	% of plants with rough surface**	% of plants with smooth surface**
	SE 6.60 CD NS	SE 3.18 CD 9.81	SE 3.60 CD 10.81
Assorted	19.24	34.40	65.60
RRII 105	12.40	15.20	84.80
RRIM 600	29.30	31.73	68.27
PB 28/59	26.15	11.90	88.10

NS – *non significant* ** $P < 0.01$

For the proportion of plants with incomplete union at the periphery of scion and stock, the stock sources showed a range of 12.40(RRII 105) to 29.30(RRIM 600). Among the plants raised on the seedlings of PB 28/59, 26.15 percent showed incomplete joining. Among the plants raised on the stock seedlings raised from assorted seeds, the proportion of incomplete joining was 19.24 percent. The differences among the groups of plants on varying stock types were not statistically significant.

Table A 1. 3. 2. Estimates of variability for the surface characters of stock-scion union.

Characters	General Mean	MS	Variance ratio	CV %	GCV	PCV	H^2 %
Percentage of budded stumps with gap between stock and scion	21.77	283.34	1.30 NS	67.75	NS	NS	NS
Percentage of budded stumps with rough surface on the union	22.61	735.41	14.50**	31.50	51.76	60.59	73.00
Percentage of budded stumps with smooth surface on the union	76.69	649.95	6.10**	10.12	14.10	17.59	64.30

NS – non significant ** $P < 0.01$

The estimates of variability for the surface characters at the region of stock-scion union are furnished in table A. 1.3.2. Very high CV value (67.75) was recorded for the proportion of plants with incomplete sealing (with gap in between stock and scion bark.). But the variance ratio was not statistically significant. For the proportions of rough surfaced and smooth surfaced plants the variance ratios showed high significance and the CV values recorded are 31.50 and 10.12 respectively.

1.4. Comparative merits of root stock sources for further growth and test tap yield of the grafted plants.

1.4.1. Growth variations of budded stumps.

Table A.1.4.1.1. Comparison of stock sources for growth variations of budded stumps, in terms of girth (cm).

Stock sources	Stock base * SE 0.09 CD 0.27	Lower joint* of bud union SE 0.10 CD 0.30	Bud point* SE 0.08 CD 0.26	Upper joint ** of bud union SE 0.08 CD 0.24	2cms above* bud union SE 08 CD 0.25
Assorted seeds	4.19	4.34	3.76	3.91	3.55
RRII 105	3.91	4.03	3.55	3.68	3.41
RRIM 600	3.78	3.91	3.41	3.52	3.20
PB 28/59	4.22	4.30	3.83	3.99	3.56
G. Mean	4.03	4.16	3.64	3.78	3.43

* $P < 0.05$ ** $P < 0.01$

Data on the positional girth variations of budded stumps, with respect to the four types of stock sources are shown in table A. 1.4.1.1 and the estimates of variability are given in table A. 1.4.1.2. The treatment differences were significant and the significance is more strong for the girth at the upper joint of bud union. Mean girth at the stock base showed a range of 3.78 cm to 4.22 cm with maximum value for PB 28/59 and minimum value for RRIM 600. For the girth at lower joint of bud union, the range was 3.91cm(RRIM 600) to 4.34(assorted seeds) followed by PB 28/59. For the girth at bud point, at upper joint of the graft and above the graft area also RRIM 600 showed the least value and the other three seed sources were comparable to each other. Girth is the highest at the lower joint, even higher than the stock base; and the girth at upper joint is higher than that at the bud point of the respective group.

Table A.1.4.1.2 Estimates of variability for growth variations of budded stumps.

Characters	MS	CV	GCV	PCV	VR	H^2
Stock base	0.23	4.97	4.88	6.96	5.83*	49.10
Lower joint of bud union	0.25	5.41	4.80	7.21	4.92*	44.30
Bud point	0.18	5.37	4.68	7.12	4.81*	43.20
Girth at the upper joint of bud union	0.23	4.82	5.22	7.10	6.86**	54.00
2cms above bud union	0.14	5.37	4.24	6.79	4.07*	38.80

* $P < 0.05$ ** $P < 0.01$

Coefficients of variation for the girth at two positions apart from the graft area, bud point, and the upper and lower joints showed a range of 4.82(upper joint) to 5.41(lower joint). GCV values recorded a range of 4.24 (higher level apart from graft area) to 5.22 (upper joint) against a range of PCV values, 6.79(higher level apart from graft area) to 7.21(lower joint). For girth at the different points H^2 values showed a range of 38.80 to 54.00.

1.4. 2 Sprouting

Table A. 1.4.2.1 Comparison of the budded stumps raised using different stock sources for sprouting percentage and time taken for bud break.

Root sources	Percentage of sprouting at each observation made at two weeks interval				Percentage of dried plants
	Two weeks SE 5.15 CV 64.70 CD-NS	Four weeks SE 5.12 CV 34.12 CD-NS	Six weeks SE 4.73 CV 38.85 CD- NS	Eight weeks* SE 5.19 CV 44.60 CD 7.69	SE 2.73 CV 57.48 CD -NS
Assorted	21.20	36.00	26.00	8.80	8.00
RRII 105	15.60	34.80	28.00	11.20	10.40
RRIM 600	18.30	34.75	27.54	5.80	8.57
PB 28/59	16.08	28.61	22.58	17.25	15.50

NS- non significant * $P< 0.05$

Two weeks after planting, the first observation on sprouting was taken. The percentages of plants sprouted in each group, at two weeks interval are shown in table

A.1.4.2.1 The sprouting percentages for the first observation showed a range of 15.60 to 21.20; but the differences among plants, varying for stock sources were not significant. Observations continued up to eight weeks at two weeks interval. For the second and third observations also the treatment differences showed narrow ranges, 28.61percent (PB 28/59) to 36.00 percent (assorted seeds) for second observation and 22.58 percent (PB 28/59) to 28.00(RRII 105) percent for the third observation. The differences were not statistically significant. The differences for fourth observation, eight weeks after planting, were significant. The values for percentage sprouting recorded a wide range of 5.80 percent (RRIM 600) to 17.25 percent (PB 28/59). Dried plants, percentage of total number of budded stumps planted, recorded a range of 8.00 (assorted seeds as stock source) to 15.5 (PB 28/59 as stock source); the values for RRII 105 and RRIM 600 stock sources being 10.40 and 8.57 respectively. For sprouting time PB 28/59 was significantly inferior and the other three types were comparable to each other.

1.4.3. The Growth variations of polybag plants.

The growth variations of grafted plants in polybag are depicted in table A. 1.4.3.1. and the estimates of variability among the stock sources , for the different characters are given in table A. 1.4.3.2. For plant height, stock girth and scion base girth of grafted plants, grown in polybags up to two to three whorl stages, the differences among stock sources were significant; of which the difference for plant height was significant at 1% level.

Table A.1.4.3.1. Comparison of stock sources for growth variations of polybag plants, in terms of girth and height (cms).

Stock sources	Scion height** SE 1.67 CD 5.02	Stock girth* SE 0.11 CD 0.31	Scion base girth* SE 0.012 CD 0.32	Girth at stock-scion joint SE 0.10	% Increase of stock girth SE 1.94
Assorted seeds	29.07	4.84	2.62	4.94	15.43
RRII 105	24.78	4.54	2.64	4.67	16.22
RRIM 600	22.59	4.37	2.19	4.51	15.82
PB 28/59	19.96	4.68	2.34	4.81	10.94
G. Mean	24.10	4.61	2.45	4.74	14.60

* $P < 0.05$; ** $P < 0.01$

The scion height showed a range of 19.96cms to 29.07 cms for the plants raised on different stock sources with lowest value for PB 28/59 and the highest value for assorted seeds. Girth at stock portion, scion base and stock- scion joint recorded the values ranging from 4.37cm to 4.84 cms, 2.19cm to 2.64 cms and 4.51cms to 4.94 cms respectively; with the lowest values for the plants raised on RRIM 600 as stock. Percentage increase of girth in polybags, from the budded stump stage, recorded a range of 10.94 to 16.22 with minimum value for the plants raised on PB 28/59 as stock source and maximum value for the group with RRII 105 as stock source.

The CV values showed a range of 4.84 to 29.63 among the variables of growth with maximum value for the percentage increase of stock girth followed by scion height. The variance ratio for the difference due to stock source was significant for scion height , stock girth and scion base girth. For the variables of growth, GCV ranged from 3.18 to 14.39 with maximum value for scion height and minimum value for stock- scion joint. PCV values ranged from 5.79 to 31.42 with maximum value for percentage increase of stock girth. Heritability values of the growth characters in polybag ranged from 11.00 to 46.00 with maximum value for scion height followed by scion base girth. Minimum value was recorded for percentage increase of stock girth.

Table A. 1.4.3.2.Estimates of variability for the growth variations of polybag plants

Characters	MS	CV	GCV	PCV	VR	H^2 (%)
Scion height	74.27	15.53	14.39	21.17	5.30**	46.00
Stock girth	0.18	5.07	3.66	6.26	3.62*	34.00
Scion base birth	0.24	9.65	7.87	12.40	4.32*	40.30
Girth at stock-scion joint	0.17	4.84	3.18	5.79	3.16	30.10
% increase of stock girth	30.32	29.63	10.43	31.42	1.62	11.00

* $P < 0.05$; ** $P < 0.01$

1.4.4. Growth variations of field plants, after one-year growth in the field.

The comparative data of field establishment and growth characters of field plants (grown over a period of one year in the field after planting with polybag plants) with respect to the plants raised on different stock sources are depicted in table A. 1.4.4.1. and the variability parameters are shown in table A. 1.4.4.2.

For the proportion of vacancy occurred, variations for stock sources showed statistical significance. Variations for the other characters were not significant. Percentage of vacancy showed a wide range of 1.38 to 11.23 among the plants raised on different stock sources. Maximum vacancy occurred for the group of plants with PB 28/59 as stock and this was the only inferior type. For all the growth characters, assorted seeds recorded maximum values immediately followed by the group with RRII 105 as stock source.

CV for vacancy was very high the value being 137.94. For the other growth characters the CV values ranged from 7.16 to 9.32. Good amount of GCVwas recorded for the vacancy .PCV values were comparatively high, ranging from 7.56 to 171.80 for all characters recorded during the first year of growth in the field.

Table A 1.4.4.1. Comparison of stock sources for establishment and growth of grafted plants in the field, after one year.

Stock sources	% 0f vacancy occurred*	Scion height (cms)	Stock girth(cms)	Girth at stock-scion joint	Scion base girth(cms)	Scion girth(cms)at 50 cms above bud union
	SE 2.48 CD=7.45	SE 9.47 CD NS	SE 0.51 CD NS	SE 0.54 CD NS	SE 0.49 CD NS	SE 0.28 CD NS
Assorted seeds	2.12	248.34	16.08	15.84	12.51	9.49
RRII 105	1.38	244.09	15.38	15.20	11.57	8.91
RRIM 600	1.38	227.18	14.83	14.61	11.19	8.55
PB 28/59	11.23	233.15	14.89	14.62	11.22	8.45
G. Mean	4.03	238.19	15.30	15.07	11.62	8.85

* P< 0.05; NS non significant

Table A. 1.4.4.2. Estimates of variability due to stock types for establishment and growth of grafted plants in the field, after one year.

Characters	MS	CV	GCV	PCV	VR
% Of vacancy occurred	115.90	137.94	102.42	171.80	3.76*
Scion height	473.77	8.89	0.94	8.95	1.06
Stock girth	1.66	7.34	1.85	7.56	1.32
Girth at lower joint of bud union	1.70	7.95	1.52	8.09	1.18
Scion base girth	1.91	9.32	3.30	9.88	1.62
Scion girth at 50 cms above	1.11	7.16	4.16	8.27	2.76

* P< 0.05

1.4.5. Growth variations of grafted plants, after two years in the field.

After two year's growth in the field, further growth and test tap yield were recorded. The data is shown in table A. 1.4.5.1 the estimates of variability are given in table A.1.4.5.2. Significant differences due to stock sources were observed for only one character; scion girth at 50 cm height from the bud union. The CV values for the different characters showed low variability due to stock variations. Comparatively good CV was recorded for test tap yield. When the variability was partitioned as genotypic and phenotypic very low GCV values were recorded for plant height and

Table A.1.4.5.1. Comparison of stock sources for growth and test-tapping yield of grafted plants in the field, grown over two years in the field.

Stock sources	Scion height(cms)	Scion girth(cms) at 10cm above bud union.	Scion girth at 50 cm above bud union*.	Number of buds per meter of budwood	Test tap yield(g/t/t)
	SE 9.18 CD NS	SE 0.25 CD NS	SE 0.22 CD 0.67	SE 0.44 CD NS	SE 0.01 CD NS
Assorted seeds	398.46	13.07	11.23	14.86	0.14
RRII 105	391.17	12.44	10.57	14.23	0.16
RRIM 600	393.81	12.12	10.38	14.14	0.13
PB 28/59	376.86	12.20	10.36	14.52	0.15
G..Mean	389.88	8.05	10.64	14.44	0.14

* $P < 0.05$; NS non significant

scion bud number. In general, the growth and test tap yield of field plants was not much influenced by the stock sources.

Table. A. 1.4.5.2. Estimates of variability for growth and test tapping yield of grafted plants, after two years.

Characters	MS	CV	GCV	PCV	VR
Scion height	468.36	5.27	0.79	5.32	1.11
Scion girth at 10cms above bud union	1.00	0.53	2.89	5.42	3.16
Scion girth at 50cms above bud union.	0.83	4.71	3.17	5.69	3.29*
Number of buds per meter of bud wood	0.52	6.88	0.05	0.07	0.53
Test tapping yield	0.00	17.05	3.67	17.49	1.24

* $P< 0.05$

1.4.6 Variations of anatomical characters due to root stock sources.

Variations of important bark anatomical characters of the bud grafted plants due to stock sources/ height positions (the stock, stock-scion interface, scion base and at 50 cm height from the bud union) are shown in table A. 1.4.6.1.

Total bark thickness

Total bark thickness was the highest at the interface and the next lower value was recorded for stock portion for the plants raised on all stock sources. With respect to both positions, plants raised using assorted seeds as stock source recorded higher values followed by those with RRIM 600 as stock source.

Table A.1.4.6.1.Comparison of field plants raised on different stock sources for bark structural characters of stock, scion and graft interface.

	Mean values for the groups of plants raised on different stock sources					SE	CV
	Positions	Assorted	RRII 105	RRIM 600	PB 28/59		
Total bark thickness (mm)	Stock	2.86	2.73	2.85	2.84	0.14	10.94
	Interface	3.11	2.91	3.02	2.84	0.21	15.77
	Scion base	2.55	2.45	2.55	2.38	0.14	12.73
	50 cm height	1.85	1.84	1.68	1.91	0.08	9.44
Soft bast thickness (mm)	Stock	1.04	1.11	1.08	0.95	0.09	18.12
	Interface	0.91	0.98	1.07	0.91	0.11	25.71
	Scion base	0.88	1.02	1.15	0.92	0.10	21.85
	50 cm height	0.85	0.77	0.67	0.82	0.08	22.77
Hard bast thickness (mm)	Stock	1.18	1.61	1.77	1.89	0.11	14.70
	Interface	2.20	1.93	1.96	1.93	0.15	16.92
	Scion base	1.67	1.43	1.40	1.45	0.01	15.00
	50 cm height	1.01	1.07	1.01	1.09	0.07	14.64
Percentage of soft bast	Stock	36.11	41.16	37.76	38.17	1.91	11.16
	Interface	29.65	33.13	36.30	33.14	2.49	16.85
	Scion base	40.16	44.12	42.86	39.17	2.13	11.44
	50 cm height	42.19	43.98	44.03	43.62	2.11	10.85
Percentage of hard bast	Stock	63.89	56.83	62.24	61.83	2.55	9.32
	Interface	70.35	66.87	63.70	66.86	2.49	8.32
	Scion base	59.85	55.88	57.14	60.83	2.13	8.15
	50 cm height	57.76	56.01	55.97	56.41	2.11	8.33
Number of latex vessels	Stock	3.40	4.60	4.05	3.80	0.29	16.54
	Interface	2.35	3.40	2.95	2.90	0.29	22.65
	Scion base	2.85	3.90	3.40	3.55	0.36	23.26
	50 cm height	2.40	2.70	2.50	2.35	0.24	21.79

Soft bast and hard bast

Soft bast thickness was the highest at the stock portion for the plants raised on the stock of assorted seeds and the seeds of PB 28/59. Scion base recorded high values of soft bast, for the plants raised on RRII 105 and RRIM 600 as stock source compared to the other groups. The soft bast, when considered as percentage of total bark thickness, interface recorded the least value for all groups. Hard bast was the highest at the interface; in terms of actual thickness and as percentage of total bark thickness, for all stock treatments. However the higher bark thickness at the interface should be attributed to high intensity of hard bast formation and resultant growth at this union part, which might be a protective mechanism acting against the stress, occurred due to graft union.

Number of latex vessel rows.

For the number of latex vessel rows also interface recorded the least value compared to the other positions for all stock treatments. For both the traits RRII 105 scion on RRII 105 stock recorded the highest value for all respective positions compared to the other treatments.

Better CV values, compared to total bark thickness, were recorded for soft bast thickness and number of latex vessel rows. The variations due to stock sources were not statistically significant for any of the characters.

1.4.7 Disease susceptibility.

In poly-bag nursery Oidium infection was low, the range for percentage of affected plants being 6.00 to 12.80. In the bud-wood nursery, during the fist year, percentage infection showed a narrow range; the range being 33.28 to 36.32 with a mean value of 35 shown in Table 1.4.7.1. During the second year the infection rate recorded slightly higher values with a range of 35.68 % to 42.92% among the treatments. However, for none of the disease observations treatment differences were significant. For the different observations good CV values were recorded also. It should be concluded that the disease occurrence is more controlled by environmental factors.

PDI rating for Oidium leaf disease for two consecutive years showed that the four treatments are on par with each other for susceptibility to this disease and stock difference has no significant influence on Oidium infection. The same inference was observed for shoot rot also.

Table A.1.4.7.1. Comparison of grafted plants, raised on varying stock sources, for susceptibility to shoot rot and Oidium diseases

Stock sources	Polybag nursery	Bud-wood nursery		
		Shoot rot	Oidium infection	
			Rating (Percentage infection)	
	Percentage of Oidium affected plants. SE 3.47 CV 78.95	Percentage of shoot rot affected plants SE 8.50 CV 38.60	First year SE 2.74 CV 17.38	Second year SE 4.35 CV 24.72
Assorted seeds	12.80	54.05	33.28	38.88
RRII 105	6.00	41.77	36.32	40.08
RRIM 600	9.75	47.08	36.16	42.92
PB 28/59	10.76	53.97	35.04	35.68
Grand mean	9.83	49.22	35.20	39.39

1.4.8. Biochemical parameters.

1.4.8.1. Total protein content and Peroxidase activity

The total protein content is depicted in Table A.1.4.8.1. The mean protein content varied from 130.4 to 194.8 mg in plants raised on different stock sources. For intra group variations, the plant groups with assorted seeds, RRII 105, RRIM 600 and PB 28/59 as stock sources showed the ranges of 124-232, 116-156, 132-208 and 118-186 mg/g respectively. Maximum protein content was recorded for the group with assorted seeds as stock source and minimum for RRII 105 as stock.

Table A.1.4.8.1. Total protein content and peroxidase activity of the plants raised using different stock sources and RRII 105 as scion

Root stock type	Total protein (mg/g)		Peroxidase (Unit/Lit.)	
	Range	Mean	Range	Mean
Assorted	124-232	194.80	100-250	168.33
RRII 105	116-156	130.40	142.86-166.67	147.62
RRIM 600	132-208	176.40	142.86-250	191.91
PB 28/59	118-186	156.80	142.86-250	176.91

The peroxidase estimated was in the range of 147.62 to 191.91 unit/lit in plants raised on different root stock sources. For within group variations the stock sources recorded ranges of 100-250 units for the plants with assorted seeds as stock; 142.86 – 166.67 units for RRII 105, 142.86-250 units for RRIM 600 and 142.86 – 250units for PB 28/59. Maximum peroxidase activity was recorded for the group with RRIM 600 seeds (191.91 units /lit) as stock source and minimum for RRII 105 stock (147.62 unit/lit.) For this trait also maxim variability within group was recorded in the plant group with assorted seeds as stock source.

1.4.8.3. Esterase activity

Esterase activity was determined by electrophoretic separation. The number of bands generated, (plate–9 fig1-4) recorded a range of 3 to 6.4 in plants raised on different root stock sources. Maximum major bands (6.4) were observed in the group with RRII 105 seeds and minimum (3) for assorted seeds as root stock source. More intense bands were observed in the plants raised using RRII 105 as root stocks (2.4), The least intense bands were observed in the plants with assorted seeds as root stock sources. Maximum broad bands (2.4) were observed in plants raised using RRII 105 seeds as root stock source. Maximum number of intermediate types of bands were also observed in plants resulted using RRII 105 as root stock source with minimum number for the plants raised using assorted seeds as root stocks The results showed that plants with RRII 105 as root stock as well as scion source, will have the highest level of esterase activity, in the scion, when compared to the plants of the same clone with other three types of root stock.

Table A.1.4.8 2. Characteristics of esterase band of plants raised using different stock sources and RRII 105 as scion.

Root stock Type	Number of bands (mean)			Width of bands (mean)		
	Total	Intense	Faint	Broad	Intermediate	Narrow
Assorted	3.0	1.0	2.0	1.2	0.06	1.2
RRII 105	6.4	2.4	4.0	2.4	1.4	2.6
RRIM 600	3.4	1.0	2.4	1.2	0.6	1.6
PB28/59	4.2	2.0	2.2	1.8	1.2	1.2

1.4.9 Genetic polymorphism.

Characterization of RAPD markers.

A total of 82, 10 mer-random primers from Operon (Operon Technologies inc U.S.A.) were screened with the DNA samples to identify informative primers that generated polymorphic patterns of PCR products .In total, 4 primers produced good DNA amplification and 30 primers generated DNA amplification with streaking patterns and the remaining 48 primers failed to amplify. The four primers ultimately selected and used for the analysis of individual plants, resulted 5 to 15 DNA fragments easily detectable with resolved bands. Those, which were reproduceable over, repeated runs with sufficient intensity, to determine the presence or absence in samples with the same relative band intensity. The size of amplified DNA fragments ranged from 500 to 3500 base pairs. In order to detect a polymorphic band linked to stock-scion interaction, DNA samples from eight compatible and eight incompatible plants along with RRII 105 as control were used for RAPD analysis with four informative primers.

Analysis of polymorphic-bands associated with stock-scion interaction

All primers produced are multibanded finger prints and each RAPD primer produced different amounts of polymorphic bands. Results indicated that the RAPD technology effectively reveals genetic difference between compatible and incompatible plants. Primers namely OPA-18, OPC-5, OPE-5, and OPE-12, produced polymorphic bands. Thus the above four primers found to help in the identification of a polymorphic band associated with stock-scion inter action in Hevea.

Primer:OPA-18.

RAPD obtained with primer OPA-18 for the eight compatible and incompatible of *Hevea brasiliensis* was shown in Plate-10: Fig: 1. One band (1.2Kb) was taken as marker, which is present in all the compatible plants taken for the study except for SS5. But this band was also present in some incompatible plants RS 5, RS 6 and RS 8.The same was absent in all other incompatible plants.

Primer OPC-5.

With this primer, one DNA fragment (1.3Kb) was noticed (Plate-. 10.Fig:2).This fragment was detected in two compatible plants and absent in the remaining compatible and incompatible (rough and smooth surfaced) plants.

Primer OPE-5.

PCR amplification with OPE-5 primer shows that majority of the DNA fragments were found to be monomorphic bands. However there are a few polymorphic bands, which were amplified below 500 base pairs. Since the band size is below 500 base pairs, it's not worth to include for analysis. (Plate- 10.Fig3:)

Primer:OPE-12.

RAPD profile of OPE-12 primer is shown in (Plate 10- Fig 4). It - was noticed that a 900 bp band was amplified with six compatible plants (-SS1, SS3, SS4, SS5, SS6, SS8) and one incompatible plant (RS 5). However this polymorphic band was absent in majority of the incompatible plants. Therefore this RAPD band may be strongly associated with stock-scion interaction in *Hevea*. However it's necessary to conduct deep studies to confirm the association of this DNA marker with stock-scion interaction in *Hevea.*

2. VARIABLITY BETWEEN INCOMPATIBLE AND COMPATIBLE GROUPS OF PLANTS.

2.1. Comparison of incompatible with compatible groups of plants for anatomical characters.

The orientation of axial tissue in the stock-scion interface

The tangential connections of tissue at the interface are clearly observed in the tangential longitudinal section of the bark (Plate-7,Fig. A). At the interface the orientation of axial tissue showed variations among the two groups of plants. Such

Table A.2.1.a. Comparison of incompatible with compatible plants for Quantitative anatomical traits.

Characters	Mean ± SE				
	Postions→ Graft type ↓	Stock	Interface	Scion base	50 cm above the bud union
Total bark thickness(mm)	Rough	2.92 ± 0.15	3.00 ± 0.16	2.56 ± 0.07	1.91 ± 0.06
	Smooth	2.68 ± 0.11	3.04 ± 0.11	2.43 ± 0.12	1.80 ± 0.05
Percentage of hard bast	Rough	63.57 ± 1.53	70.62 ± 1.71	63.11 ± 1.38	59.25 ± 1.00
	Smooth	60.32 ± 1.64	65.82 ± 1.45	54.33 ± 2.53	55.81 ± 1.63
Number of latex vessel rows	Rough	4.20 ± 0.23	3.10 ± 0.20	3.27 ± 0.25	2.43 ± 0.13
	Smooth	4.03 ± 0.31	2.83 ± 0.27	3.57 ± 0.27	2.73 ± 0.18

Table A.2.1.b Coefficients of variations and ‘t’ values.

Characters	Positions→ Graft type ↓	Stock		Interface		Scion base		50cm above the bud union	
		CV	‘t’value	CV	‘t’value	CV	‘t’value	CV	‘t’value
Total bark thickness	Rough	14.12	0.62	20.98	-0.78	11.03	0.57	11.37	0.30
	Smooth	16.38		13.72		19.27		10.93	
Percentage of hard bast	Rough	9.33	9.00**	9.36	8.99 **	8.45	10.95**8	6.31	7.61 **
	Smooth	10.45		8.51		16.76		11.31	
Number of LVR	Rough	21.01	-1.53	25.28	1.36	29.99	-1.35	20.35	-0.89
	Smooth	29.44		36.94		24.78		25.75	

** P< 0.01

variations appeared to be resultant of the level variations of the cambial zones of the three parts (stock, scion and the interface). Accordingly the number of deviations in the running direction of the conducting tissue varied and it was clear in the radial longitudinal sections, which is evident from the figure (Plate- 7,Fig·B-F). According to the level variations of the vascular cambia at the interface, stock and scion, the intensity of deflection in the orientation of the axial tissue varied and the variation has been expressed as different grades (S, W, W+,C,and W++) as shown in the figures. W++ and C curves were not present in compatible group while more than 90 percent of the plants in incompatible group showed the deflections at varying extend.

The plants with graft incompatibility symptoms, observed on visual observation, were compared with those having compatible symptoms, for quantitative characters, using Student's t test. Differences for growth aspects were observed at different stages of growth, from the budded stump stage onwards. Anatomical characters and test tapping yield were recorded from the field plants, after two year's growth in the field.

The mean values, along with the SE, for the different anatomical characters at four height positions of incompatible (rough type) and compatible (smooth type) groups of grafted plants, are shown in table A.2.1.a and the variability parameters are shown in table A.2.1.b. The characters are total bark thickness, percentage of hard bast and number of latex vessel rows. The positions studied are the stock part, stock scion interface and two height positions of the scion stem; numbered as (1) two cm below the lower joint of the graft (stock), (2) graft interface (3) two cm above the upper joint of the graft (scion base) and (4) 50 cm above the bud-union. Total bark thickness and the percentage of hard bast thickness recorded the highest values at the interface region followed by the stock portion for both compatible and incompatible grafts. For the number of latex vessel rows highest values were recorded for the stock portion for both the graft types. For bark thickness and percentage of hard bast rough type recorded higher values and the reverse was for number of latex vessel rows.

For total bark thickness and number of latex vessel rows the 't' values were not significant with respect to any of the positions, indicating that compatible and incompatible grafts are on par with each other for those traits. For the percentage of hard-bast thickness the differences between compatible and incompatible groups were highly significant with respect to all positions irrespective of the stock sources. From the results it is observed that incompatible grafts produce significantly high amount of stone cells than the compatible grafts. This should be considered as a symptom of higher intensity of stress at the interface region of incompatible grafts, compared to the compatible grafts.

Considering the three characters, comparatively better values of CV were recorded for the number of latex vessel rows with a range of 20.35 to 29.99 for incompatible group and a range of 24.78 to 36.94 for compatible group. CV value was the least for the percentage of hard bast, with respect to all the four positions, with a range of 6.31 to 9.36 for incompatible grafts and a range of 8.51 to 16.76 for compatible grafts respectively. Considering the variability within each group, a wide range was recorded for compatible group.

2.2. Comparison of incompatible with compatible graft types for positional girth variations at budded stump stage.

After sorting out the surface characters and identifying the morphological symptom of incompatibility, the girth variations at different height positions of both types of budded stumps were recorded, before planting in polybags. The data is given in table A.2.2.1. The positions are (1) two cm above the graft area (2) upper joint of the bud patch (3) the bud point (4) lower joint of the bud point and (5) 2cm below the graft area.

Of the five positions measured, first and last are the stock stem portions above and below the graft area. The other three are the upper and lower joints of the graft and the bud point. The data is helpful to examine the effect of stock incompatibility on the growth of stock seedlings, in the graft area and also at the lower and upper levels, after bud grafting. For all the positions, better growth was recorded for compatible grafts. The differences were statistically significant for the two stock positions and also for the bud point. At the joints the union growth is more complicated and the influence of other factors also may interfere.

Table. A.2.2.1. Comparison of incompatible with compatible graft types for positional girth variations at budded stump stage.

Positions	Graft types	Mean (cm)	SD	't' values
2cm above upper joint of the bud patch (stock above the graft)	Rough	3.37	0.57	-2.06*
	Smooth	3.52	0.56	
At upper joint of the bud patch	Rough	3.72	0.60	-1.29
	Smooth	3.82	0.56	
At the bud point	Rough	3.55	0.61	-2.37*
	Smooth	3.74	0.65	
At lower joint of the bud patch	Rough	4.15	0.73	-1.51
	Smooth	4.29	0.73	
2cm below the lower joint of the bud patch (Stock base)	Rough	4.10	0.68	-2.24*
	Smooth	4.20	0.71	

*P < 0.05

2.3. Comparison of incompatible with compatible graft types for positional girth variations at polybag stage.

Comparison of the two groups of grafts for positional girth variations and for plant height, after four month's growth in polybag nursery and just before field planting, is shown in table A.2.3.1. For girth at the scion base and at the stock-scion - joint and also for plant height, incompatible grafts recorded lower values. The same girth values were recorded for stock base of the two groups. For none of the characters at polybag stage the differences were statistically significant.

Table A.2.3.1 Comparison of growth characters of incompatible and compatible graft types in polybags.

Polybag characters	Graft types	Mean (cm)	SD	't' values
Scion height	Rough	27.90	9.81	-0.23
	Smooth	28.19	10.28	
Scion base girth	Rough	2.57	0.67	-0.28
	Smooth	2.59	0.60	
Girth at the lower joint of stock and scion	Rough	4.80	0.79	-0.50
	Smooth	4.85	0.77	
At 2cm below the lower joint of the bud patch (Stock)	Rough	4.74	0.81	0.06
	Smooth	4.74	0.78	

2.4. Comparison of incompatible and compatible graft types, in the bud wood nursery, for growth characters

Table. A.2.4.1. Comparison of incompatible and compatible graft types, in the bud wood nursery, for growth characters. a. First year observations

Characters	Graft types	Mean (cm)	SD	't' values
Girth at 2cm below the lower joint of the bud patch (stock)	Rough	15.18	2.47	-1. 62
	Smooth	15.70	2.48	
Girth at the lower joint of Stock and scion	Rough	14.85	2.40	-1. 98*
	Smooth	15.47	2.51	
Scion base girth	Rough	11.63	2.21	-1. 32
	Smooth	12.01	2.19	
Girth at a height of 50 cm above the bud union	Rough	8.79	1.67	-1. 54
	Smooth	9.11	1.62	
Plant height.	Rough	229.74	56.41	-1. 19
	Smooth	238.50	58.59	

*P < 0.05

The data pertaining to the two groups of plants for growth in the field after the first and second year's growth and the data on test tap yield are furnished in tables A.2.4.1.a and b respectively. After the first year growth the growth characters recorded are girth of the stock; girth at the stock-scion joint, at the scion base, and at

50 centimeter above the bud union; and also the plant height. For all the characters the incompatible group recorded lower values than the values of the respective characters of compatible group. The difference was statistically significant, at 5% level, for the girth at stock-scion joint only.

Table. A. 2.4.1. Comparison of incompatible and compatible graft types, in the bud wood nursery, for growth characters. b. Second year observations

Characters	Graft types	Mean (cm)	SD	't' values
Girth at a height of 10 cm above the bud union.	Rough	12.30	1.76	-1.75
	Smooth	12.69	1.72	
Girth at a height of 50 cm above the bud union	Rough	10.56	1.67	-1.39
	Smooth	10.85	1.62	
Plant height	Rough	379.04	65.44	-0.87
	Soft	386.58	70.41	
Number of buds per meter of bud wood	Rough	14.60	2.90	-0.95
	Smooth	14.25	2.77	
Test tap yield (mg)	Rough	0.10	0.05	-7.36**
	Smooth	0.16	0.07	

** P< 0.01

After two year's growth, the observations taken from the bud wood nursery are scion characters such as scion girth at two positions (10cm and 50 cm above the bud-union), plant height, and number of buds per meter and test tap yield. In the observations after two year's growth in the field also incompatible grafts recorded comparatively lower values for all the characters under study. The difference for test tap yield was highly significant.

3. CORRELATIONS

3. 1. ASSOCIATION AMONG NURSERY CHARACTERS

3.1.1.Correlations among seed characters, germination percentage and growth characters at juvenile stage.

Table A.3.1.1.Correlations among seed characters, germination percentage and growth characters at juvenile stage.

Characters	r- value	Characters	r - value
X1 X2	0.92**	X3 X4	0.00 NS
X1 X3	0.28 NS	X3 X5	0.21 NS
X1 X4	-0.18NS	X3 X6	-0.31 NS
X1 X5	0.24 NS	X3 X7	0.51*
X1 X6	-0.30 NS	X4 X5	0.35
X1 X7	0.00NS	X4 X6	0.46*
X2 X3	0.30 NS	X4 X7	0.05
X2 X4	-0.14 NS	X5X6	-0.60**
X2 X5	0.30 NS	X5 X7	0.28
X2 X6	-0.38NS	X6X7	-0.37
X2 X7	0.01 NS	-	-

*P< 0.05 ; ** P< 0.01

Seed volume (X1), Seed weight (X2), Germination percentage (X3), Dry wt. of root X4), Dry wt. of shoot (X5), R/S ratio (X6), Plant height (X7)

The associations among seed characters, germination and growth characters are shown in table A. 3.1.1. Seed volume and seed weight showed positive correlation at highly significant level. The relationship of both the characters with germination percentage were positive but were not at significant level. More over the relationship of seed volume and seed weight indicated a negative relationship with root weight as well as with root/shoot ratio. Seed germination showed significant positive association with plant height.

3.2. Associations of anatomical characters with respect to different positions of the grafted plant.

Table A.3.2.1.Correlations of two important bark characters, the bark thickness (X1) and number of latex vessel rows (X6) with respect to the different positions of the bud grafted plants, at the stock portion (P1), stock-scion interface (P2), scion base(P3) and at 50 cm height from the bud union (P4), after two year's growth in the field

Characters	P1X1	P2X1	P3X1	P4X1	P1X6	P2X6	P3X6	P4X6
P1X1	1	0.48**	0.82**	0.14	0.15	-0.05	-0.15	-0.11
P2X1		1	0.41**	0.15	-0.15	0.07	-0.05	-0.21
P3X1			1	0.14	0.10	-0.12	-0.19	-0.02
P4X1				1	-.17	-0.14	-0.09	0.17
P1X6					1	0.61**	0.38*	0.36*
P2X6						1	0.49*	0.03
P3X6							1	0.30

* $P < 0.05$; ** $P < 0.01$

Associations of two important bark anatomical characters, with respect to four positions of the bud - grafted plants, after two years growth in the field, are shown in table A.3.2.1. The characters are bark thickness and number of latex vessel rows. The four positions are, (1) the stock portion (2) stock- scion interface (3) scion base and (4) 50 centimeters above from the bud union. For bark thickness, associations among stock portion, stock-scion interface and scion base are highly significant while bark thickness at stock portion showed no significant association with this trait at 50cm height from the bud union or with the number of latex vessel rows at any position. Bark thickness at the interface showed significant association with the bark thickness at scion base while this trait did not show any significant association with this trait at 5ocm height or with the number of latex vessel rows at any position. The number of latex vessel rows at stock portion showed significant associations with the same trait at other positions. For this trait at the interface, association with the same trait at scion base was statistically significant while it's relationship with the same trait at 50cm height was not significant.

Table A. 3.2.2.Correlations of test tap yield with latex vessel rows and bark thickness at scion base.

Characters	r-value
Yield vs latex vessel rows	0.29NS
Latex vessel rows and bark thickness	- 0.19 NS
Yield vs total bark thickness	0.30 NS

Yield showed positive associations with bark thickness and number of latex vessel rows; but the associations were not statistically significant. Latex vessel rows showed a negative relationship with bark thickness, which was also not significant.

3.3. Associations among the growth characters at different growth phases

Associations among the growth characters at different growth phases, for example the budded stump stage, polybag stage, and field plants grown over one year and two years after planting in the field, and the associations of such characters with test tap yield were estimated to examine the possibility of early prediction for desirable traits. The data is shown in table A. 3.3.1.

Correlations among the growth characters of budded stumps, polybag plants and field plants raised from the same budded stumps are shown in table A.3.3.1. An association of such traits with test tap yield of two-year-old field plants also is furnished in this table. With respect to the budded stumps, girth at the bud point showed very high association with the stock base girth ($r = 0.8641$; $P < 0.01$) and this trait showed good correlation with the stock girth of polybag plants also ($r = 0.7368$; $p < 0.01$). The girth of stock at budded stump stage highly influenced the girth of stock and scion stem at further stages of growth in polybag and later in the field. The results showed that the growth of stock and scion are highly interrelated; the respective traits between growth stages are inter related and the terminal and radial growth are also interrelated. That means the vigor of stock will influence further growth of stock and scion in polybag as well as in the field. All associations were positive. The degree of relationships of stock girth at budded stump stage with the girth at further growth stages reduced gradually but the relationships were maintained at significant level.

Table A.3.3.1 Associations among the growth characters at different growth phases, budded stump stage, polybag stage, and field plants grown over one year and two years after planting in the field, and the associations of such characters with test tap yield.

		Correlation coefficients				
		Girth at budded stump stage		Growth characters at polybag stage		
Growth phases	Characters→ ↓	Bud point	Stock girth at the base	Scion height	Scion base girth	Stock girth
Girth at budded stump stage	Bud point	1	0.86**	0.38**	0.45**	0.74**
	Stock stem above the graft area	0.84**	0.94**	0.36**	0.50**	0.81**
Growth characters of poly-bag plants	Scion height	0.44**	0.46**	1	0.70**	0.54**
	Scion base girth	0.45**	0.51**	0.73**	1	0.60**
	Girth at bud union	0.72**	0.82**	0.48**	0.57**	0.91**
	Stock girth	0.74**	0.83**	0.45**	0.52** -	1
Bud-wood nursery-1st year	Stock girth	0.35**	0.39**	0.51**	0.50**	0.45**
	Girth at the stock-scion joint	0.35**	0.38**	0.53**	0.10	0.46**
	Scion base girth	0.42**	0.36**	0.48**	0.51**	0.42
	Scion girth at 50cmheight	0.40**	0.34**	0.51**	0.48**	0.38**
Bud-wood nursery-second year	Scion girth at 10 cm height	0.33**	0.35**	0.46**	0.45**	0.36**
	Scion girth at 50cm height	0.33**	0.36**	0.47**	0.45**	0.37**
	Number of buds / meter of bud-wood	0.21**	0.17*	0.36**	0.15*	0.14*
	Test tap yield	0.19**	0.25**	0.15	0.25**	0.19**

* P < 0.05 ** P< 0.01

3.4. REGRESSIONS

Based on the associations of stock stem girth and girth of the bud point at budded stump stage with the growth characters of stock and scion at further growth stages (growth in polybag and growth in the field, and also with the test tap yield), regression equations were derived for estimating the extent of relationships among the characters/growth phases. Such estimates are useful for early prediction of desirable traits. The statistical parameters of such regressions are shown in table. A.3.4.1.

Table A.3.4.1. Regressions of the growth characters and test tap yield of field plants on the growth characters at budded stump stage and polybag stage.

Independent characters	Dependent characters	Constants	Regression Coefficients	R^2	T-Stat
BS X2	BW2 X 3	11.10	0.91	0.04	3.26**
BS X4	BS X 2	0.43	0.797	0.75	26.70**
BS X4	PB X 2	1.10	0.91	0.68	22.48**
PB X2	BW1X1	5.89	1.23	0.19	7.44**
PB X1	BW1 X2	7.22	1.79	0.26	9.27**
BS X4	BW2 X4	0.04	0.02	0.06	3.96**
BS X 4	BW 2 X 1	7.27	0.84	0.13	5.94**
BS X 4	BW1X1	9.61	1.35	0.15	6.47**

** $P< 0.01$

Regression equations

1. W2X3 = 11.10+0.91 (BSX2)
2. BSX2 = 0.43+0.79 (BSX4)
3. PBX2 = 1.10+0.91 (BSX4)
4. BW1X1= 5.89+1.23 (PBX2)
5. BW1X2= 7.22+1.79 (PBX1)
6. BW2X4= 0.04+0.02 (BSX4)
7. BW2X1= 7.27+0.84 (BSX4)
8. BW1X1= 9.61+1.35 (BSX4)

BSX2 = Girth (cm) at bud patch portion.

BSX4= Girth (cm) at stock base.

PBX1= Scion base girth (cm).

PBX2=Girth (cm) at the bud union at polybag stage .

BW1X1= Lower joint of bud patch after one year growth in the field.

BW1X2= Scion base girth (cm) of field plants, one year after planting with polybag plants.

BW2X3= Number of buds per meter of bud wood.

BW2X4= Test tap yield (g/plant/tap) of two year old field plants(two years after planting with polybag plants) .

The regression equations for eight combinations were derived. The regressions were highly significant for all the eight combinations. The regressions of bud point girth at budded stump stage and the scion base girth at polybag stage recorded high R^2 values, 74.66 and 67.62 percent respectively. The R^2 values were negligible for the regressions of (1) number of buds/meter bud wood on girth of the bud point, at bud wood stage and on budded stump stage and (2) test tap yield of field plants on stock base girth at budded stump stage.

B. OBSERVATIONS ON MATURE TREES UNDER TAPPING.

1. VARIABILITY

1.1. Comparison of tapping side with untapped side of the tapping trees, for bark thickness and bark anatomical characters.

Data on the bark thickness and six anatomical characters were recorded from the bark collected from three height positions of the tapping side as well as from the untapped sides of mature trees, under tapping for the previous one year following ½ S d/3 system of tapping, with a view to study the response of trees to tapping stress with respect to the bark structural traits. Mean values with respect to the positions, for the respective characters were computed. A comparative data is shown in table B.1.1.1.

Table B.1.1.1 Comparison of tapping side with untapped side for bark thickness and bark anatomical characters after one year tapping following 1/2Sd/3 tapping.

Characters	Sides Tapped/untapped	Mean	SD	CV	't'
Total bark thickness (mm)	Tapped	5.44	0. 49	9.00	0.66
	Untapped	5.35	0. 47	8.79	
Percentage of hard bast	Tapped	71.95	5.08	7.07	0.71
	Untapped	70.92	5.26	7.41	
Number of latex vessel rows	Tapped	13.28	1.26	9.50	2.13**
	Untapped	12.62	1.16	9.23	
Latex vessel density(nos/2.5mm	Tapped	5.54	0.36	5.54	1.91
	Untapped	5.35	0.42	7.77	
Width of latex vessels(μ)	Tapped	22.00	2.17	9.84	0.06
	Untapped	21.95	4.74	21.60	
Ray height/width ratio	Tapped	7.87	1.16	14.77	1.30
	Untapped	8.29	1.37	16.56	
Percentage of tanniferous cells /unit area.	Tapped	46.91	3.36	7.17	3.23**
	Untapped	43.32	5.07	11.70	

** $P< 0.01$

Table B.1.1.2 Bark thickness (mm) on tapping side and untapped side, at different height positions from the bud union, (at six monthly intervals of growth) of the trees under tapping.

Time of observation → / Height positions↓	Sides	Bark thickness (mm) ± SE as on the time given				
		April, 2000	October, 2000	April, 2001	October, 2001	April,2002
150 cm	Tapping	5.18 ± 0.11	5.66 ± 0.11	5.90 ± 0.08	6.57 ± 0.10	8.99 ± 0.12
	Untapped	4.32 ± 0.08	5.38 ± 0.10	5.93 ± 0.08	6.43 ± 0.11	9.10 ± 0.12
125 cm	Tapping	5.76 ± 0.14	5.47 ± 0.14	4.93 ± 0.08	6.58 ± 0.13	8.73 ± 0.14
	Untapped	4.74 ± 0.11	6.22 ± 0.14	6.23 ± 0.07	6.74 ± 0.11	9.67 ± 0.15
90 cm	Tapping	6.07 ± 0.12	6.64 ± 0.11	6.51 ± 0.08	5.09 ± 0.11	7.17 ± 0.13
	Untapped	4.77 ± 0.09	6.38 ± 0.11	6.60 ± 0.08	7.04 ± 0.11	10.24 ± 0.18
60 cm	Tapping	6.45 ± 0.12	7.04 ± 0.14	7.11 ± 0.08	6.64 ± 0.19	9.16 ± 0.20
	Untapped	5.04 ± 0.14	6.48 ± 0.12	7.31 ± 0.09	7.56 ± 0.11	10.43 ± 0.17
0(Scion base)	Tapping	7.19 ± 0.17	6.97 ± 0.14	7.77 ± 0.09	7.96 ± 0.12	10.48 ± 0.13
	Untapped	5.41 ± 0.12	7.36 ± 0.12	7.85 ± 0.09	8.12 ± 0.11	11.18 ± 0.15

For all the seven characters studied, tapping side recorded slightly greater values than the untapped side. Of this the difference for two characters, number of latex vessel rows and percentage of tanniferous cells were statistically significant. For the total bark thickness, percentage of hard bast, latex vessel density, latex vessel width and the height /width ratios of phloem rays the differences were not statistically significant. The results indicated a stimulation of growth, especially high formation of laticifer rows as an initial response of tapping stress.

Positional variations of bark thickness were recorded from tapping side and untapped side, for further two years at six monthly intervals, following a higher intensity of tapping, ½ S d/2. The data collected from five positions are shown in tables B.1.1.1 and B 1.1.2 respectively.

Bark thickness at each position on tapping side was lower than that at the respective position of untapped side indicating retardation of bark growth on continuation of tapping.

1.2. Positional variations for growth in terms of girth.

Girth recording also was carried out at six monthly intervals with respect to the different height positions. From the girth data percentage increase of girth at the different height positions, over two years, were computed. The data are furnished in table B. 1.2.1.

Table B.1.2.1. Positional variations of girth and mean girth increment (over two years) of the trees under tapping.

Positions on the tree	Mean girth (cm) ± SE		Coefficient of variations for girth		Mean girth increment over two years (cm) With % increase in brackets
	As on 2000 April (Initial)	As on 2002 April (Final)	2000 April (Initial)	2002 April (Final)	
150cm above the bud union	52.41 ± 0.37	56.95 ± 0.54	6.81	9.28	2.27 (8.66%)
125cm above the bud union	54.37 ± 0.36	58.89 ± 0.51	6.59	8.58	2.27 (8.31%)
95cm above the bud union	55.19 ± 0.37	61.08 ± 0.51	6.52	8.15	2.95 (10.07%)
60cm above the bud union	60.44 ± 0.39	62.44 ± 0.53	6.67	8.32	2.26 (7.85%)
Base of the scion at the bud union	65.35± 0.43	70.06 ± 0.59	6.40	8.27	2.06 (7.20 %)

The girth increment over two years varied from 2.06 to 2.95 centimeters at different height positions of the tapping trees with minimum increase at the base and maximum increase at 95 centimeter height above the bud union; in terms of actual increase as well as percentage increase. Another observation is a slight increase of CV values for the girth after two year's growth, at all height positions, compared to the initial girth.

The rate of increase of bark thickness at different height positions of tapping side and untapped side, along with the girth increase over two years of tapping are shown in Fig.2. On the untapped side there is no much variation of growth rate among the height positions. This side showed high rate of bark growth than the tapping side at all positions.

On tapping side, position 2, 3 and a few trees in position1 include the tapped area where bark regeneration is under progress at different levels. Position 0 (scion base) and position 4 are below and above the tapped area; of which position 4 showed better rate of bark growth. Position 0 at the tapping side recorded lower rate of bark growth.

When the girthing rate was taken into consideration, the values for different heights recorded a narrow range, from 7.2 to 10.07 with maximum value for the position at 95cm height from the bud union and minimum value for 0 position, the scion base. The rate of girth increase showed a negative relationship with the rate of bark growth at tapping side.

1.3. Quantity of intraxylary phloem and primary xylem points.

The number of primary xylem points and intraxylary phloem points present in one year old stem of mature tapping trees, untapped trees of the same clone and same age, in the same plantation, and two year old plants were recorded and compared. The data is given in table B. 1.3.1.

Table B.1.3.1. Comparative data on the number of intraxylary phloem and primary xylem points in tapped and untapped mature trees and untapped plants of two year old.

	IXPP (Nos)		PXP (Nos)	
Source	Mean	CV	Mean	CV
Tapped (mature)	67.89	19.77	91.06	8.53
Untapped (mature)	42.88	30.81	76.88	15.39
Young (untapped)	25.44	20.15	74.77	15.25

High numbers of intraxylary phloem points were recorded for the tapped trees compared to the untapped mature trees and young plants, with the lowest numbers for young plants. For primary xylem points also tapped trees recorded the highest value; but the differences among the three groups were not so wide. The coefficients of variations for the intraxylary phloem showed greater values than the corresponding values for primary xylem points.

The primary xylem points recorded the mean values 91.06, 76.88 and 74.77 numbers for tapped, untapped and young trees respectively. The values of intraxylary phloem points recorded for the three groups are 67.89, 42.88 and 25.44 respectively. Variability, within group, was comparatively more for the quantity of intraxylary phloem points than for primary xylem points.

1.4. Intraclonal variations for the growth and yield factors of mature trees, under tapping.

The distribution of experimental trees, , mean and variability parameters, with respect to the different characters studied are shown in table B.1.4.1. The data on girth and bark thickness represent the respective characters recorded at the beginning of the experiment. For bark thickness the average of tapping side and untapped side was computed.

The sample distribution is almost normal for all characters except for a little deviation for bark thickness at 150cm above the bud union (X6) and for DRC in the second year (X12).

Variability, for the different characters showed a range of 6.46 to 58.47 for the different characters. High CV values for intraclonal variation, above 50%,were recorded for the mean girth increments at the scion base (highest) and at 150cm height from the bud union(second value from the top), followed by the summer variations of latex volume and DRC (above 30% and below 50 %). Low values of CV were recorded (below 10%) for girth at scion base and that at 150cm above the bud union; and DRC for first and second years and a little higher values of CV (above 20%and below 30%) were recorded for bark thickness at scion base and at 150 cm height.

Table B.1.4.1 Intraclonal variations for the growth and yield factors of mature trees, under tapping.

Characters	Sample distribution based on mean ± SD			Mean	SD	CV
	Mean<=SD (A)	Mean>=SD (B)	Between A&B			
X1	18	16	60	65.16	4.21	6.46
X2	16	17	61	2.60	1.52	58.47
X3	11	13	70	52.42	4.04	7.71
X4	11	14	69	2.30	1.22	52.90
X5	14	18	62	7.06	1.62	22.90
X6	23	6	65	5.18	1.07	20.70
X7	16	22	56	80.73	8.17	10.13
X8	6	8	46	11.23	1.26	11.23
X9	16	15	63	98.17	23.05	23.48
X10	13	9	57	102.31	24.47	23.91
X11	16	18	60	32.32	2.21	6.84
X12	17	5	57	35.18	2.62	7.44
X13	18	18	54	35.02	12.50	35.69
X14	11	13	64	16.88	5.51	32.62

X1-Girth at scion base (cm)

X2-Mean girth increment at scion base, over two years of tapping (cm).

X3-Girth at 150cm above the bud union (cm).

X4-Mean girth increment at 150 cm above bud union, over two years of tapping (cm).

X5-Bark thickness at scion base (mm).

X6-Bark thickness at 150 cm height above the bud union (mm).

X7-Girth at stock portion of tree trunk (cm).

X8-Bark thickness at stock portion of tree trunk (mm).

X9-Latex volume in the first year (ml).

X10-Latex volume in the second year (ml).

X11-DRC in the first year (%).

X12-DRC in the second year (%).

X13-Summer variation, drop, in latex volume (%).

X14-Summer variation, rise of DRC (%).

Table B.1.4.2 Categorization of TPD affected trees, for different characters recorded before the onset of external symptoms of TPD.

TPD Trees (nos)	Category with respect to the different characters													
	X1	X2	X3	X4	X5	X6	X7	X8	X9	X 10	X 11	X 12	X 13	X 14
2*	M	M	H	M	H	L	ND	ND	H	H	M	M	L	L
4	M	H	M	M	M	L	ND	ND	H	M	M	M	-	-
16*	M	M	M	M	M	M	L	L	M	M	L	M	M	M
24	M	H	M	H	H	M	H	L	M	M	H	L	-	-
47*	M	M	M	H	M	L	M	H	L	L	M	H	M	M
53	M	M	M	M	M	M	M	M	M	L	M	L	-	-
144	M	H	M	H	M	L	M	M	L	M	M	L	-	-
159**	H	H	H	M	H	M	H	H	M	L	H	M	M	-
249**	H	H	M	H	M	M	H	H	H	L	H	H	L	-
313	M	L	M	M	M	H	L	H	L	-	-	-	-	-

* Partial dryness; ** partial dryness and precoagulation (coagulation of latex in the cup before collection).

From the start of the experiments, over a period of two years, ten trees succumbed to TPD. The trees were categorised as low, medium and high for the 14 characters and the information is given in table B.1.4.2. The girth values represent the respective trait in the beginning of the experiment that is after imposing one year tapping adopting 1/2S d/3 and after shifting to 1/2S d/2 system. The result showed that for the TPD affected trees, girth and girth increment at 150cm height as well as at scion base are medium to high with an exception to the girth increment at scion base of only one tree. Bark thickness at scion base was also high to medium for the trees associated with low to medium bark thickness at 150cm height from the bud union. Latex volume or DRC did not show any such specific patterns while the summer drop for latex volume and rise in DRC was not high for partially dried trees.

2.Correlations among growth characters and yield of mature trees under tapping.

The possible associations among 14 characters including the girth and bark thickness of stock, scion base and at a height level 150 cm above the bud union (5cm above the opening height), percentage increase of girth at the two height levels of scion stem over two years of tapping, latex volume (annual mean) for two consecutive years, dry rubber content (annual mean) for two consecutive years and summer variations of latex volume and dry rubber content (DRC) are shown in table B.2.1 and B.2.2.

2.1. Correlations among growth characters.

Table B.2.1. Correlations among growth characters.

Characters	X2	X3	X4	X5	X6	X7	X8
X1	0.95	0.61**	0.139	0.11	0.19	0.51**	0.18
X2	1	0.26*	0.22*	0.45**	0.26*	0.15	0.05
X3		1	0.06	0.19	0.39**	0.18	0.29**
X4			1	0.04	0.10	0.05	0.09
X5				1	0.56**	0.20	0.12
X6					1	- 0.01	0.36
X7						1	0.08

* P< 0.05 **P< 0.01

The correlations among growth characters are shown in table B. 2.1.the characters X1 to X8 are as shown below.

Girth at scion base (X1).

Mean girth increment at scion base, over two years of tapping (X2).

Girth at 150cm above the bud union (X3).

Mean girth increment at 150 cm above bud union, over two years of tapping (X4).

Bark thickness at scion base (X5).

Bark thickness at 150 cm height above the bud union (X6).

Bark thickness at stock portion Girth at stock portion of tree trunk (X7).

Bark thickness at stock portion of tree trunk (X8).

The girth at scion base showed high associations with the girth at 150 cm height and also with stock girth at highly significant level (P<0.01). The mean girth increment at scion base influenced the girth and girth increment at the higher height level and also the bark thickness at both height levels of the scion trunk. Associations of this character with girth and bark thickness of the stock portion of tree trunk were not statistically significant. Girth at the distal position of the bud union showed significant associations with bark thickness at the same position and the bark thickness at stock level. Bark thickness at the two height levels of scion trunk were significantly associated while those traits did not keep significant associations with the girth or bark thick ness of the stock portion.

2.2. Correlations among growth characters, latex volume and dry rubber content.

Table B.2.2. Correlations among growth characters, latex volume and dry rubber content.

	X9	X10	X11	X12	X13	X14
X1	0.52**	0.51**	0.32**	0.25*	0.08	0.13
X2	0.11	0.11	-0.05	0.22*	-0.12	-0.22*
X3	0.40**	0.44**	0.29**	0.22*	-0.04	0.10
X4	0.06	0.12	0.15	0.21*	0.06	-0.16
X5	0.09	0.10	-0.14	0.04	-0.12	-0.10
X6	0.17	0.24*	0.23*	0.32*	-0.05	0.02
X7	0.08	0.14	0.05	-0.01	0.13	-0.10
X8	0.14	0.12	0.31**	0.39**	-0.01	0.05

* $P< 0.05$ **$P< 0.01$

Explanation of X1 to X8 are shown below table B.2.1.

The associations of growth characters of stock and scion parts of the tree trunk are shown in table B.2.2. The girth at both height levels of the scion stem showed significant associations with latex volume and DRC of both years while those traits did not keep any significant associations with the summer variations of latex volume or DRC. The girth increment at scion base showed significant positive association with DRC in the second year and negative association with DRC variation; no such associations were kept with the DRC in the first year. The girth increment at the higher height level also kept positive relationship with the DRC in the second year. The bark thickness of scion, at 150cm height, and stock showed significant positive associations with DRC in both years and the first character kept a positive relationship with the latex volume, in the second year also. The bark thickness at scion base did not keep any significant associations with any of the yield factors.

2.3 Correlations among yield factors.

The correlations among yield factors are furnished in table B.2.3. The relationships among volume and DRC, in both years, showed mutual associations at highly significant level. The latex volume in the first year showed significant negative association with summer variation (% drop) of latex volume while it showed a positive relationship with summer variation (% rise) of DRC. The latex volume in second year showed significant positive associations with DRC in first and second years and also with the summer rise in DRC. The association of DRC in first year with the same trait in the second year was also significant.

Table B. 2.3. Correlations among yield factors.

	X9	X10	X11	X12	X13	X14
X9	1	0.78**	0.35**	0.30**	-0.20*	0.27*
X10		1	0.31**	0.21*	-0.21	0.22*
X11			1	0.46**	0.13	0.18
X12				1	-0.00	-0.02
X13					1	0.13

* $P< 0.05$ ** $P< 0.01$

Latex volume in the first year (X9).

Latex volume in the second year (X10).

DRC in the first year (X11).

DRC in the second year (X12).

Summer variation, drop, in latex volume (X13).

Summer variation, rise of DRC (X14).

1.Work site

2. Seed bed

3. Seedling Nursery

4. Brown shoot nursery

Fig 2. Polybag Nursery

Fig 1. Polybag plant

Fig 3. Green shoot Nursery

PLATE 3 GREEN BUDDING

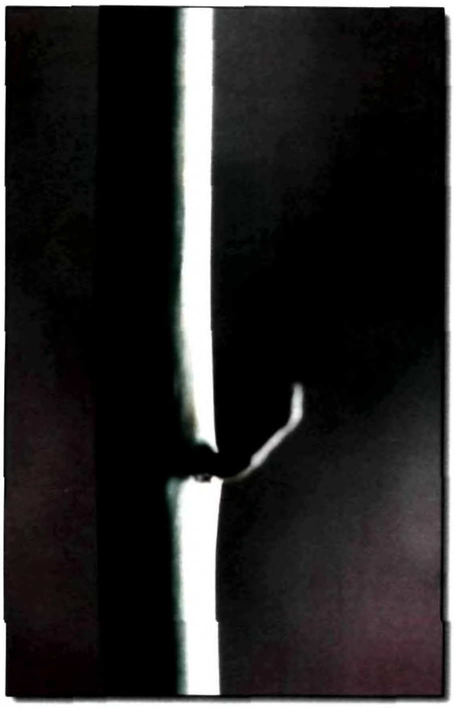


Fig 1.Bud Stick

Fig 2. Scion bud (preparation)

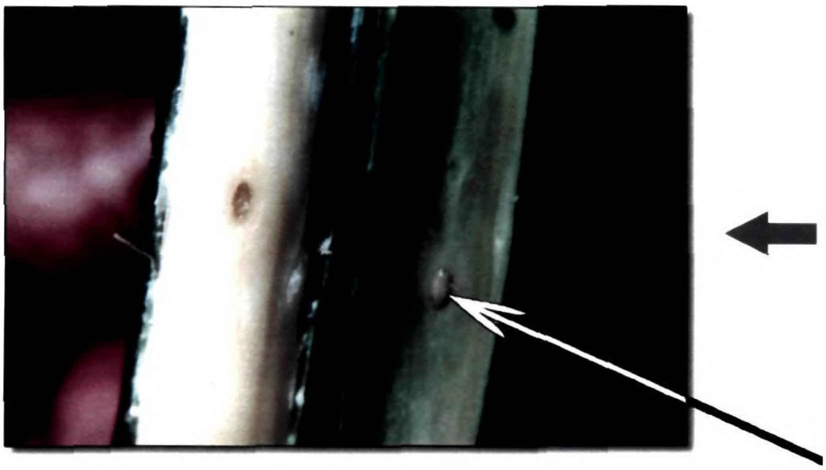


Fig 3. Bud patch

Fig 4. Budding pannel

PLATE 4 MORPHOLOGICAL SYMPTOMS OF INCOMPATIBILITY

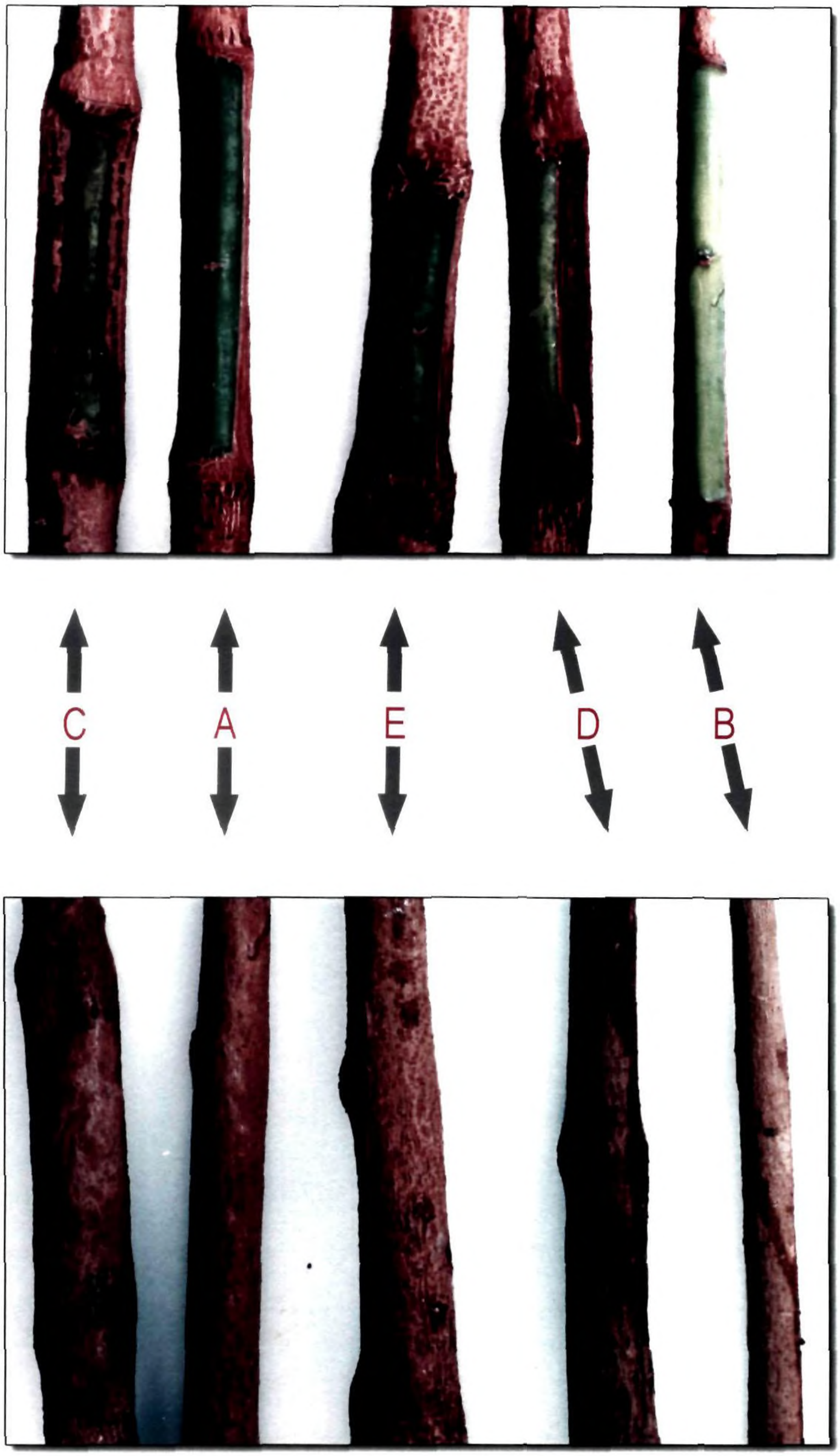


Fig 1.Grafted side

Fig 2.Opposite Side

A & B : *Complete and Compatible*

C : Complete and Incompatible

D & E : Incomplete & Incompatible

PLATE 5 *HEVEA* BARK STRUCTURE

Fig A

Fig B

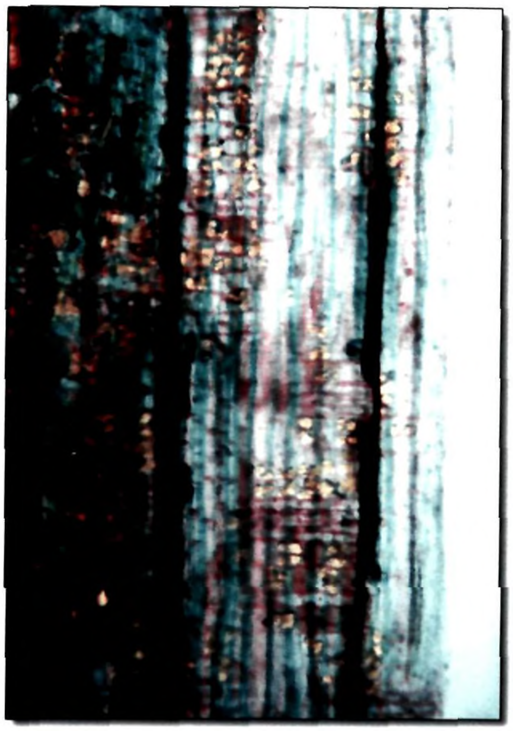
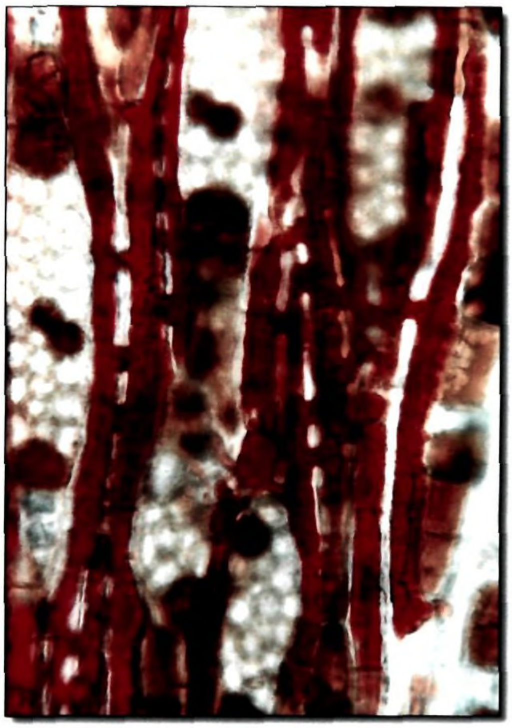


Fig C

Fig D



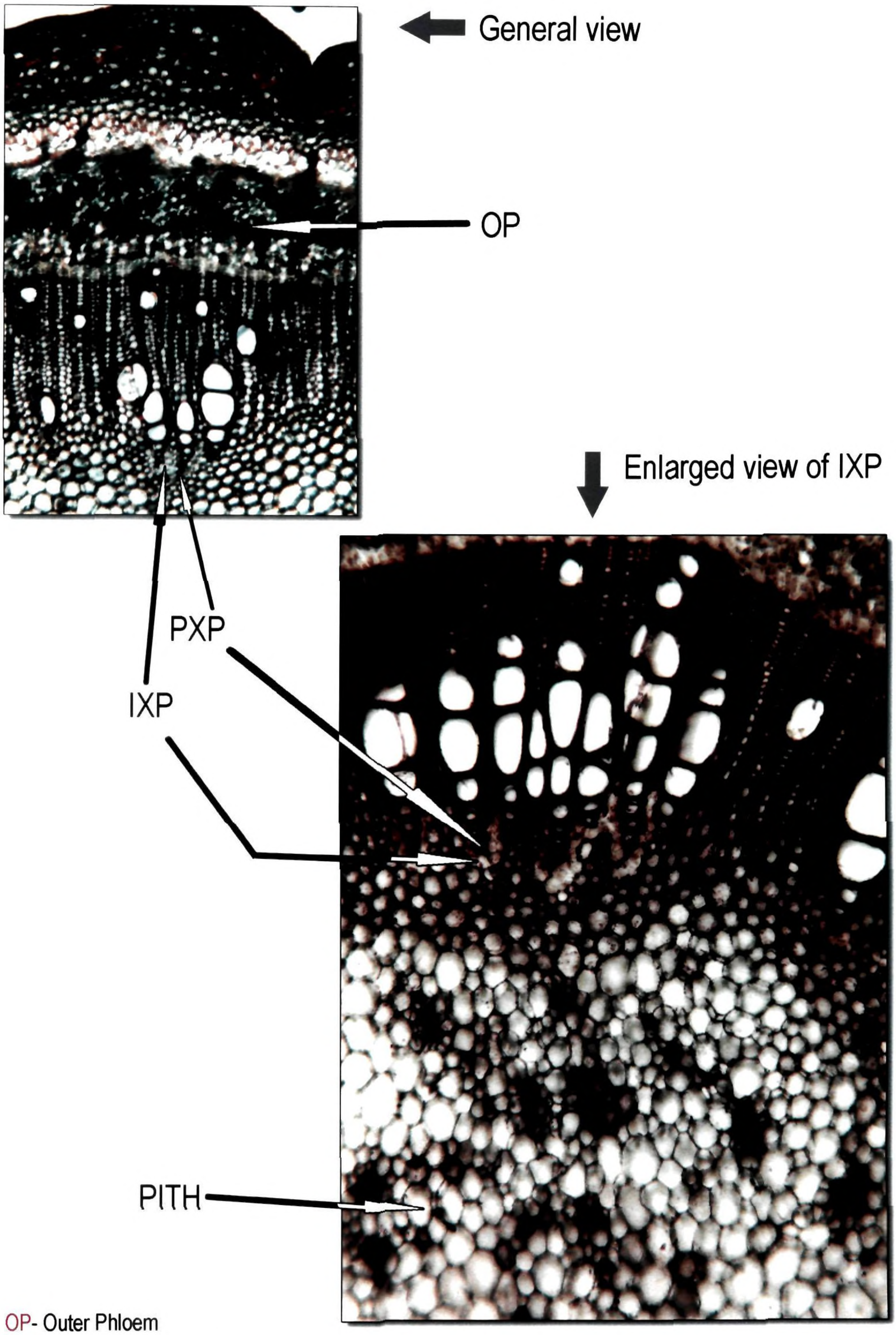
A.Radial Longitudinal Section

B.Enlarged view of the phloem region

C.Tangential Longitudinal Section at the sieve tube layer

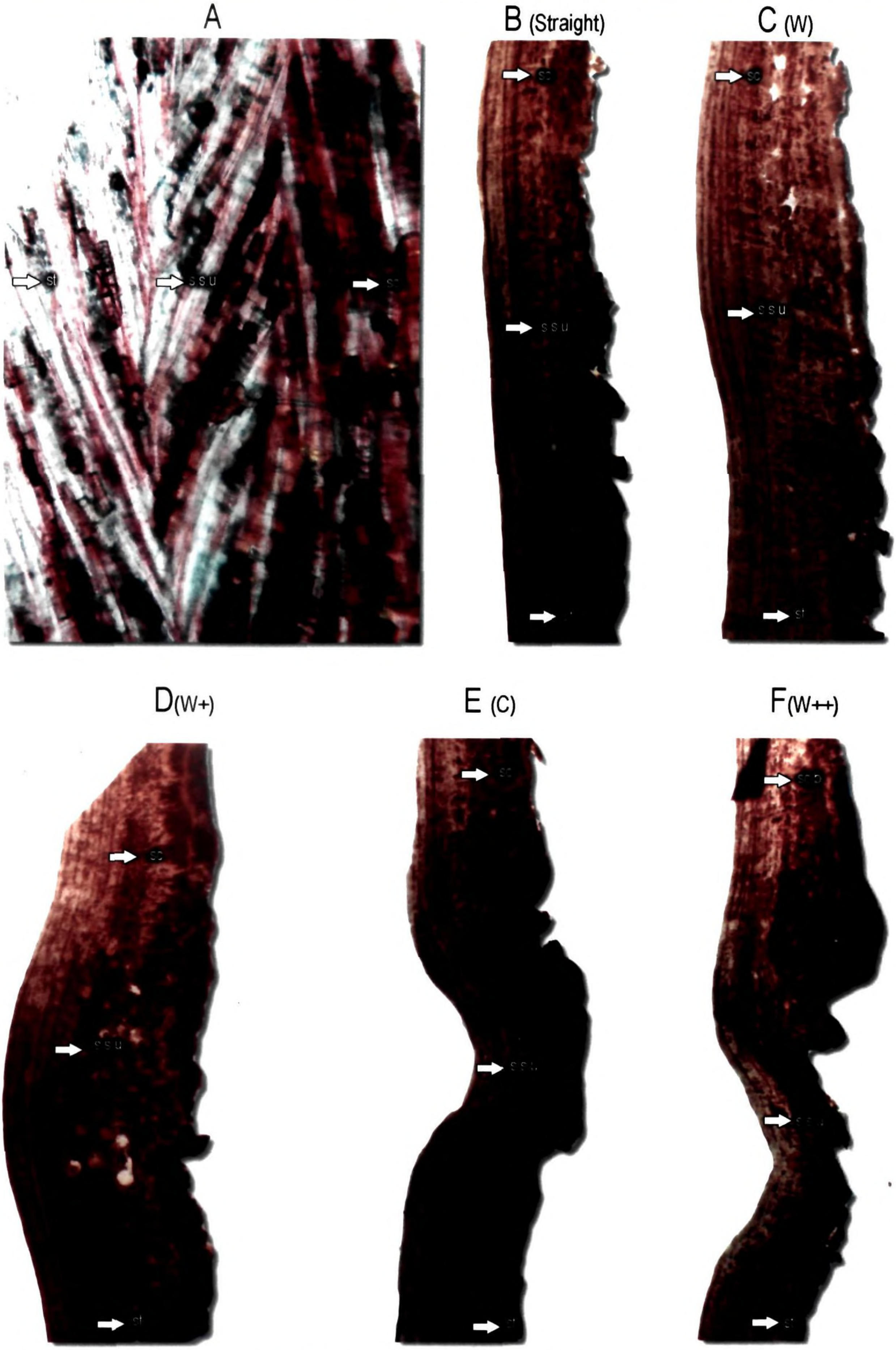
D.Tangential Longitudinal Section at the laticifer layer

PLATE 6 CROSS SECTION OF A STEM



OP- Outer Phloem
IXP- Intra Xylary Phloem
PXP- Primary Xylem Point

PLATE 7 BARK STRUCTURE AT THE UNION INTERFACE



A.Tangential Longitudinal Section,B-F.Different Types of Joint in R.L.S.
(Extent of deviation of the tissue noted by the sign in brackets)
st-Stock,ssu-Stock-Scion Union,sc-Scion.

PLATE 8 TAPPING PANEL DRYNESS

Fig 1

Fig 2

Fig 3

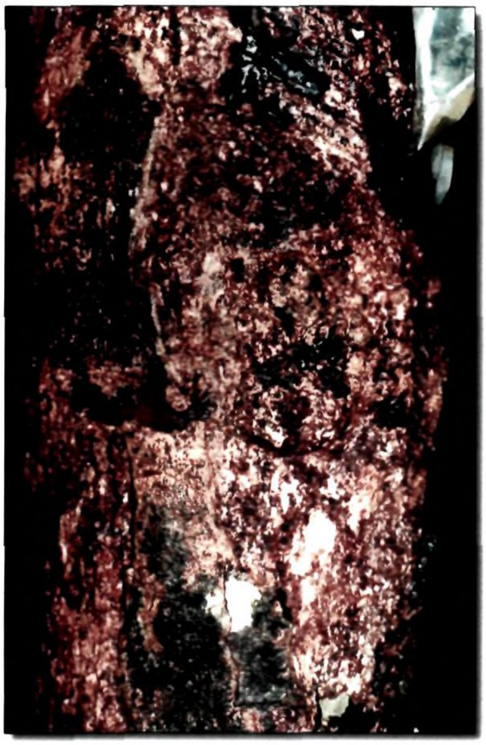


Fig 4

1.Partial dryness and deflection of trunkshape
2.Flaky & Pealing bark with renewal
3.Bulge below the tapping cut
4.Completely dry panel with reduced growth of trunk at the base

PLATE 9 ZYMOGRAMS OF ESTERASE

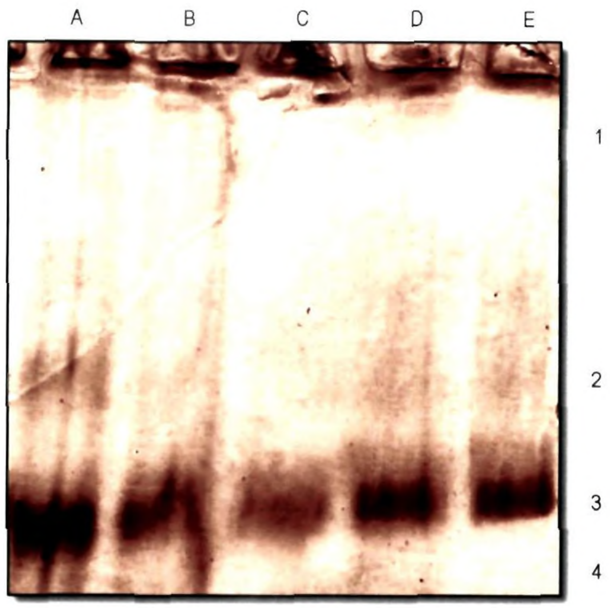


Fig 1.ASSORTED

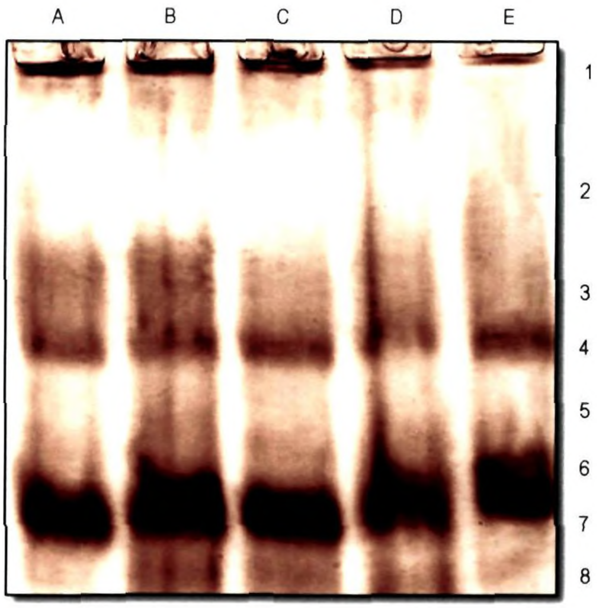


Fig 2.RRII 105

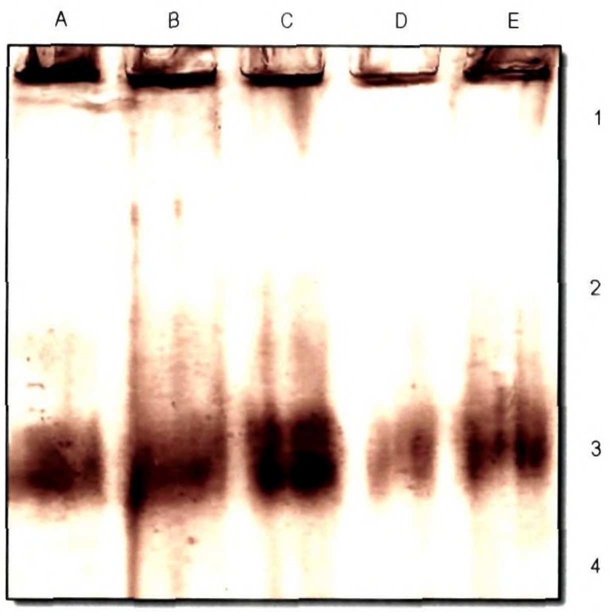


Fig 3.RRIM 600

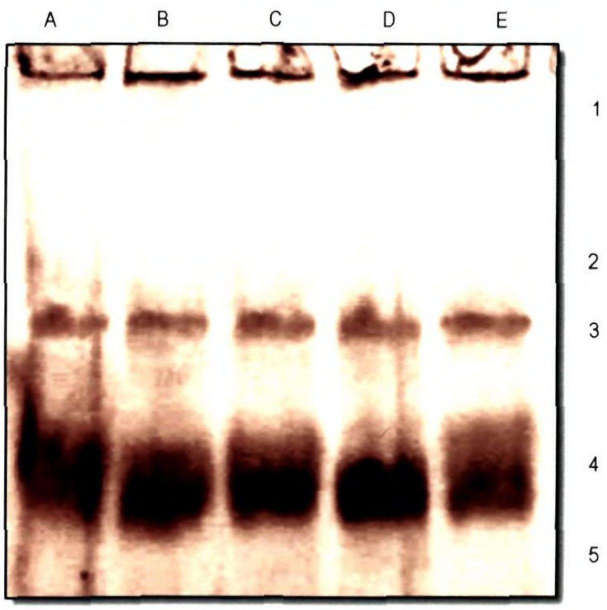


Fig 4.PB 28/59

Fig 1-4.Stock types
A-E.The 5 grafted plants per group
1-8.Band Numbers

PLATE 10 RAPD Profiles

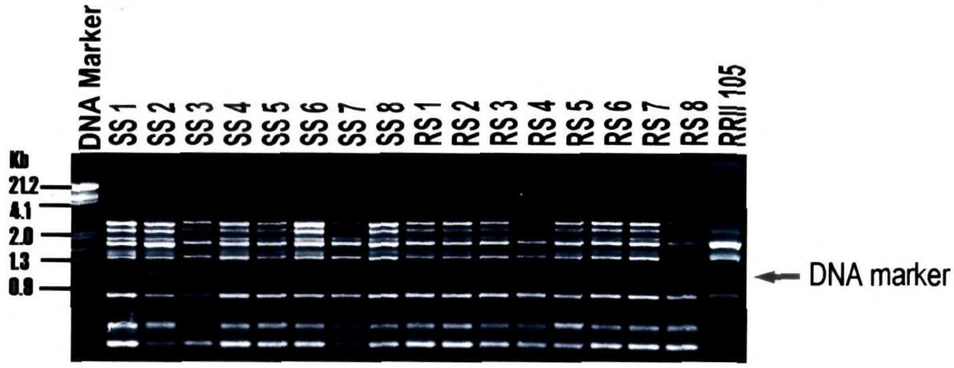


Fig1. RAPD Profiles generated using Primer OPA -18

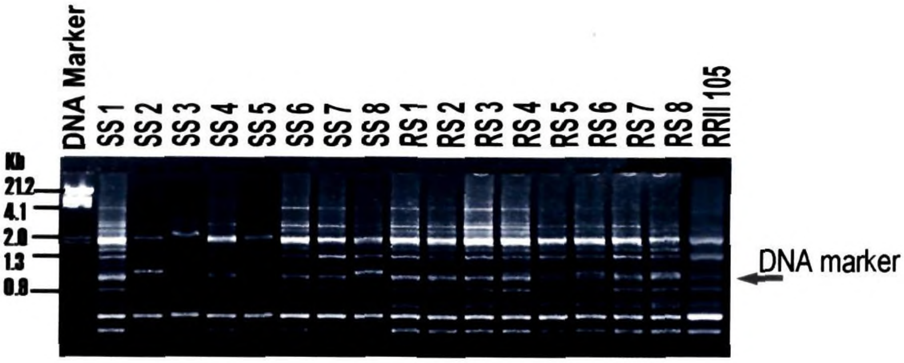


Fig 2. PCR Amplified products with Primer OPC -5

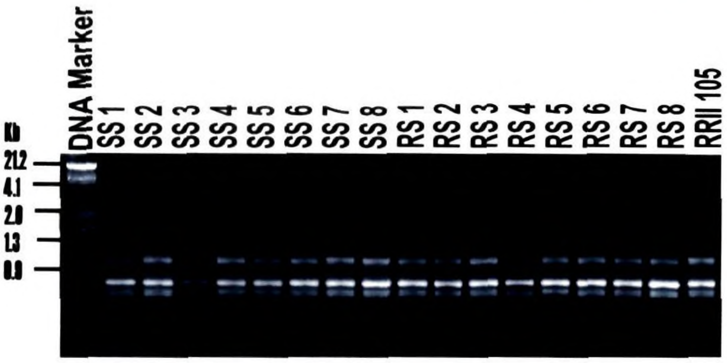


Fig 3. RAPD banding pattern produced by Primer OPE -5

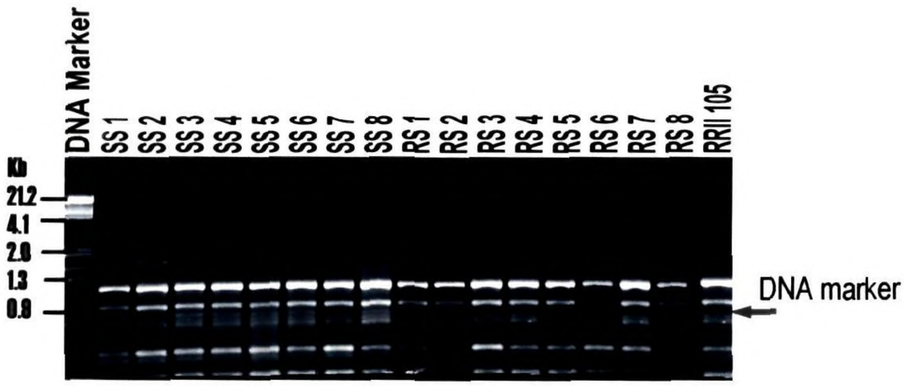


Fig 4. PCR banding pattern generated by Primer OPE -12

SS1-SS8 : Stock samples of smooth surfaced plants
RS1-RS8 : Stock samples of rough surfaced plants
RRII 105 : Control plant
DNA Marker : λ DNA (Double digested with EcoRI and Hind III)

Fig.1. Vigour in terms of number of leaf flushes

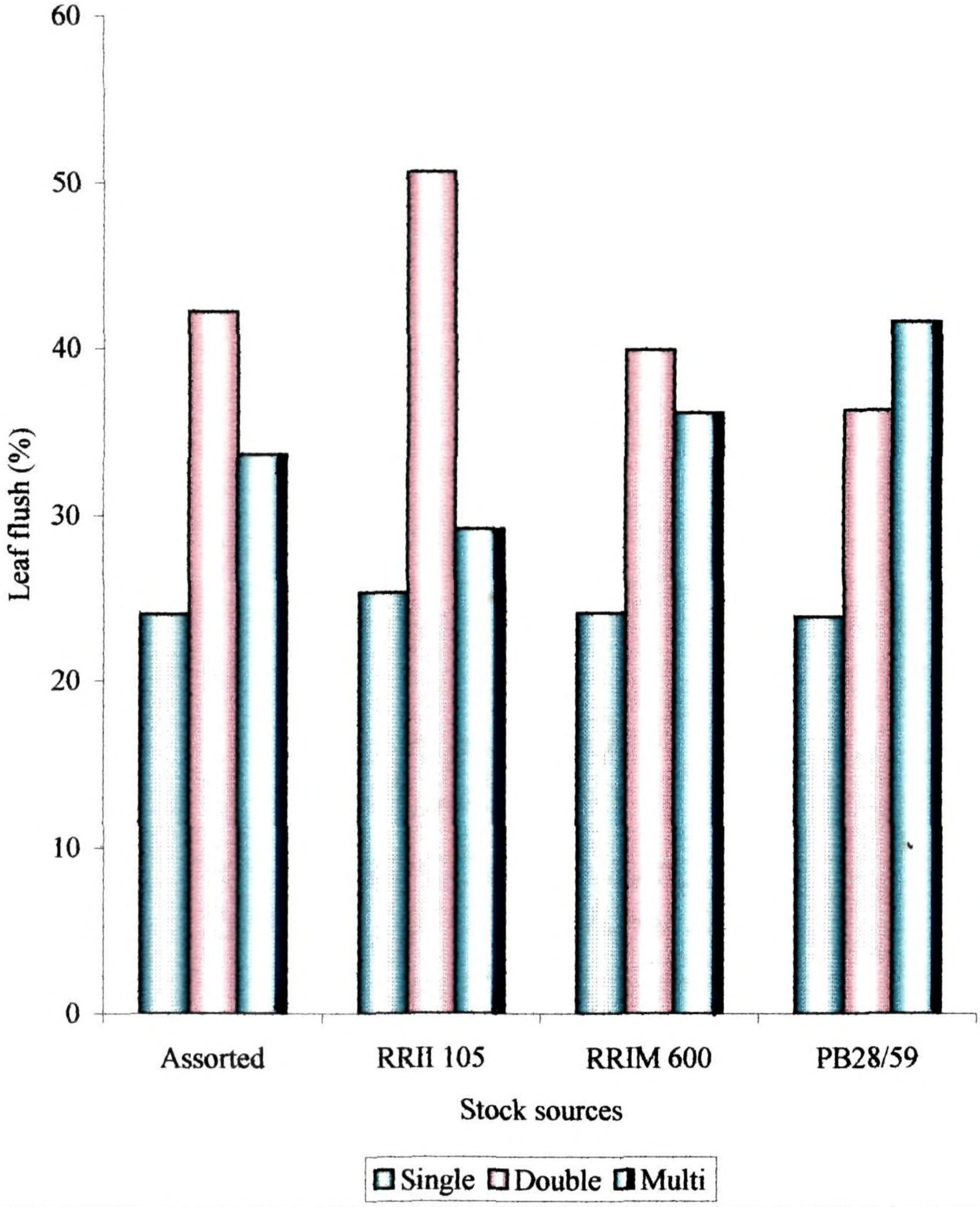
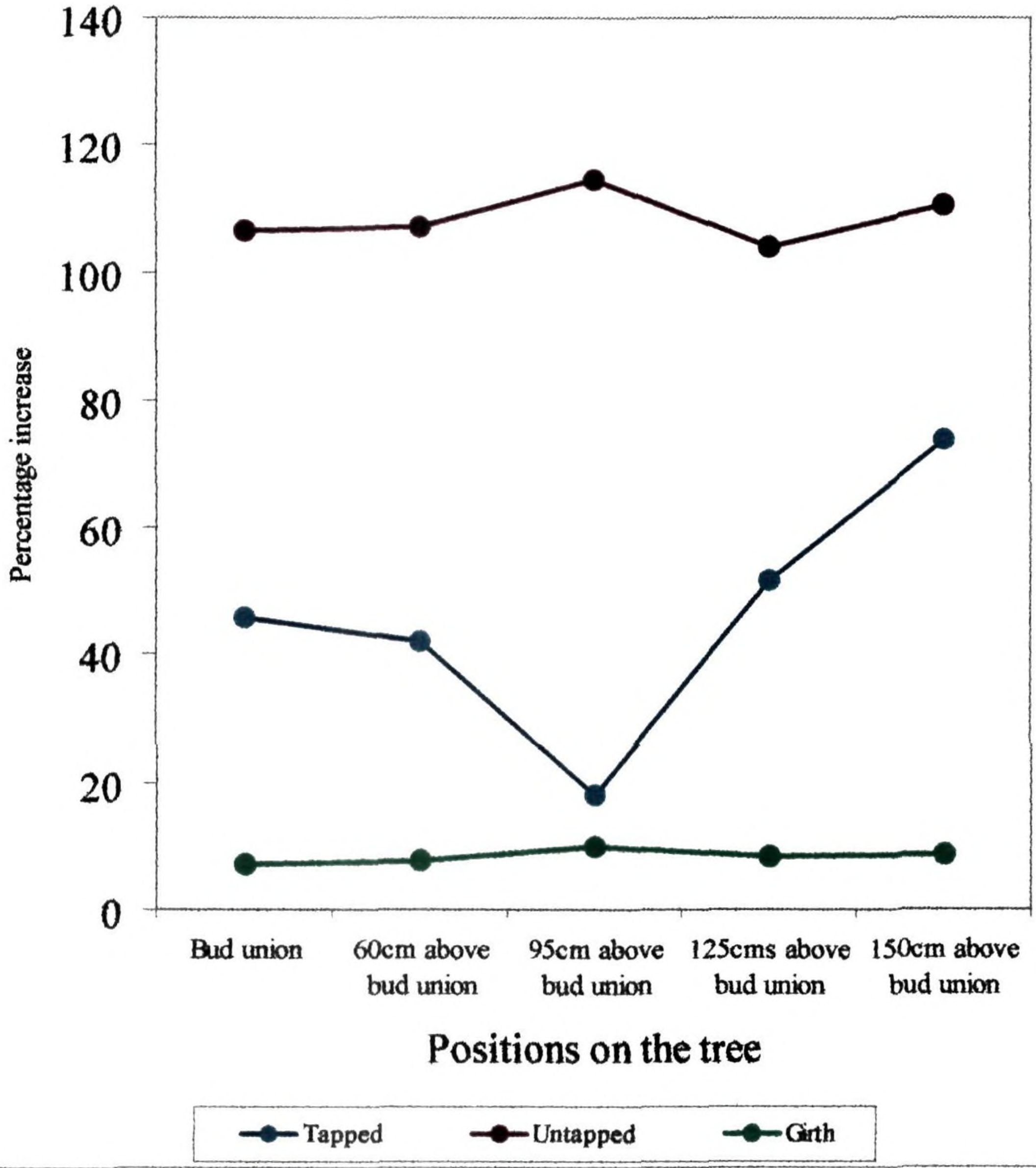


Fig.2. Percentage increase of bark thickness (both sides) and girth after first year of tapping



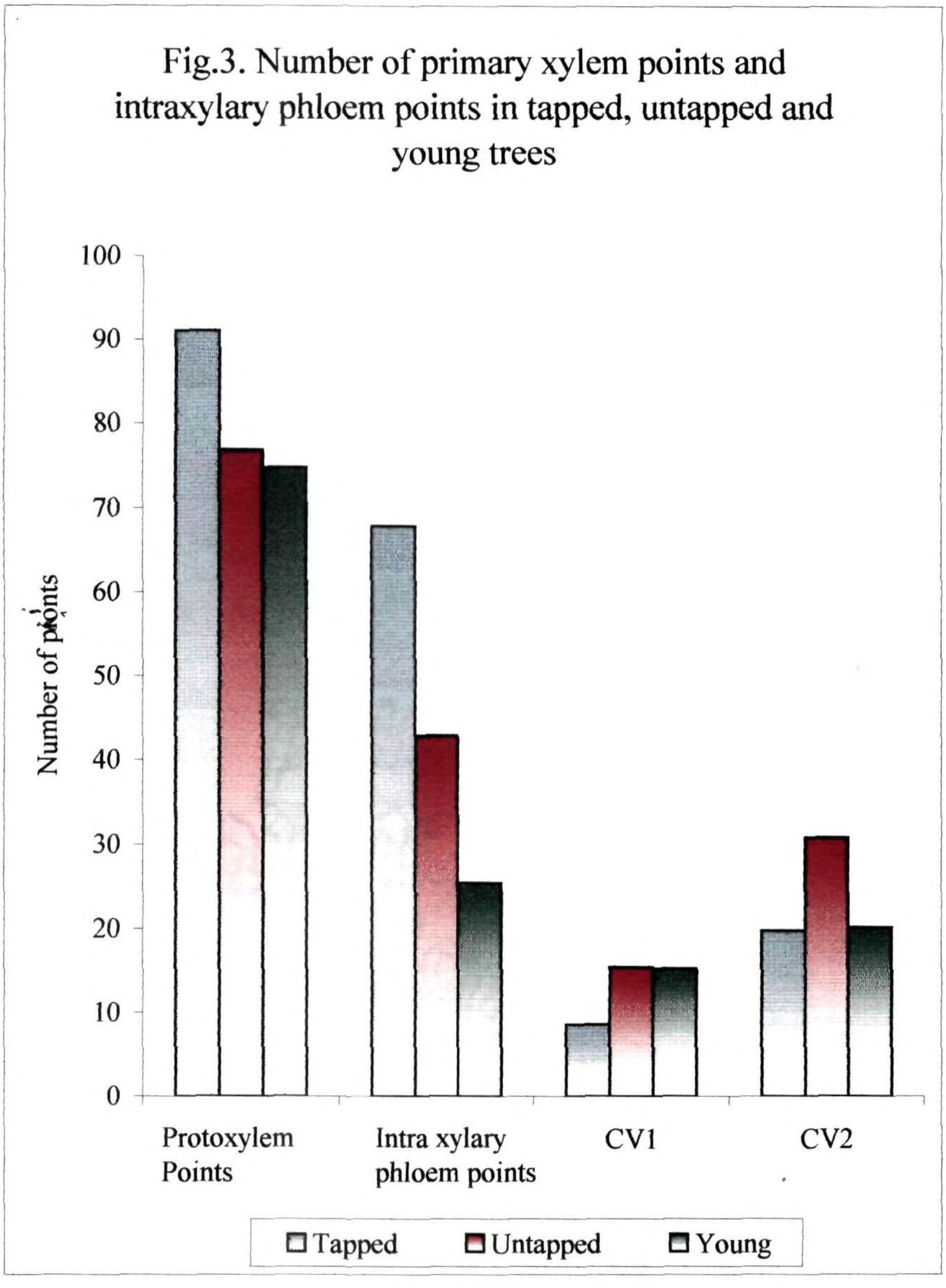


Fig.3. Number of primary xylem points and intraxylary phloem points in tapped, untapped and young trees
100
90
80
70
60
50
40
30
20
10
0
Number of points
Protoxylem Points
Intra xylary phloem points
CV1
CV2
Tapped
Untapped
Young

DISCUSSION

5. DISCUSSION

In *Hevea*, generative as well as vegetative multiplication is possible. During the early periods of plantation industry generative propagation was under practice. Later the technique of bud grafting was perfected (Dijkman, 1951) and clonal propagation came in to practice. For bud grafting, stock seedlings are produced from seeds of the same species and are grown in special nurseries.

The fruits, when mature and dry, dehisce and seeds fall off. Fruit ripening coincides with the Southwest monsoon. Fruit dehiscence requires sunshine and hence seed-fall time is after the monsoon. If monsoon prolongs seed viability is lost. Rubber seeds loose viability very rapidly. The only test of seed viability is germination. The seeds should be put for germination as soon as they are collected. Pod rot disease, caused by *Phytophthora* sp. is a big hazard in central Kerala of South India and hence seeds are collected, on commercial basis, from Kanyakumari District of Tamilnadu. Now a days assorted seeds are conventionally used for raising stock seedlings.

The budding process adopted in rubber is a method of forket method of patch budding (San, 1972). In this method a patch of seedling (stock) bark is replaced with a patch of almost the same size of bark from the scion plant. The bud patch gets attached to the stock permanently and becomes a part of it. The stock is then cut of, a few centimeters above the grafted portion and the cut end is waxed to avoid water loss. The budded stumps are used directly for planting or they are planted in polybags and grown up to 2-3 whorl stage or 4-6 whorl stage before field planting. The scion bud develops into a shoot and it is expected that the resultant plant will exhibit the characters of the scion. Depending on the colour and age of the scion stick from which scion bud is collected and also the age of stock seedlings used three types of buddings are common (1) brown budding (2) green budding and (3) young budding. As the names indicate, scion bud is collected from brown stage for brown budding. For the other types, scion bud is collected from younger green shoots (Marattukalam and Mercyikutty, 2000). For young budding very young stock plants are used. Each type of budding has it's own advantages / disadvantages. Growing green budded stumps in polybags, before field planting, is an accepted method now.

During the early years of clonal propagation monoclonal seeds were being used for raising stock seedlings. It was noticed that the root system has some influence on the growth and productivity of the resultant plant (Ng, *et al.*, 1981). This topic has been a subject of study in the Rubber Research Institutes of Malaysia (Buttery, 1961) and India and the results showed that the growth and yield is some what affected due to stock variations. Some work has been undertaken by Sobhana *et al.*, (1998) on the influence of stock on the physiological characters of rubber and Krishnakumar *et al.*, (1992) and Sobhana *et al.*,(2000) observed intraclonal variations, at enzyme level in bud grafted plants. Based on genetic differences between TPD affected and un affected plants, using RAPD technique, with a very limited number of plants, Sobhana *et al.*,(1999) suggested the possibility of tapping panel dryness due to genetic conflict between stock and scion where she has not suggested any further evidences to prove it. Still the nature and extent of such variations on growth and yield, the nature or symptoms of stock-scion incompatibility, and its impacts on other problems in the plantation industry are unexplored subjects.

In the present work, a comprehensive study was carried out to assess the variability aspects, at morphological, anatomical, biochemical and genetic levels, of assorted and monoclonal root-stock seeds and seedlings, grafting success, incompatibility symptoms and it's impacts on growth and other agronomic characters and test tap yield, the nature of incompatibility and it's possible impacts with a view to develop some parameters for nursery selection for compatible grafts. This study extends to important associations and also regression analysis for the relationships among important agronomic characters. This work involve (1) a nursery experiment using assorted seeds and monoclonal seeds of three popular rubber clones as root stock sources and RRII 105, the most accepted clone in India, as scion. In this study, the observations are centered on the variability of budded plants due to stock sources for important characters, at various stages of it's growth. The incompatibility symptoms, it's nature and impacts are discussed. (2) Intra clonal variability and associations of important characters of mature trees under tapping, which were raised on seedlings of assorted seeds.

Root stock seeds and seedlings.

Seed characters.

In rubber, fruit set is good. But loss of seeds due to pod rot disease and rapid loss of viability are the major factors (Premakumari, 1975) affecting the availability of root- stock seeds in central Kerala. In Kanyakumari region the rainfall pattern is more distributed than that in central Kerala (Nazeer, 1990; Nazeer,*et al.*,1992). So both the factors are favourable for seed production. Assorted seeds are readily available in this region and it is more convenient to make it available on commercial basis. Germination is the most reliable test for seed viability. The feasibility of using commercial seeds as materials for raising stock seedlings has not been much studied and there is no specific selection criteria for seed selection, except the conventional method of discarding very light seeds by tossing on the floor. Isolated studies on seed weight and seedling vigour (Saraswathyamma and Nair, 1976) have not provided any substantial information on the variability available among the monoclonal seeds and the locally available assorted seeds, or with in the same group, for the seed characters such as size and weight and for the percentage of germination. The present study showed that the monoclonal seeds of different popular clones such as RRII 105, RRIM 600 and PB 28/59 expressed less variability within the respective clone when compared to the variability within the assorted seeds but the difference is not very wide which might be attributed to a limited range of genetic base. It should be concluded that comparatively higher uniformity can be achieved, for the characters under study, from the monoclonal seeds while the assorted seeds are not so inferior in this respect to avoid it's use as stock source.

Seed germination as well as seed weight and seed volume differed significantly among the clones. Further analysis implicated that those traits have high genetic influence and are highly heritable. For seed weight and seed volume RRII 105 was significantly superior to the other materials, including the assorted seeds while PB 28/59 was significantly inferior to the other three seed types for germination percentage. Considering the associations among seed characters, it was observed that seed weight or seed volume have no significant association with seed germination and so seed germination is not dependent on the other two traits. Seed weight and seed volume are mutually associated while none of those traits has significant association with any of the seedling characters also. A significant association between germination percentage and plant height implicates that germination percentage is the sign of growth potential which will be reflected on the plant height also. For germination percentage assorted seeds are not significantly inferior to any of the monoclonal seeds though the difference was close to the significant level. So it should be concluded that assorted seeds are not far inferior to the other seed types for germination and juvenile growth of the resultant seedlings. In the results, a general superiority of RRII 105 over the other types of monoclonal seeds and assorted seeds for the seed characters was indicated.

PB 28/59 is a very popular clone at Kanyakumari region (Mercykutty *et al.*, 1995) and there is good chance of mixing of this clone in the commercial seeds coming from Kanyakumari. Whether there is any specific reason for a reduced viability of seeds of this clone has yet to be checked. Delayed seed fall (Premakumari, 1975) or Oidium infection can affect seed viability. However the results of the present study indicate the advantage of improving the viability of commercial seeds from Kanyakumari by discarding the seeds of this particular clone from the seed lots to be used as root stock source in seedling nurseries. It should be taken care of to avoid mixing of the seeds of this clone, as it is a popular clone at the seed collection area.

Seedling growth.

Significant differences for the growth and biomass production could not be observed among the four types of seedlings at juvenile age. Shoot weight was the highest for RRII 105 and plant height was the highest for RRIM 600. Significant difference among monoclonal seeds of few clones for seedling growth have been reported earlier (Saraswathyamma *et al.*, 1984) but no further report on the study came out. In this study the seedlings generated from the four stock sources did not show any such relationships at young age. For the number of leaf flushes formed by two month's growth, some differences among the seed types were evident.

Seed germination, seedling establishment and attainment of seedling growth to get maximum number of buddable seedlings at the time of budding are the three important requirements in the nursery before bud grafting is carried out. For green budding, the seedlings that have attained a diameter of 2.5 centimeters or above, at the time of budding, are considered as good (Marattukalam and Mercykutty, 2000). For such traits the differences due to seed types were highly significant. Fairly good CV values were recorded for the seedling characters assuring good amount of variability to be exploited. Considerably good percentages of genotypic coefficient of variation (GCV) were recorded for all the three characters indicating high influence of genetic factors in the expression of those characters. The phenotypic coefficient of variation (PCV) for the number of buddable seedlings, in terms of girth, was comparatively high which recorded medium heritability indicating the higher influence of environmental factors on this trait. The other two characters recorded high values of heritability indicating that such traits are genetically controlled and stock selection can improve the production, establishment and growth of stock seedlings. From the results it should be concluded that the productivity of rubber nurseries can be improved through selection of seed source for raising stock seedlings. RRIM 600 is not reported as a good stock source (Alexander, 1987) while PB28/59 and GT1 were under recommendation in Malaysia.

Considering the production of transplantable germinated seeds and seedling establishment, obtained in this study, PB 28/59 was the only inferior type while the assorted seeds stood on par with the other two monoclonal seed types. The inferiority of PB 28/59 for the number of transplantable germinated seeds should be attributed to the low germination percentage as the transplantable seeds, as percentage, did not show such variations among the four types and stood as second from the top and RRIM 600 was inferior to the other three types. Lethality at germination and juvenile age might be the reason for good percentage of seedling establishment. The magnitude of CV, within assorted seeds, is not very high for the two characters, which assured that the heterogeneity of commercial seeds, now available at Kanyakumari region is not very high though it is greater than monoclonal seeds. Availability of less number of clones in the plantations should be the reason.

It should be considered that assorted seeds, as rootstock source, gain advantage of easy availability and it's heterogeneity to reduce the monogenic nature of grafted plants. Monoculture is not a good policy in planting practice of any crop (Varghese *et al.*, 1990). For the maximum production of seedlings of buddable girth at green budding season, one policy to be suggested is to avoid the seeds of PB 28/59 from the lot of assorted seeds, commercially available from Kanyakumari region. Regarding this aspect among the monoclonal seeds, seeds of RRII 105 are a superior type. The area planted with RRII 105 is being extended in the plantations (Joseph and Haridasan, 1990; 1991) and hence the chances of improving the quality of commercial seeds are also more.

Budding success.

Grafting means joining of more than one piece of living tissue by means of tissue regeneration so that the united parts grow as a single entity. It is a horticultural technique that dates back to antiquity (Anndrews and Marquez, 1993). When grafting or budding is done following standard methods, there are different factors, which will affect the success of the operation as detailed by Roberts, (1927). A cordial and effective existence of the partners is possible only if the healing process is properly completed which is termed as budding success. Grafting errors like uneven cuts, delayed waxing, use of desicated scion etc will cause grafting failure. Lachaud, (1975) had written a complete review of such aspects. Lack of skill of the grafter and insufficient proximity of stock and scion tissue can be the reason for bud failure. Some researchers suggested incompatibility (Garner, 1979; Deloire and Hebant, 1982) as reason for low budding success

Low grafting success for bud grafting is not a problem in *Hevea* and more than 80percent success is obtained in commercial practices (Marattukalam and Mercykutty, 2000; Reju, *et al.*, 2002). So also bud grafting is approved as a conventional method of propagation in this species. In bud grafting, success depends up on the bark "slipping" or the peeling property of the bark, which indicate active division, and growth of vascular cambium. The newly formed cells are easy to separate from one another and so the bark "slips". Peeling quality of the stock seedlings as well as the scion stick at the time of budding is a very important factor affecting the budding success (Marattukalam and Mercykutty, 2000) and the total number of seedlings selected from the nursery for budding depends on this factor also. In *Hevea,* maturity of terminal leaf storey is a sign of good peeling quality.

Comparative merits of the stock types, with respect to the total number of buddable seedlings and budding success obtained, were recorded in the present study. For the number of successful grafts produced per lot of seeds sawn, PB28/59 was significantly inferior to the other seed types. No such variations occurred when the budding success was considered as percentage. For all the treatments more than 90 percent budding success was recorded. Hence the inferiority of PB 28/59 for the number of buddable plants and budding success might be due to low germination percentage. Good GCV values were recorded for those characters indicating the involvement of genetic factors for the expression of such traits while better values of PCV and medium heritability values indicated the chances of variation due to environmental variations. A very good result for the grafts of RRII 105 with seeds of the same clone as stock source, in terms of total number of stock seedlings produced and number of successful grafts obtained, should be attributed to genetic compatibility.

From the above results, it should also be observed that assorted seeds, available at present, are not significantly inferior to the monoclonal seeds of PB

28/59, RRIM 600 or RRII 105, now available in the seed source of South India, for germination, seedling growth, production of buddable seedlings, in green budding time, and also for budding success. Considering all the nursery traits mentioned above, the general performance of the monoclonal seeds of RRII 105 was superior to the other seed types.

Observations on surface morphology of the graft types.

The successfully grafted plants are usually maintained in the nursery till it is uprooted and planted in polybags, or sometimes directly in the field. For planting purpose the plants are uprooted and stumped following the standard methods (Marattukalam and Mercykutty, 2000) and the planting material prepared is known as budded stumps. Sprouting of the buds takes place only after stumping and planting. Before that, the growth activity of the grafted plants involves two processes. One is terminal and radial growth of the stock part and the other is radial and tangential growth in between the stock and scion, which strengthen the union

Morphological marker for stock-scion incompatibility

Two observations were made from the budded stumps; one is based on the morphology of the graft area to study (1) the nature of surface texture of graft area of resultant plants and (2) to observe the perfection of sealing process with regard to the differing stock sources. Such aspects have been topics of interest for researchers

(Lapins, 1959) in other tree crop species. In rubber no symptoms have been identified as markers for sorting out compatible and incompatible grafts.

Treatment differences for the percentages of plants with corky rough surface with prominent scar and those with smooth and level surface were significant at I percent level. Stock plants raised from assorted seeds produced 34.4 percent of corky rough surfaced grafts followed by 31.73 percent recorded for RRIM 600 and the two types were on par with each other for this trait. Rough surface at the peripheral junction of stock-scion union is a clear symptom of stock-scion incompatibility (Garner, 1979). Very high percentages of grafts with smooth surface were recorded for the groups of plants with monoclonal seeds of PB 28/59 and RRII 105 as stock and the two stock sources were on par with each other for this trait. The result showed that monoclonal seedlings of PB 28/59 and RRII 105 are compatible stock sources for acceptance of RRII 105 as scion. Cell to cell recognition has been suggested as a possible mechanism of graft incompatibility (Yeoman *et al.*, 1978; Jeffree and Yeoman, 1983). A wound response is involved in graft union and wound-sealing process requires redifferentiation of parenchyma for the wound sealing process for compatible graft union (Sachs, 1981). But the smooth functioning of the grafted plant on physiological point of view should satisfy so many internal and external factors.

For the percentage of plants with incomplete union, differences among the seed types were not significant but the treatment means showed a wide range with the lowest value for RRII 105. It should be assumed that the interference of some factors related to the expertise in grafting technique have involved in this trait and such involvement has only less affected in the case of stock raised from genetically more related seeds. For the percentage of grafted plants with gap, left in between the stock and scion, the variance ratio was not significant; but for this trait the CV value was very high indicating high variability for this character. The results show that the incomplete joining of stock and scion is not mainly due to the influence of environmental factors or due to budder effect. In patch budding, the size of bud patch should be almost the same size of the cut (Andrews and Marquez, 1993) made on the stock. The expertise of grafter (budder) in preparing the correct size of bud-patch can be the best possible reason to interfere with complete joining of the sides of stock and scion. Desication of scion tissue can be another reason. If so, much improvement in the production of perfect grafts can be expected through proper training, for the budder.

For the proportions of plants with rough surface of the graft area with scar and that with smooth surface of the union, the variance ratios were highly significant. Lower value of CV recorded for the smooth surfaced plants indicated higher stability of the character. Higher CV value was recorded for the percentage of rough surfaced plants indicating higher variability within this group. Both the traits recorded high values of heritability, showing that genetic factors are involved in the expression of those characters. Genetic incompatibility is reported as a multigenic effect (Copes,1970, 1978,Salesses and Al kai, 1985). Very high values of GCV and PCV for the percentage of rough surfaced plants, compared to the smooth surfaced plants showed that the high variability for the former trait was generated due to genetic factors and the expression of this trait can vary much due to environmental variations also. The high variability can be due to stock- scion interaction, also controlled by the genetic incompatibility of stock and scion. Hence this character is a clear symptom of stock- scion incompatibility in rubber.

Comparative merits of root stock sources for further growth and test tap yield of the grafted plants.

When two plants or plant parts join together and grow as a single unit, anatomical and physiological interactions and adjustments are imperative. Some times such adjustment may be desirable or it may produce undesirable effects. When the effects are towards desirable side or to perpetuate the characters of the scion tissue the respective stock is considered as compatible. The compatibility effects for important traits such as sprouting of grafted bud (Nelson, 1968), field establishment, further development and growth of scion tissue (Hartmann and Kester, 1972) growth competition between stock and scion and resultant impacts on the growth and productivity of scion plant (Beak bane and Roger, 1956), at different environment (Hartmann and Kester, 1972) and growth stages may vary. There are several reports on the stock effect on plant size due to the interaction of stock and scion. This is said as definite root stock influence on scion vigor as well as in combination with other factors. Differences in scion performance of strong and weak stock can be greatest when the plant is growing under adverse soil conditions (Lee, 1989). Many examples of the effect of rootstock on size of the scion variety are available in the case of apple (Bunyard, 1920 ,Hatton,1927). In citrus also marked effect of rootstock on scion vigor is reported and studies with Orange varieties (Bitters, 1950,1951;Gardner, 1963) showed that the rootstock used markedly affected tree size as well as fruit size and yield. Some observations on such aspects, with respect to the vigor of budded stumps, sprouting of scion bud, survival and growth of polybag plants, field establishment, growth, some anatomical and biochemical parameters, important leaf diseases and test tap yield of field plants were carried out and comparative merits of the root stock sources for such aspects were examined in this study.

Positional girth variations of budded stumps.

When the budded stumps developed using different stock sources are considered for, positional girth variations, the treatment differences are significant for all variables and the significance is more strong for girth at the upper joint of bud union. Girth value is the highest at the lower joint, even higher than the stock base girth; and the girth at upper joint is higher than that at the bud point of the respective group. The increased girth might be due to the union growth at the joints. The stock base girth is low for the stock, raised from RRII 105 and RRIM 600 while that from the assorted seeds and the monoclonal seeds of PB 28/59 are on par with each other. For the girth at the lower joint, bud point and the upper joint RRIM 600 recorded the least girth values. Excessive bark thickening and over production of sieve cells are reported as symptoms of incompatibility (Copes, 1980) but it is not suggested as a reliable indicator (Garner, 1979 and Westwood, 1988). In general RRIM 600 showed poor growth of stock seedlings after bud grafting as well as it's poor girth increase for the bud joining.

Low values of CV recorded for the different characters showed low magnitude of variability among the stock sources. However the variability is statistically significant and hence useful. Heritability values, ranging from 38.8 to 54 percent, were recorded for the girth at different positions with the maximum heritability for girth at upper joint and minimum for the girth above graft area. The result showed that the growth process for bud union involve good genetic control than the genetic contribution for the girth of stock seedlings. The union growth is also influenced by environmental factors.

Sprouting of scion bud

The spouting percentages for the first observation showed a range of 15.60 to 21.20; but the difference was not significant. For the second and third observations also the treatment differences showed narrow ranges and the differences were not statistically significant. The differences for fourth observation, eight weeks after planting, were significant indicating that the time taken for bud breaks varied, among the treatments, significantly. The results indicated delayed sprouting for the budded stumps raised using the stock seedlings from PB 28/59. For sprouting quality PB 28/59 as stock source, was significantly inferior and the other three types were comparable to each other. Late bud break is noticed as an external symptom of incompatibility by Nelson,(1968) in his survey of horticultural plants for incompatibility. Internal symptoms usually precede external symptoms. Moor and Walker (1981b) defined incompatibility as physiological intolerance at cellular level.

Growth in polybag nursery.

For plant height, the plants raised on the stock seedlings of assorted seeds were comparable to the plants with RRII 105 as stock source. It was superior to the other two groups of plants while the latter two were comparable to each other. For stock girth, the plants raised on assorted seeds and PB 28/59, as stock source recorded better performances than the other two types, with RRIM600 and RRII 105 as stock sources. The treatment differences for stock vigor at budded stump stage was somewhat maintained in the polybag also. For scion base girth, the plants raised using RRIM 600, as stock source was the most inferior type. For this trâit assorted seeds and the monoclonal seeds of RRII 105 were on par with each other. For the girth at stock-scion joint and for percentage increase of stock girth, the differences due to stock sources were not statistically significant. But the absolute value recorded for percentage girth increase of the stock part in polybag was very low for the group with PB 28/59 as stock, compared to the values recorded for the other three types and highest value was recorded for the plants on monoclonal seeds of the same clone, RRII 105.

Out of the five characters recorded from the polybag plants, the scion characters recorded comparatively higher values of CV, showing good variability. The highest CV value was recorded for the percentage increase of stock girth in polybag, but for this trait the variance ratio was not significant; and high PCV value for this trait showed high influence of environment for the expression of this character. Better CV values recorded for the girth of scion characters (scion height, and scion base girth) due to stock variations, compared to the stock girth should be attributed to the stock – scion interaction. High heritability value, recorded for scion height, indicated better influence of genetic factors on this trait. The effect of root sock on plant height has been well utilized in apple. Based on the vigor of the scion plant subsequently appearing, the stock varieties were grouped as very dwarf, semi-dwarf, vigorous and very vigorous by Hatton (1927; 1930). The girth variability at the interface showed more similarity to the girth variability of stock than the girth variability of scion base indicating better contribution from the stock (Hartmann and Kester, 1972). Comparatively less heritability values were recorded for stock girth and the girth at stock-scion joint, of poly bag plants compared to such traits at budded stump stage.

Observations on field plants.

Using the experimental materials (polybag plants), field plants were raised in a spacing recommended for bud wood nursery (Potty, 1980). Observations were taken on growth, diseases, test tap yield, biochemical and molecular parameters.

Growth performances and test tap yield.

Differences on the percentage of vacancy occurred due to stock sources was statistically significant. PB 28/59 was the only stock inferior to the other stock types with respect to this character. For all the other characters the plant types were on par with each other. High variability was recorded for the percentage of vacancy occurred when the polybag plants were planted in the field. The character showed good GCV value indicating the involvement of genetic factors in the expression of this character. Very high value of PCV showed that environment also has good influence on this trait.

Occurrence of vacancy in the field and subsequent gap filling is a serious problem in rubber cultivation. The improvement in propagation methods led to the production of polybag plants to be used as planting material, instead of budded stumps. This development was a solution to the problem of high vacancy in the field during subsequent years; but still it is an expensive item in adverse climatic conditions during planting season, or when the root system is disturbed on long distance transport of polybag plants. Since this trait is influenced due to stock, judicious selection of stock will make improvement in this field to reduce cost of production and also to raise uniform plantations. There were some reports on the limitations and economic loss due to graft incompatibility as stock effect .In pinus *radiata* more than 50 percent of the grafted trees showed incompatibility responses Sweet and Thulin, (1973). The tree growers were forced to change from grafted to seedling orchards due to this problem. In *Hevea*, as tree crop vacancy filling is an expensive operation. It is still observed that the cost of cultivation can be reduced by reduction of vacancy by stock selection. Since this trait showed high GCV value there is ample scope for stock selection for low vacancy.

Disease susceptibility.

Shoot rot and *Oidium* infection are the major diseases of *Hevea*. PDI rating for *Oidium* leaf disease for two consecutive years showed that the four treatments were on par with each other for susceptibility to these diseases and stock difference had no influence on the infection rate. *Hevea* plants grown in the nurseries showed similar responses for powdery mildew intensity (John *et al.*, 2000). The disease intensity was not controlled by the stock effect but due to seasonal changes climatic effect and leaf phenology. Tree species typically express trait differently in different environments (Carson and Carson, 1989) and genotypic ranking may change with the environment the pathogen may also vary in virulence in different environment.

Biochemical parameters

Total protein content

The total protein content within clone variability was the highest for the group with assorted seeds as stock source while the plants with monoclonal seeds as stock sources, showed more uniformity, with maximum uniformity for the group with RRII 105 as stock source, probably due to genetic similarity between stock and scion. Lower protein content was observed for the plants with genetically similar stock .The possibility for reduction in biosynthetic capacity of such plants cannot be ruled out.

Peroxidase activity

Peroxidases are ubiquitous enzymes found in virtually all green plants, which often increase as a response to stress, and one of the principal roles of peroxidase appears to be cellular protection from oxidative reactions imposed on all photosynthetic plants (Siegel, 1993). Under stress conditions plants produce good amount of peroxidase.

Considering the peroxidase activity, maximum variability within group was recorded in the plant group with assorted seeds as stock source. The plants raised on monoclonal stock sources showed more uniformity with maximum uniformity for

RRII 105 as stock source. This may be due to the genetic similarity of the stock and scion. The lowest peroxidase level for the plants with RRII 105 as stock source should be attributed to the extreme compatibility of graft union as suggested by Andrews and Margues (1993).

Esterase activity

Enzyme systems are ideal gene markers, which are involved in various physiological functions. The results showed that plants with RRII 105 as rootstock as well as scion source, had the highest level of esterase activity, when compared to the plants of the same clone with other three types of root stock sources. The high activity might be due to the homologous nature of the stock and scion. The variation observed in the esterase activity was independent of the total protein content. For this trait also assorted seeds generated more variability in the scion plant when compared to the monoclonal stock sources. Sreelatha , *et al* (1993) observed Sharpe and well resolved banding patterns of esterase and peroxidase in the leaf extracts of *Hevea brasiliensis.*

Krishnakumar *et al;* (1992) observed polymorphic isozymes expressions among different plants of RRII 105 raised using assorted seedling as root stock. Isozymes variations in different clones, raised on assorted seedlings as root stock were observed by Sobhana ,*et al* (2001). The amount of total protein content and the level of peroxidase and esterase activity varied due to root stock effect. Biochemical parameters in the scion part are highly influenced by stock-scion interaction.

Genetic polymorphism

RAPD analysis

RAPD technique developed by Williams *et al.*, (1990) relies on the differential enzymatic amplification of small DNA fragments using PCR with single oligonucletide primers (usually 10- mer). Polymorphisms result from either chromosomal changes in the amplified regions or base changes that alter primer--binding sites. RAPD has its advantages over RFLP, as it requires only small amounts of DNA and polymorphisms can be visualized through agarose gel electrophoresis avoiding the need for Southern hybridization using hazardous radio labeled materials. This technique is relatively simple and more rapid. The RAPD markers are usually dominant because polymorphisms are detected as the presence/ absence of bands.

The RAPD technique is based on the development of an *in vitro* procedure called Polymerase Chain Reaction (PCR) for the primer directed enzymatic amplification of a specific DNA fragment (Saiki *et al.*, 1988). The PCR technique relies on the capability of a thermo stable DNA polymerase called Taq DNA polymerase, isolated from a thermophilic bacterium Thermus *aquaticus* to remain active even at 96^{o} C, which is essential for the denaturation of template DNA. The reaction is based on the annealing and extension of two oligo nucleotide primers that flank the target regions in duplex DNA. After denaturation of the DNA, achieved by heating the reaction mix, each primer hybridizes to one of the two separated strands such that extension for each 3' hydroxyl end is directed towards the other. This extension was carried out by Taq DNA polymerase in presence of deoxynucleotide triphosphates. Thus each cycle of PCR involves three steps i.e. denaturation, annealing and extension. During each cycle the quantity of the amplified fragments get doubled. RAPD studies have efficiecently been done to investigate genetic relationship between and within populations (Gen-Lou Sun *et al.*, 1999., Duarte *et al.*, 1999,Bai *et al.*, 1998),to evaluate the clones(Scheepers *et al.*,1997; Hicks,1998), to study the inheritance and gene introgression (Garcia *et al.*,1995;Gomez *et al.*, 1996) and to identify molecular markers for various characters (Borovkova, *et al.*, 1995) . Thulaseedharan, *et al.*, (1994) observed variation in RAPD profile of TPD resistant and susceptible seedling trees and two polymorphic DNA markers conferring tolerance to tapping panel dryness. Varghese, *et al.*, (1997) observed fairly high genetic variability among Wickham clones using RAPD markers.

Growth performances and test tap yield in the second year.

Among the observations on growth characters, taken during the second year of planting, significant differences due to stock sources were observed for only one character; scion girth at 50-centimeter height from the bud union. Stock effect on the scion plant vigor during subsequent growth is a well-established fact. Several reports on the application of such effects are available in horticultural studies (Vaile, 1938). In the present study the CV for the different characters showed low values indicating low chance for improvement through stock selection. Comparatively good CV was recorded for test tap yield but this trait did not show any significant differences due to stock sources. When the variability was partitioned as genotypic and phenotypic very low GCV values were recorded for plant height and number of buds per meter of bud wood. In general, the growth and test tap yield of field plants was not much influenced by the stock sources.

Comparison of stock sources for structural traits.

The productivity of *Hevea* is highly related with bark structural characters such as bark thickness, softbast/hard bast proportion, and number of latex vessel rows. The bark is more affected in plants (Copes, 1980) due to incompatibility than the wood. Structural events taking place from the beginning of stock- scion union are important factors to decide compatible union and also to control subsequent effects on the growth and productivity of the grafted plant. The incompatibility sometimes produces delayed effects on growth through reduced translocation (Argles, 1937) or differential starch distribution (Breen and Moraoka, 1975).

Breakage at the graft union, (Garner, 1979; Eleftheriou, 1985) due to very few or practically no functional connection or interlocking fibers in between stock and scion are clear evidences of incompatibility in some cases. High deposit of tannin or suberisation of bark tissue are observed as signs of incompatibility (Santamour, 1988b). The bark structural differences occurring in the plants due to stock sources were examined on quantitative basis. Structural differences between stock and scion parts and also at the interface were recorded.

When considered as stock wise, bark thickness was more for the plants raised on assorted seeds, as stock, followed by those with RRIM 600 as source of stock.

When considered as position-wise, the interface recorded maximum bark thickness. Further observations showed that the proportion of soft bast is the least at the interface and the least number of latex vessel rows were present at the interface region indicating that the higher bark thickness at the interface was due to high amount of cork tissue. The situation recalls the situation reported in other crops (Proebsting, 1926) in the case of incompatible grafts which show weak development of vascular connections between stock and scion (Proebsting, 1928; Deloire and Hebant, 1982) leading to physiologically delayed incompatibility which may be the causal factor leading to TPD. It should also be considered that very low proportion of plants showed incompatibility symptom, on the graft area, among the plants raised on RRII 105 as stock source compared to those with assorted seeds/RRIM 600 as stock source. Comparatively better proportions of soft bast tissue with higher number of latex vessel rows for the plants raised on RRII 105 stock source was an interesting result though the statistical significance for the differences, due to stock sources, was interrupted by some other factors. The study was conducted in central Kerala where the situation is ideal for rubber cultivation and this clone performs well while the establishment, growth and productivity of RRII 105 is not so good in non-traditional areas. It is suggested that use of monoclonal seeds of the same clone, as stock source may improve the performance of RRII 105 in such areas. The incompatibility effect is seriously affected at adverse situations (Ko, 1999).

Variability, due to incompatibility.

The stock effect on scion characters of *Hevea* have been a subject of study (Seneviratne *et al,* 1966) and a few reports on the intra clonal variations of growth and yield (Ng *et al,* 1981) and physiological parameters, are available. But the compatibility /aspect is an unexploited area. No report is available on any specific symptoms of compatibility to be utilized as a selection parameter. In the present study, the surface texture of the graft area was identified as a symptom to select out compatible/ incompatible grafts. In some plants the graft area was smooth without prominent scar at the union part which should be attributed to good acceptance of scion tissue and easy wound sealing of the gap between stock and scion which are factors affecting stock-scion union (Argles, 1937). Others showed rough surface with prominent scar. Variability for the occurrence of compatible and incompatible types, among the plants raised on the different stock sources, was statistically significant also. From the pooled data, compatible and incompatible plants were sorted out, irrespective of the stock sources and the mean values of the two groups, for important characters, were compared to examine the feasibility of using such morphological symptoms as incompatibility and to evaluate it's impacts on other agronomic traits. The structural differences in the internal orientation of tissue at the graft area was observed and compared.

The orientation of axial tissue in the stock-scion interface.

As observed in other crops (Stoddard and McCully, 1979,1980; Moore and Walker, 1981a and Gebhardt and Goldbach, 1988) a callus bridge of parenchyma cells forms rupturing and invading the necrotic layer formed at the cut ends. During this process, the tensile strength of the graft increases due to physical cohesion between stock and scion and union of the two strengthens. Then new vascular cambium is differentiated from the parenchyma cells bridging the stock and scion and finally the secondary tissues are formed. Hence the direct contact of the vascular cambia of stock and scion is not a necessity for the graft union (Lachaud, 1975). Still a good alignment of the three cambia will provide greater mechanical strength (Esau,1965; Kollmann,*et al.*, 1985) to the union. Thus the three main points of contact in the orientation of the vascular tissue at the interface are (1) stock with interface (2) at the interface and (3) interface with scion. The deflections can be at one or more points in varying shape and so also are the different grades .

The distribution pattern of different grades of deflections in the two groups, were examined. W++ and C curves (deflections at high intensity) were not present in compatible group while more than 90 percent of the plants in incompatible group showed the deflections at varying extend. Breen, (1974) suggested damage of Phloem tissues to be more severely affected than xylem tissues due to incompatibility. According to Copes, (1980) the symptoms include phloem and cortex degeneration a typical axillary xylem parenchyma lack of axillary parenchyma in the phloem and necrosis of cortex cells (Copes, 1980). Increased peroxidase activity in both partners and deposition of lignin and polyphenols at the graft union, impeding formation of vascular connections and translocations is reported by Deloire and Hebant, (1982).

Comparison of incompatible with compatible plants

Incompatibility effects on the vigor of budded stumps

At all the positions measured for growth in terms of girth, better growth was recorded for compatible grafts. The differences were statistically significant for the two stock positions and also for the bud point. At the joints the union growth is more complicated and the influence of other factors may interfere with the growth at the joints. The union growth depends up on so many factors such as size of the gap in between the stock and scion tissue, genetic differences between the two partners and also the environmental factors at the time of budding (Lee, 1989, 1994 ; Ko, 1999). However the enhanced growth at the bud point for compatible grafts is a good sign of compatibility and a useful parameter to be exploited for selection of budded stumps for the growth potentials of stock and scion.

Growth variations of incompatible and compatible grafts in polybags

Growth variations of the two groups of grafts in polybag nursery was made just before field planting, For none of the characters the differences were statistically significant at polybag stage. For girth at the scion base and at the stock-scion -joint and also for plant height, incompatible grafts recorded lower values. In polybags the soil composition and atmospheric conditions are almost uniform and ideal. The incompatibility effects are seriously expressed at adverse situations (Andrews and Marqez , 1993). That should be the reason why the differences observed did not reach to a significant level. From the results it should also be concluded that it is better to do selection at budded stump stage, for compatibility/ incompatibility effects on the growth of scion part.

Growth variations and yield trend of field plants.

For all the growth characters the incompatible group recorded lower values than the values of the respective characters of compatible group. In the first-year observations, the difference was statistically significant, at 5% level, for the girth at stock-scion joint only. The difference between compatible and incompatible groups for test tap yield was highly significant. From the results it should be concluded that plants resultant of the incompatible grafts are inferior for further growth and development of both the partners and also for juvenile trend of rubber yield. Lack of significance for the differences of growth characters, in the field, should be attributed to the ideal situations, for rubber cultivation, in central Kerala and less amount of soil heterogeneity due to nursery spacing. It is a well-known fact that RRII 105 is inferior to RRIM 600 and GT 1 for its growth and establishment and also for the productivity (Alexander, 1987) in nontraditional areas in India where the climate is not ideal for rubber cultivation.

Quantitative anatomical characters.

Total bark thickness and the percentage of hard bast thickness recorded the highest values at the interface region and for the number of latex vessel rows highest values were recorded for the stock portion for both the graft types. For bark thickness and percentage of hard bast, incompatible plants recorded higher values and the reverse was for number of latex vessel rows. Thickening of bark and suberisation is associated with incompatibility (Copes, 1980). For total bark thickness and number of latex vessel rows the differences for mean values were comparatively low. The differences were not significant with respect to any of the positions, indicating that compatible and incompatible grafts are on par with each other for those traits. For the percentage of hard-bast thickness the differences between compatible and incompatible groups were more and was highly significant with respect to all positions irrespective of the stock sources. From the results it is observed that incompatible grafts produce significantly high amount of cork tissue and stone cells than the compatible grafts. This should be considered as a symptom of higher intensity of stress at the interface region of incompatible grafts, compared to the compatible grafts. Considering the three characters, comparatively better values of CV were recorded for the number of latex vessel rows with a range of 20.35 to 29. 99 for incompatible group and a range of 24.78 to36.94 for compatible group. CV value was the least for the percentage of hard bast, with respect to all the four positions, with a range of 6.34 to 9.36 for incompatible grafts and a range of 7.61 to 16. 76 for compatible grafts respectively. Considering the variability within each group, a wide range is recorded for compatible group. Hence it should be concluded that the external symptom is expressed at extreme conditions and the external symptom is reliable.

Correlations and regressions for early prediction of planting material

Associations among seed characters and nursery characters.

The degree of relationships among characters is a useful tool to predict useful agronomic traits indirectly through the close associations of such characters with other easily predictable traits. In rubber test tap yield (Annamma *et al.*, 1989), early vigor and bark anatomical characters of scion plant (Premakumari *et al.*, 2002) have been suggested as early prediction parameters in clone selection. Such associations between the two partners due to stock variations is an unexplored area though some intraclonal variations at isozyme level have been suggested. Some predictions could be made on the basis of the results of this study.

The results observed for the associations among seed characters and nursery characters did not provide any useful associations to predict seed viability before germination or for prediction of seedling vigour. Germination is the best criterion for seed viability and terminal growth of the seedlings. The correlations showed that the root / shoot ratio is more influenced by the shoot biomass than the root biomass but the association between dry weight of shoot and plant height was not statistically significant.

Correlations among stock and scion portions of the grafted plants, with respect to anatomical characters.

The results showed that stock has high influence on scion characters and the influence is more pronounced at the scion base, in the case of bark thickness than it's hold at higher height positions of the scion stem. The stock, interface and scion base keep a close and significant association. Several studies on the stock- scion interaction in grafting is available (Beakbane and Roger, 1956) in tree crops. It is reported that the phloem tissue and its function is more affected, due to grafting, than the xylem (Breen, 1974). Different types of incompatibility symptoms and effects such as translocated incompatibility (Mosse, 1962) and delayed incompatibility (Argles, 1937) are reported. The stock is contributing more for the formation of interface callus and so the characters at the interface is more controlled by the stock than the scion (Hartmann and Kester, 1972). The results implicate the possible influence of stock-scion interaction on the occurrence of tapping panel dryness.

Associations among growth characters and test tap yield.

For early prediction of growth and yield, correlation studies on growth and yield and various anatomical and physiological parameters have been used in rubber. Such studies are mostly limited to the scion part of bud-grafted plants at different ages. In the present work it was extended to the level of stock scion interaction and the growth variations of stock, stock-scion joints and the growth at the bud point of grafted plants have been utilized for estimating the trend of further growth and development of the scion plant, developed by bud grafting. With respect to the budded stumps, girth at the bud point showed very high association with the stock base girth ($r = o.8641$; $P < 0.01$) and this trait showed good correlation with the stock girth of polybag plants also ($r = 0.7368$; $P < 0.01$). The girth of stock at budded stump stage highly influenced the girth of stock and scion stem at further stages of growth in polybag and later in the field. So the growth of stock and scion are highly interrelated. The specific trait at one stage shows good correlation with the same trait at subsequent stages of growth. Terminal and radial growth are interrelated. That means the vigor of stock will influence further growth of stock and scion in polybag as well as in the field. All associations were positive. The degree of relationships of stock girth at budded stump stage with the girth at further growth stages were maintained at significant level.

Early selection of compatible type of planting materials

The economy of rubber cultivation is very relevant as rubber is a commercial item reasonably contributing to the Indian economy. Any chance of reducing the cost of cultivation of this perennial crop is welcome; especially when the rubber growers are suffering from price fluctuations (Ipe, 1988). Early selection as well as the selection for yield and growth potentials is the thrust area of rubber research. Based on the significant associations of stock stem girth and girth of the bud point at budded stump stage with the growth characters of stock and scion at further growth stages, regression equations were derived. Such estimates are useful for early prediction of desirable traits.

The regression of growth characters and test tap yield on some of the characters at the budded stump stage / polybag are useful for early prediction of growth at polybag stage and in field also for predicting the yield trend of field plants. The regressions were highly significant for all the eight combinations. The regressions of bud point girth on scion base girth of the budded stump stage was highly significant and recorded an R^2 value of 74.66; the girth at stock-scion joint of polybag plants depends on the girth at bud point of the budded stump, the R^2 values for the relationship being 67.62 percent. According to the equations, a cordial and enhanced growth activity at the bud point, after graft union, can be predicted from the base girth of the stock plant. This trait will explain nearly 75 percent of the growth variations at the bud point. Growth at the bud point can be taken as a good parameter to decide the growth at the stock- scion joint of the polybag plant for the strength of the union, which will explain nearly 68 percent of the variations of growth in polybag. Such selection may reduce the cost of rubber cultivation by reducing the percentage of weak plants in polybag nursery, assuring the productivity of polybag nursery.

Another strong relationship was the dependency of scion base girth of the field plants on scion base girth of polybag plants, which was also highly significant. Based on this relationship polybag plants can be selected for field planting, on the basis of scion base girth, for further good growth in the field. Now the practice of deciding the vigor of polybag plants is on the basis of number of leaf flushes (Marattukulam and Mercykutty 2000). Tappability criteria are girth (Sethuraj, 1977; 1980), which are the most suitable criteria for good growth vigor in the field. Good girthing in early stages of growth in the field is desirable for a balanced growth in the field, to avoid expensive practices like branch induction for enhanced vigor and balanced growth. A strong relation ship of scion base girth, with girth at the stock-scion joint of the field plant assured compatible union and a strong vascular connection between stock and scion promising a good alliance promoting a functional coordination and possible reduction of wind damages. In rubber cultivation wind damage is a serious problem compelling to outcast certain high yielding clones from clone recommendation (Yee,*et al* 1969; Rubber board , 1999). The present study contributed a lot of information to ensure the use of proper stock and selection of planting materials at budded stump stage.

B. Observations on mature trees

The nursery study, from seed to field plants, contributed valuable information on the importance of seed selection in productivity of nursery, in growth and rubber production and in biochemical activities of grafted plants and the possible impacts. The study also provided a wide look into the new horizon of rubber research to collect information on the nature of stock-scion incompatibility; incompatibility symptoms, as morphological marker; it's structural basis and possible functional impacts on growth and yield trend and the variability due to stock sources. Observations on mature trees aims at (1) estimating the intraclonal variations within a population of nearly 100 tapping trees of RRII 105 for growth and productivity and also for some important structural traits (2) to study the localized effect of tapping stress on bark growth and girthing (3) to study the chances of early prediction of TPD occurrence before the expression of morphological symptoms or cessation of latex flow (4) to estimate the associations among the variables.

1.VARIABILITY

Comparison of tapping side with untapped side of the tapping trees, for bark thickness and bark anatomical characters.

The bark anatomical characters were recorded from the trees, which were under tapping for the previous one year following ½ S d/3 system of tapping. Hence the trees were under tapping stress. The data presented in this work with respect to each character is the mean value computed from the values collected from three height positions, the scion base, 95cm height from the bud union and 150cm height from the bud union, of the sample trees. For all the seven characters studied, tapping side recorded slightly greater values than the untapped side. It is very interesting to see a stimulating effect of growth on the tapping side. Growth retardation due to tapping (Wycherley, 1975,1976) and latex regeneration before the next tapping (Narayanan, et *al* 1974; Sethuraj and George, 1980) within 48 hours has been suggested. From the results it should be assumed that tapping causes growth stimulation. Endogenous ethylene production (Abeles, 1973) associated with tapping is also reported. Production of endogenous ethylene can be a consequence of tapping stress (Lieberman, 1979). An immediate effect of growth stimulation can be attributed to the production of endogenous ethylene. Stimulation of growth activity associated with endogenous ethylene production is normal (Yang and Pratt, 1978) but report on such growth stimulation is scanty in *Hevea* though enhanced flow of latex is reported (Abeles, 1973; Leiberman, 1979). On the contrary increased bark thickness associated with reduced number of latex vessel rows (Gomez, 1982) and repeated periderm formation (Obouayeba 1993) in ethylene stimulation experiment have been reported. Such effects were observed after prolonged application of ethylene for years. The immediate effect of tapping should be attributed to the protective mechanism of the tree against wounding. Tissue hardening and periderm formation can be a subsequent effect leading to thickening of bark. Significantly high proportion of tanniferous cells supports the situation of increased stress on tapping side.

Subsequent effect of higher intensity of tapping on bark growth and girthing of trees.

During the initiation of the experiment, in April, 2000, bark thickness on tapping side was greater than that on untapped side at all the five positions. During the period of two years under higher intensity of tapping, bark growth was retarded which is evident from the values of bark thickness recorded subsequently.

Positional variations of girth were also recorded at six monthly intervals. From the data on girth and percentage girth increase at the different height positions, over two years, it was observed that minimum increase is at scion base and maximum increase at 95 centimeter height above the bud union; in terms of actual increase as well as percentage increase. Another observation is a slight increase of CV values for the girth after two year's growth, at all height positions, compared to the initial girth. The results implicate a positional difference in sensitivity to tapping stress, reflected as growth response.

On the untapped side there is no much variation of growth rate due to height positions. This side showed high rate of bark growth than the tapping side at all positions.

On tapping side, position 2, 3 and in a few trees in position 1 are tapped area where bark regeneration is under progress at different levels. Position 0 (scion base) and position 4 are below and above the tapped area respectively; of which position 4 showed better rate of bark growth. Position 0 at the tapping side recorded the least rate of bark growth indicating maximum stress. The high growth retardation effect at 0 position should be attributed to stock scion union.

When the girth increment rate was taken in to consideration, the values for different height levels recorded a narrow range, from 7.2 to 10.07 with maximum value for the position at 95cm height from the bud union and minimum value for 0 position, the scion base. The rate of girth increase showed a negative relationship with the rate of bark growth at tapping side while it showed a positive relationship with bark thickness at untapped side. Two conclusions are derived from this result. One is that the normal activity of xylem to phloem differentiation is not much disturbed in Untapped side and xylem to phloem proportion increased on tapping side as tapping intensity increased. The second conclusion is that there is tree-to-tree variation for the sensitivity to tapping stress. The tendency for localized production of wood tissue in response to increased tapping depth in Hevea is a known fact (Bobilioff, 1923., Dijkmann, 1951; De Jonge and Warrier 1965).

Formation of internal phloem.

Occurrence of internal phloem in the xylem tissue is reported in some species of Euphorbiaceae (Metcalfe and Chalk, 1950 and Esau, 1969). Premakumari, *et al.*, (1985 b) reported the occurrence of intraxylary phloem in Hevea. Premakumari and Panikkar,(1988) on further study found positive correlation between the number of intraxylary phloem points and the rate of girth retardation under tapping and later studies (Premakumarie, 1992a) proved some relationship of the quantity of such internal phloem formation (number of phloem points) with the chances of tapping panel dryness in Hevea. In the present study, it was observed that tapping trees produces high quantity of intraxylary phloem compared to the untapped trees of the same age, in the same plantation, while the quantity of primary xylem did not show such difference between tapping trees and untapped trees. In tobacco, girdling activated the internal phloem to take on the functions of external phloem (Zamski and Tsivion, 1977) for transporting assimilates. In Microtyloma the internal phloem is of secondary origin and is present only in older parts. In the present study it was observed that the intraxylary phloem points are very few in numbers in two year old young plants when compared to mature trees, especially in comparison to the trees under tapping, while no such a wide variation was observed for the number of primary xylem points. So it should be assumed that the formation of intraxylary phloem in *Hevea* is associated with a disturbance in downward translocation of metabolites due to the girdling effect subsequent to tapping process. High CV values for the number of intraxylary phloem points than the CV values for the number of primary xylem points shows that the latter trait is a more stable one while the former trait is variable due to external factors. Tapping and consequent girdling effect can be the most probable external factor to hinder phloem translocation and to trigger the formation of intraxylary phloem in *Hevea.*

Intraclonal variations of growth and latex flow characters.

The estimates of intraclonal variability for the different characters of tapping trees showed that CV values for the mean girth increments at scion base (highest) and at 150cm height from the bud union (second value from the top) after high intensity tapping over a period of two years, are very high, while the CV values for girth at the respective positions, before the onset of high intensity tapping, are low. The increased CV for girth increment during the period under high intensity tapping should be attributed to the differential response of trees to tapping stress. The other two variables, which recorded high rate of intraclonal variations, are summer variations of latex volume and dry rubber content. For such traits also interference of drought and other factors such as wintering, flowering and refoliation associated with summer climate are involved. That again ascertains the differential sensitivity of trees to the external stress. Such sensitivity should be attributed to the stock variations or to the microclimate. Premakumari (1992a) observed that genetic factors are involved in the rate of summer variations of latex volume and DRC.

Tapping panel dryness.

Tapping panel dryness in *Hevea* is considered as a physiological disorder affecting the laticiferous system first and later extending to the other tissue leading to morbid anatomy of the bark and even the wood. The productivity of a plantation is seriously affected due to this disorder, especially at high intensity of tapping (Sethuraj, 1988; Vijayakumar *et al.*, 1991) and high yielding clones are more prone to this physiological syndrome (Premakumari et *al.*, 1991).

A lot of work has been conducted on the symptoms and various external and internal factors are suggested as causes leading to the situation, as shown in the detailed review of this thesis. The symptoms are variously expressed such as partial or complete drying of the tapping cut leading to cessation of latex flow (Sanderson and Sutcliffe, 1921); Cracking and flaking of the bark (Rands, 1921), trunk deformation and abnormal trunk vigor at the base with abnormal cambial activity as slowing down or accelerating. Krishnakumar *et al.*, (1999) observed higher peroxidative activity in TPD affected plants. Peroxidase enzyme is known to promote a variety of biological reactions including biosynthesis and degradation of growth regulators (Grambow and Langenbeck-Schwich, 1983). Peroxidase enzyme system enhances the biosynthesis of ethylene (Machachova and Zmrhal, 1981; Abbas, 1997). Production of free radicals and active oxygen species damaging membrane systems, including lutoids and consequent disturbance in lutoid stability and premature in situ coagulation of latex on the panel of TPD affected trees is suggested by Crestin (1985). The source of all information collected so far is the affected tree, the back history of which is not known. The available information centers on tapping intensity and yield but the fact that only certain tree in a plantation succumb to the disorder and there is no method to identify such trees earlier remain unrevealed. A positive association of number of latex vessel rows in clones, at the opening time, with percentage incidence of TPD later (Premakumari *et al.*, 1992 b) indicate the involvement of clonal characteristics of phloem tissue in TPD occurrence.

In the present work 14 characters, including growth aspects and yield factors, recorded during the experiment and prior to any external expression of TPD symptoms by any trees, were considered as parameters. The experimental trees were subject to high intensity tapping. The trees, which later succumbed to TPD, were categorized for the various characters as low, medium and high. The results showed a general trend with respect to the growth characters such as bark thickness, girth and girth increment but latex volume and DRC showed differing pattern among the TPD trees. The results implied the possibility of identifying suspected TPD trees, on the basis of localized effect of bark growth and girthing, before the symptom is advanced to the stage of external expressions such as flaky bark or cessation of latex flow.

Correlations among growth characters and yield of mature trees under tapping.

The girth at scion base showed high associations with the girth at 150 cm height and also with stock girth at highly significant level ($P<0.01$). This association assured the interaction and adjustment between stock and scion for the expression of growth of both parts. The results further implicates that the scion base keeps in good track with the bark growth as well as girthing at higher level of the scion than the growth at stock part while the root stock showed good associations with the growth at higher levels of scion stem. The mean girth increment at scion base influenced the girth and girth increment at the higher height level and also the bark thickness at both height levels of the scion trunk. Associations of this character with girth and bark thickness of the stock portion of tree trunk were not statistically significant. Girth at the distal position of the bud union showed significant associations with bark thickness at the same position and the bark thickness at stock level. Bark thickness at the two height levels of scion trunk were significantly associated while those traits did not keep significant associations with the girth or bark thick ness of the stock portion. The results implicate that the vigor of stock has good influence on the vigor of scion stem; but this relationship is somewhat disturbed at scion base in tapping trees, which should be attributed to the possible block of translocation gradient at scion base due to weak union of stock and scion. This should be considered as a delayed incompatibility generated under tapping stress. Such block at scion base as a delayed physiological effect of graft incompatibility is reported in other crops (Proebsting, 1926, 1928).

When the associations of growth characters of stock and scion parts of the tree trunk are taken in to consideration, the girth at both height levels of the scion stem showed significant associations with latex volume and DRC of both years while those traits did not keep any significant associations with the summer variations of latex volume or DRC. The influence of girth (Licy,*et al* 1992; Licy, 1997) girth increment (Premakumari, 1992a) and bark thickness on rubber yield is reported earlier (Sethuraj, 1981). The bark thickness of scion, at 150cm height, and at stock portion showed significant positive associations with DRC in both years. The positive association of bark thickness at stock portion with DRC should be through the relationship of this trait with the bark thickness at higher height level of scion stem. The bark thickness at scion base did not keep any significant associations with any of the yield factors which is the point of interest for discussion. The scion base is a stress point where various stress reactions occurs, as reported in other crops (Proebsting,1928; Hartmann and Kester, 1972) and the proportion of hard tissue vary according to the stress affected at the stock-scion joint. The adjustments taking place at the interface and scion base might be interfering with the positive associations of growth and yield.

The relationships among latex volume and DRC may vary according to various factors. Under reduced latex flow, due to high plugging process, volume and DRC may show negative relationships (Grantham, 1925). Such reports are there in stimulation studies (Thanah *et al.*, 1996) in late dripping trees (Premakumari,1992a). The intraclonal associations estimated between volume and DRC, showed positive associations in both years indicating that the latex volume obtained during the period under study was due to enhanced biosynthetic activity; not due to enhanced dilution reaction. The relationships of latex volume with summer variations of latex volume and DRC, in both years, also support the same contention. Hence it should be concluded that a shift from ½ S d/3 system to ½ S d/2 system did not lead to DRC fall. According to the results of the present study, variations of latex volume and DRC due to tapping intensity is not the causal factor for the occurrence of tapping panel dryness.

SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSION

Clonal propagation by bud grafting is widely adopted in rubber. Assorted seeds are popularly used as the source of stock seedlings. Advanced planting materials are green budded stumps grown in polybags. The stock sensitivity is an unexplored area though intraclonal variations for important agronomic characters are observed. A comprehensive nursery study was planned to compare four types of seeds as stock sources to test the feasibility of assorted seeds as root stock source for the multiplication of RRII 105,the most popular clone in India. Nursery performances, compatibility/ incompatibility aspects, chances of early selection of planting materials for good growth and productivity, intraclonal variability of growth and yield aspects of mature trees under tapping stress, and tapping panel dryness are considered for this study.

Seeds of four stock types, assorted and monoclonal types were collected from Kanyakumari region for raising stock seedlings in the Central Nursery at Karikkattoor. For lab study as well as for studies on germination, seedling nursery, polybag nursery and field planting of grafted plants, CRD design with five replications were adopted. Observations on mature tapping trees were taken from a plantation in the campus of the Regional Engineering College Pampady.

This study reveals the following findings. The magnitude of intraclonal variations in seed characteristics such as volume, density and germination percentage of the stock sources exhibited significant differences and such characteristics are genetically controlled and highly heritable. Seed volume and seed weight did not show any significant hold on the seedling characters. Monoclonal seeds and seedlings showed more uniformity than the assorted seeds. Seed germination is the best indicator of seed viability and good seedling growth .For seed characteristics and seedling characters monoclonal seeds of RRII 105 were superior to PB 28/59. Assorted seeds are comparable to the monoclonal seeds of RRIM 600.

Considering the productivity of rubber nursery, in terms of production of maximum number of transplantable germinated seeds, production of maximum number of seedlings with buddable girth and peeling quality and production of maximum number of successful grafts, seeds of RRII 105 is the best stock source followed by assorted seeds. PB 28/59 is the inferior type of stock source and this effect is attributed to low germination percentage.

With respect to the grafted plants grown in the field, observations were made for the vigor of budded stumps, sprouting of the scion bud, survival and growth of polybag plants, field establishment, growth, and for anatomical, biochemical and molecular parameters. Data was collected on important leaf diseases and test tap yield of field plants for up to two years of growth.

Morphological symptoms of stock –scion incompatibility were identified in rubber for the first time. Surface morphology of graft union was the basic criteria as external symptoms for identification of incompatibility. Rough and corky surface of the graft area with prominent scar on the periphery with bulged and undulating surface was identified as morphological symptom of stock-scion incompatibility in rubber. Using such symptoms the incompatible grafts can be identified and discarded before polybag planting and thereby the cost of preparation of polybag plants can be reduced. In the present study assorted seeds and monoclonal seeds of RRIM 600, as stock sources, produced significantly high proportions of incompatible grafts while monoclonal seeds of RRII 105 and PB 28/59, as stock, produced very low proportions of incompatible grafts, in terms of morphological symptoms. Considering the productivity in nursery and incompatibility problems together, monoclonal seed source of RRII 105 is the best stock source for the multiplication of RRII 105 clone. This might be due to the genetic similarity between both partners.

Morphological expression is a consequence of growth reactions to the stock – scion incompatibility. Highest GCV value was recorded for the proportion of rough surfaced grafts. The high magnitude of variability should be attributed to the stock – scion interaction which is genetically controlled. Undulating surface topography of the bark is another symptom, which indicates differential cambial activity at the union part, which may result in weak connection leading to reduced strength at the union region. The consequences expected are a high incidence of wind damage, which is a serious problem in rubber plantations, and disturbance in translocation flow from scion to stock.

Deflections of vascular tissue, high amount of cork tissue, high rate of sclerification and low amount of vascular connections at the interface region were observed as anatomical symptoms of incompatibility. The deflections at the interface may cause a blocking effect leading to delayed physiological impacts like bark damage and abnormal growth, especially in adverse situations the symptoms expressed in some trees under tapping stress. The differences in bark thickness between stock and scion, also contributes to the incompatibility effect. The vascular connection at the interface was also observed in the tangential longitudinal section of the bark.

Biochemical expression of incompatibility was high level of peroxidase activity indicating stress at the graft region. The incompatibility symptoms of anatomical and biochemical nature were also less pronounced in grafted plants raised on the seedling of RRII 105 which should be attributed to the genetic similarity between stock and scion.

Delayed sprouting, low percentage of field establishment, high amount of sclerified tissue at the stock –scion interface, highly deflected and impeding type of vascular tissue at the interface of grafted plants and low yield potential, indicated by test tap yield of field plants, were observed as impacts of incompatibility.

Variations in scion growth, in terms of plant height, and girthing of stock and scion due to stock variations were observed at different growth stages. Scion height recorded highest heritability value indicating better influence of genetic factors. Bark thickness was highest for the plants raised on assorted seeds, and maximum bark growth was recorded at the interface. Since the increased bark thickness recorded for this group was contributed by high proportion of hard tissue, it should be considered as consequence of stress due to stock scion incompatibility Good amount of soft bast tissue with more number of latex vessel rows was observed in plants raised on RRII 105 stock and was a desirable quality suggesting better performances.

A preliminary attempt was made, for the first time, to explore the genetic polymorphism among the stock seedling of compatible and incompatible grafts to study the genetic involvement to control the stock –scion compatibility. One genetic marker was identified. The primer OPE-12 amplified one polymorphic band at 900 bp present in majority of the compatible stocks and absent in majority of incompatible stocks. This band probably belongs to part of a gene, which may control the stock – scion compatibility. This showed the opportunity for selection of compatible stock sources for specific scion clone and for further studies of stock- scion compatibility at molecular level.

Correlation study, with respect to anatomical characters of stock and scion showed that stock has high influence on scion characteristics and is more pronounced at the scion base. The stock contributes more for the callus formation and so the growth activity for graft union is more controlled by stock. Data on growth characteristics of scion part of grafted plants implicates the influence of stock –scion interaction. High sclerification at the interface and scion base may lead to translocational stress at scion base, which may later contribute to the occurrence of tapping panel dryness under tapping stress.

Some inferences, from the estimates of associations and regressions are (1) selection of grafted plants for good establishment and growth in the field can be made at budded stump stage. (2) Stock girth has significant hold on such traits. (3) Selection of polybag plants, on the basis of plant base girth, will assure good girthing of scion plant in the field. Regression equations have been derived. The regressions were highly significant and are useful for early prediction of desirable traits. Disease susceptibility of field plants is not much influenced by the stock differences.

The observations on mature trees under tapping showed that the magnitude of intraclonal variation is very high for girth increment on tapping compared to the variation for girth. High variation for girth increment should be attributed to differential growth responses of trees to tapping stress. The rate of girth increase showed a negative relationship with the rate of bark growth at the tapping side and positive relationship at the untapped side indicating the negative impacts of tapping stress on bark growth. Stem anatomical studies showed that the formation of intraxylary phloem in *Hevea* is associated with a disturbance in downward translocation of metabolites due to girdling. Tapping and the consequent girdling effect can be the most probable external factor to hinder phloem translocation and trigger intraxylary phloem formation. On characterization of trees which succumbed to tapping panel dryness, it was observed that TPD occurrence is more related to the variations of growth attributes than with the variations of yield attributes. Extra energy rendered for bark regeneration on continuous basis, on continuous tapping, and disturbed translocation at stock scion joint in incompatible grafts is suggested as the main factor leading to tapping panel dryness.

The present study contributes valuable information to enhance the productivity of nurseries, as well as for early selection of planting materials for good survival, growth and productivity in the field. Selection of compatible grafts for field planting, using the parameters at the morphological and molecular level as described in this study may control the occurrence of tapping panel dryness which is a serious hazard in rubber plantations.

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