PHYSIOLOGY OF ROOTING AND STOCK-SCION INTERACTION IN *HEVEA*

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The University of Kerala
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By

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CERTIFICATE

Certified that the thesis entitled "**Physiology of rooting and stock-scion interaction in *Hevea***" is an authentic record of the original research work carried out by Mrs. P.Sobhana, Plant Physiologist, Rubber Research Institute of India, Kottayam, under my supervision and guidance during the period 1993-1998. It is further certified 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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DECLARATION

I hereby declare that this thesis entitled "**Physiology of rooting and stock-scion interaction in *Hevea***", submitted by me for the degree of Doctor of Philosophy in Botany of the University of Kerala, is a bonafide record of the research work done by me at the Rubber Research Institute of India, Kottayam, under the supervision of Dr. M.R.Sethuraj, Director of Research (Retd.), RRII, Kottayam. I further declare that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles of any University or society to me.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Symbols and Abbreviations

APS	- Ammonium per sulphate
Ca	- Calcium
CEC	- Cation exchange capacity
CER	- Carbon dioxide exchange rate
CO_2	- Carbon dioxide
C	- Celcius, centigrade
cm	- centimetre
CV	- Coefficient of variation
DNA	- Deoxy ribo nucleic acid
EDTA	- ethylene diamine tetra acetic acid
e.g	- *exempli gratia*, for example
et al	- *et albi*, and else where, *aliae* or *alia*, and others.
etc	- *et ceteri*, and the other, and so forth
Fe	- Ferrum, iron
g	- gram(me)
gs	- stomatal conductance
ha	- hectare
i.e.	- *id est*, that is
IAA	- indole acetic acid
IBA	- indole butyric acid
K	- Kalium, Potassium
kg	- kilogram
l	- litre
m	- metre, minutes
meq	- milliequivalent
Mg	- Magnesium
mg	- milligram
ml	- millilitre
mm	- millilmetre
mM	- millimolar
Mn	- Manganese

N	- Nitrogen, normal (normality)
NR	- Natural rubber
NS	- not significant
NAA	- naphthalene acetic acid
O	- Oxygen
P	- Phosphorus
PAGE	- polyacrylamide gel electrophoresis
per cent	- *per centum*, by the hundred
ppm	- parts per million
RNA	- ribo nucleic acid
rpm	- rotations per minute
RRII	- Rubber Resaerch Institute of India
RRIM	- Rubber Research Institute of Malaysia
S.E	- standard error
TCA	- trichloro acetic acid
TEMED	-N,N,N'N' tetramethyl ethylene diamine
Tris	-tris hydroxyl methyl amino methane
viz.	- *videlicet*, namely
UV	- ultra violet
%	- per cent, percentage
$\propto$	- alpha
μ	- micro
n	- nano
s	- seconds

Chapter 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Natural rubber (NR) present in numerous plant species commonly known as laticiferous plants has been an essential product for mankind since several centuries. Although latex is present in thousands of plant species (Polhamus, 1962; George *et al.*, 1980) NR content in all these species is not adequate to cultivate them as commercial crops. Among these latex yielding plants, only a few species, viz, *Hevea brasiliensis* Muell. Arg. (Para rubber), *Parthenium argentatum* Gray (Guayule rubber), *Ficus elastica* Roxb. (India rubber) and *Castilla elastica* are important. But *Hevea* is the only economically viable cultivated source of NR.

The genus *Hevea* belongs to the family Euphorbiaceae and ten species have been reported so far (Webster and Pardecooper, 1989). Occurrence of latex in the plant parts is a feature common to all the species of this genus.

But among these ten species only *Hevea brasiliensis,* known as para rubber tree, serves as the major source of natural rubber.

Hevea brasiliensis had its origin in Brazil and was grown as wild trees in the Amazon river basin of South America. The present rubber plantation industry in tropical Asia started with the introduction of a few thousands of seeds from Brazil by Sir Henry Wickham in 1876 through Kew Gardens in the UK (Dijkman, 1951).

The economic product of the tree is latex present in the latex vessels which are found as an extensive network of articulated anastomosing structures in the phloem region. Latex is taken from the bark of the trees by a process of controlled wounding termed tapping. The latex thus obtained by regular tapping of the mature trees contains 29-42 percent rubber.

Rubber occupies one of the top positions in the Plantation Industry of our country. As per the available information (Rubber Statistics, 1996), 0.52 million hectare area is under rubber cultivation in India, about 86% of which is in Kerala. NR production in India was around five lakhs tones during '94-'95. Though in terms of total production India ranks 4th among the major NR producing countries, in the case of productivity India stands first. But India remains a net importer of NR and for achieving self-sufficiency in NR production various strategies are put forth by the Rubber Board of India, Ministry of Commerce, Government of India, viz, expansion of area under rubber

cultivation by planting rubber in unconventional areas of the country, enhancing productivity from the existing plantations by adopting various scientific techniques, replanting old and uneconomic plantations with high yielding clones, etc.

Out of the 8.81 lakhs of rubber growers in the country, about 85 percent are small growers, who have holdings of less than 0.5 hectares. Hence this crop provides livelihood for a large population of the country and is a major source of employment. Moreover, Rubber Plantation Industry is contributing substantially to off-set some of the socio-economic problems like deforestation, depletion of soil, environmental pollution, etc.

During early periods of rubber cultivation unselected seeds were used as the planting materials. High variability and low yield of the seedling trees necessitated crop improvement programmes in *Hevea.* Vegetative propagation through budgrafting with desirable clones and further breeding programmes through hybridisation have been attempted.

Budgrafting, the commercially accepted practice of vegetative propagation in *Hevea* throughout the rubber producing countries have definite advantages since large quantities of planting materials can be produced within a short period and is economic and easy to perform. But stock-scion interaction exhibited among the budgrafted plants reduce the homogeneity of the plants even in monoclonal plantations. This may result in variability in yield and growth of the trees. Any factor which affects yield needs

detailed investigations. The heterogeneity among the seedling rootstocks raised from open pollinated seeds may be the reason for the intraclonal variations observed in growth, yield, susceptibility to diseases, etc. in monoclonal plantations. One of the constraints for studying stock-scion interaction in *Hevea* is the non-availability of sufficient homogeneous rootstock materials. In this context, own-rooted plants of *Hevea* will be of immense value in understanding the stock-scion interaction in various aspects. Hence studies were conducted to standardise air-layering technique, one of the methods for obtaining own-rooted plants. Physiological and biochemical basis of rooting, hormonal influence on rooting, etc. were also examined. Keeping in view of these, a review of the literature covering different propagation techniques, physiology of rooting, effect of hormones on rooting, stock-scion interaction, etc is given below.

1.2 PROPAGATION

The very existence of different plant species possessing unique characteristics depends on the techniques adopted for their propagation. The method of propagation adopted for a plant depends on various factors like the breeding system, botanical characteristics of the species, state of improvement of the plant, etc. In general, methods for plant propagation are broadly classified into two categories (a) propagation by seeds (sexual) and (b) vegetative propagation (asexual). In plants, where it is difficult to raise true to type plants by seed propagation generally vegetative

propagation is adopted.

Hevea brasiliensis is a cross pollinated tree crop and hence the seeds obtained are highly heterozygous in nature (Dijkman, 1951). Since it is the major source of NR and a very important cash crop one has to make the choice of the planting material on the basis of practical considerations and economic returns. In addition, it is a perennial tree and the economic life lasts for more than thirty years and therefore, planting material should be selected with maximum care.

Efforts for evolving high yielding strains / hybrids of *Hevea brasiliensis* through artificial pollination of clones with desirable characteristics have been made at different Rubber Research Institutes in most of the rubber producing countries. The new hybrids after assessing their yield potential and other desirable characteristics have been released for commercial planting. Preserving the genetic identity during mass multiplication of these new hybrids is highly essential. This can be achieved only through vegetative propagation, because *Hevea brasiliensis* is a cross pollinated crop.

Cuttings, layers and budgraftings have long been practiced as the techniques for vegetative propagation in many horticultural crop plants and tree species (Hartman and Kester, 1976). *In vitro* micropropagation is also used nowadays for mass multiplication of high value horticultural crop plants, forest trees and ornamental plant species (Sita and Vaidyanathan, 1979;

Gupta *et al.*, 1980; Gurumurthi and Stanley Jagadees, 1992; Jambhale and Patil, 1996).

The success of any propagation method primarily depends on the establishment and development of a desirable root system. Root system is a very important part of a plant since various basic functions of the plant are being carried out by it and their significance during germination and early establishment of the plants is all the more crucial. Anchorage, absorption and translocation of water and mineral nutrients, production of growth regulators, storage of food reserves, etc. are the major functions of the root system (Rom, 1987). Among these various functions anchorage plays a vital role especially in perineal tree crops. The root growth and its pattern are controlled mainly by the genetic make up of the cultivar or clone. Environment, especially soil environment also influences growth of the root system. For a perennial tree crop like rubber, a root system which ensures proper anchorage as well as well developed laterals for absorption of mineral nutrients is essential for the healthy growth and establishment of the trees and prevention of uprooting due to severe winds.

1.2.1 Methods of vegetative propagation

1.2.1.1 Rooting of stem cuttings

Rooting by stem cuttings is considered as one of the most economic method of clonal propagation and is an extensively studied area.

Hence in the present review, only relevant literature on rooting of cuttings of *Hevea brasiliensis* had been given.

Earlier attempts to propagate *Hevea* clones by rooting of cuttings was successfully carried out by Tinley (1960). Production of these rooted clonal cuttings became possible by the introduction of mist propagation technique. But earlier observations indicated that these plants were not suitable for commercial planting due to the lack of tap roots. Hence attempts were made by Yoon and Leong (1975) to induce pseudo-tap roots in the stem cuttings by some horticultural manipulations. Allowing one of the roots in the stem cutting to grow like a tap root by pruning all the other roots was tried for this purpose. Even this technique was reported to be not successful for commercial planting.

1.2.1.2 In vitro micropropagation

In vitro micropropagation also can be successfully employed for producing genetically identical plant materials in *Hevea* clones. Studies on shoot tip culture of *Hevea* clones conducted at Rubber Research Institute of India were successful (Sinha *et al.*, 1985; Sobhana *et al.*, 1986; Asokan *et al.*, 1988). Studies on micropropagation by Paranjothi and Gandhimathi (1975 a) and Carron *et al.* (1988) could regenerate plants only from seedling explants.

1.2.1.3 Air-layering

Air-layering is considered as an effective means of vegetative propagation in many plant species. This technique is being widely employed for perpetuating genetically identical lines or clones for tree improvement programmes and also to raise true to type plants for commercial exploitation especially in forest trees (Khosla *et al.,* 1979). This technique has several advantages over rooting in stem cuttings. The layered branches get sufficient food reserve from the parent plant as long as it is not severed . The continuous supply of the food reserve and growth hormones will enable these layers to produce a balanced and well developed root system (Chauhan and Dua, 1982). Studies on vegetative propagation of *Ochreinauclea missionis* by stem cuttings and air-layering showed that air-layering resulted in comparatively better results with regards to rooting (Jose *et al.,* 1995). Moreover, in *Hevea,* a good mist propagation system is essential for maintaining the turgidity of the stem cuttings throughout the period of root induction which accounts additional expense (Leong *et al.,* 1976). In *Hevea,* information available on vegetative propagation by air-layering is meager (Rubber Research Institute of Malaysia, 1959 b). Attempts to induce rooting in scion stem by marcottage were however, not successful (Yoon and Ooi, 1976). No further reports on this line of work in *Hevea* seems to be available in the literature.

1.3 Factors influencing rooting

As is evident from the literature several factors affect the success of air-layering, viz, age of the stock plants, rooting medium, wrapping materials, type of wood, time of the year in which air-layers are made, growth regulators, etc.(Hartman and Kester, 1976; Nanda and Kochar, 1984; Dutta and Mitra, 1991; Heller *et al.*, 1994; Ranaware *et al* ., 1995; Sabyasachi and Mishra, 1996). Nutritional as well as biochemical and physiological status of the parent plant at the time of air-layering also influences the rooting.

1.3.1 Rooting response of different species / clones

As mentioned, the rooting ability of a species depends on the inherent genetic characters. Clonal differences in rooting response in stem cuttings of *Hevea* were reported earlier by Tinley (1960) and Yoon and Leong (1975). Variations in rooting ability were noticed in different species or even within species (Couvillon, 1985).

Differences in response to air-layers even within species were noted in other plants also. Different cultivars of mango responded differently to air-layers as reported by Ram and Sirdri (1982).

1.3.2 Effect of age on rooting

Age of the mother plant influences the success of rooting in air-layers. In general, rooting was more in juvenile than adult plants (Hartman

and Kester, 1976). Studies in cashew (Bian, 1976; Rao *et al.*, 1988; Rao and Satyanarayana, 1989), jackfruit (Desai and Patil, 1984), peaches (Couvillon, 1985), *Eucalyptus* (Hartney, 1980) further support the above findings. Earlier studies on rooting in stem cuttings of *Hevea* also gave a clear indication that rooting success was influenced by the age of the mother plant ie, juvenile plants, especially seedlings gave higher rooting percentage than mature plants (Tinley, 1960; Yoon and Leong, 1975; Leong *et al.*, 1976).

Endogenous phenol level difference was suggested as one of the reasons for variations in rooting ability associated with age of the mother plant in cashew (Rao and Satyanarayana, 1989). They also reported differences in various factors governing rooting viz, hydrolysable carbohydrates, nitrogen content, endogenous auxins and inhibitors in plants at different ages (more about influence of phenols on rooting is given in sections 1.3.4 and 1.3.5).

Juvenile cuttings rooted easily in *Ficus pumila* whereas only mature cuttings treated with IBA exceeded 30% rooting. Highest vascular cambial activity and shoot RNA levels occurred in juvenile material during maximum rooting periods, while lowest RNA was observed in mature shoots (Davies, 1984).

1.3.3 Effect of rooting medium and wrapping materials on rooting

The rooting medium seems to be a very important factor in the rooting success of layers. Maintaining properly moistened rooting medium

throughout the period of root induction is a basic requirement for successful layers. Different rooting media have long been tried by several workers. They include saw dust, coconut husk, soil, fibers, sphagnum moss, vermiculite, etc. (Hartman and Kester, 1976). A mixture of saw dust-cattle manure, top soil mixture (1:1:1 by volume) was reported to be the best among the ten rooting media tested in cashew (Bian, 1976). A good rooting medium should provide not only sufficient moisture for root initiation and growth but also ensure optimum aeration. Sphagnum moss which is one of the best materials for keeping moisture content for a long time and providing sufficient aeration has been tried for successful layers in many plant species (Hartman and Kester, 1976). Survival of the rooted layers was found to be more in layers with moss and soil + FYM mixture (Bhatt and Chundawat, 1982).

The wrapping materials also have some influence on rooting. Earlier trials to enclose the rooting medium around the girdle include materials like metal or wooden boxes, paper cones, rubber sheeting, etc. Nowadays, polyethylene sheets are commonly used as the wrapping material since it possess lot of advantages such as permeability to gases, low transmission of water vapour, durability to withstand weathering for long periods, etc. Sphagnum moss and vermiculite wrapped with polythene and then with gunny sack markedly improve the root formation in mango air-layers (Prasad and Singh, 1972). Effect of time of ringing and rooting media on

rooting and survival of air-layers of guava was studied by Bhatt and Chundawat (1982) and found that wrapping with black polythene was advantageous. Black and white polythene were compared as wrapping materials and black was found to be better with regard to increased root number and root length (Desai and Patil, 1984). The response of three wrapping materials viz, black plastic, clear plastic and aluminium foil was assessed in layers of rubber plants, *Ficus elastica* by Herrin and Carter (1995) and found that all the wrapping materials responded similarly.

1.3.4 Biochemical factors influencing rooting

Biochemical characterisation of easy-to-root and difficult-to-root cultivars of different plant species was reported by many workers (Stolz, 1968; Kumar *et al.*, 1985;). Among the different biochemical parameters, substantial amount of work was carried out on the relationship of carbohydrate level of the plant tissue and rooting success (Nanda and Anand, 1970; Couvillon, 1988; Rao and Satyanarayana, 1989; Rio *et al.*, 1991; Hambrick *et al.*, 1991). A positive relationship between soluble sugars and rooting success was demonstrated by these authors while starch content was reported to be negatively correlated with rooting in many cases. Seasonal variations in rooting response was attributed to the changes in the carbohydrate level especially reserve food materials like starch (Nanda and Anand, 1970). Inadequate photosynthesis in the stem cuttings planted in rooting bed during induction of roots was suggested as the reason for the requirement of higher level of carbohydrate at the time of

cutting (Couvillon, 1988).

Rooting ability and endogenous phenolic content exhibit positive correlation in many plant species (Hartman and Kester, 1976). Rao and Satyanarayana (1989) demonstrated that differences in endogenous phenolic contents was responsible for the variations in rooting ability in cashew. Accumulation of rhizocaline above the girdle, which is believed to be an effective agent in initiating root formation by the influence of auxin was reported earlier by Went (1938). Balakrishnamurthy and Rao (1988) showed that high amounts of phenols in the tissue with endogenous and exogenous auxins forms a root promoting substance (rhizocaline) inducing profuse rooting.

Though not as important as the above described biochemical parameters, amino acids were also reported to have some influence on rooting. Amino acids were utilised for root induction process thereby showing depletion in its concentration (Suzuki and Kohno, 1983; Madhusudanan, 1987). Hambrick *et al.* (1991) reported a negative correlation of amino acid level with rooting.

Hard-to-root cultivars had higher levels of IAA oxidase and peroxidase activities and lower pigment content than easy-to-root cultivar. Further, enhanced shoot capacity to utilize available carbohydrates, reduced lignification and reduced activities of the above enzymes were noticed as a result of etiolation stimulated rooting (Veeraraghavathatham *et al.*, 1985).

1.3.5 Effect of growth regulators on rooting

The role of growth regulators on rooting is an extensively studied area (Nanda *et al.*, 1970; Sadhu *et al.*, 1972; Hartman and Kester, 1976; Gurumurthi *et al.*, 1984; Nanda and Kochar, 1984; Puri and Nagpal, 1988). It is well established that both endogenous as well as exogenous auxins influence root initiation and further growth of the roots. The first observed naturally occurring rooting hormone in plants is indole acetic acid (IAA). Later synthetic auxins which are similar in action as that of IAA are formulated. Among these indole butyric acid (IBA) and naphthalene acetic acid (NAA) are found to be more effective than other compounds and these two growth regulators are now being widely used to promote rooting in stem cuttings and layers.

Enhanced rooting in air-layers of certain horticultural crops like ber, acid lime, jambolana, mango, fig, cashew, pomegranate, etc. by IBA and NAA application was reported by many scientists (Bhujbal, 1972; Chandrababu *et al.*, 1982; Suryanarayana and Rao, 1982; Banerjee *et al.*, 1982; Ram and Sirohi, 1982; Nunez-Elisia *et al.*, 1992). In most of these cases the quality of roots was also reported to be superior in auxin treated layers.

The requirement of exogenous growth regulators varies in different plant species and even within species. In *Hevea*, stem cuttings even response to low concentrations of growth regulators varies in different clones (Tinley, 1960). Experiments to induce rooting in stem cuttings of one year old

plants of *Hevea* were carried out by Castro *et al.* (1990) by girdling or not with copper wire thirty days before cutting and dipped or not in IBA 200 ppm plus KOH. It was reported that girdling had no effect on rooting and dipping caused phytotoxic symptoms.

Most of the work on enhancement of root growth by growth regulators in *Hevea* were carried out in budded stumps (Pakianathan *et al.*, 1979) Treatments of the tap roots of the budded stumps with IBA, NAA and furadan stimulated production of lateral roots. Application of a formulation consisting of 2000 ppm IBA, 1% KNO_3 and 5% Captan-50 resulted in several-fold increase in dry weight of lateral roots compared with the untreated stumps after two months of treatment (Jaafar and Pakianathan, 1979).

It was observed by Chhonkar and Singh (1967) and Acharyya and Dash (1972) that IBA treatments were superior to IAA and control in every respect, ie, percentage of success, average number of roots per layer, length of the roots and average diameter of roots in cashew layers.

Some of the plant species like *Acacia catechu, Michelia champaka, Artocarpus heterophyllus,* etc. failed to root by air-layering without the application of growth regulators (Mukherjee and Chatterjee, 1978; Channaveerappa and Gowda, 1984; Nagpal and Puri, 1986). The beneficial effects of IBA on rooting in layers of jackfruit and champaka were reported by the above authors. Air-layering studies in jackfruit by Sabyasachi and Mishra (1996) showed that IBA at 7500 ppm applied to hardwood stems produced the

highest percentage of rooting while lowest rooted air layers (26%) in softwood stems to which no auxin was applied.

All the rooting characters were higher in layers with the application of IBA in various plant species (Chatterjee, 1978; Patil and Chakrawar, 1979; Channaveerappa and Gowda, 1984). Growth regulator treatment not only enhances the percent of rooting but also exerted its favorable influence on enhancing the root quality of the adventitious roots in layers. Higher levels of IBA (10000 ppm) was effective in increasing the rooting response, root number per layer and root length in *Aegle marmelos.* Survival of the rooted layers also increased by growth regulator treatment (Misra and Agrawal, 1975; Mukherjee *et al.,* 1986). In custard apple also higher levels of IBA (10000 ppm) resulted in increased rooting success of layers (Pathak *et al.,* 1991). Still higher concentrations of IBA and NAA (20000 ppm) gave 100% rooting in air-layers of water apple *(Syzygium javanica* L.) as reported by Dutta *et al.* (1982). On the other hand low concentrations of IBA and NAA (100 ppm) were effective in increasing rooting success of *Butea monosperma,* an important lac host plant (Kumar, 1989). Patel and Pasaliya (1995) tried the effect of IBA, NAA and IAA applied immediately after ringing or 10 or 20 days later on rooting in air layered shoots of guava and found that NAA at 9000 ppm applied immediately after ringing gave the highest number of primary and secondary roots.

The different hormones and their concentrations influencing the

rooting response show variations depending on plant species or cultivars and different seasons (Bhandary and Kologi, 1960; Bid and Mukherjee, 1969; Anand and Haberlein, 1975). IBA (50 ppm) was the most effective treatment for root induction in *Melia azidarach* during February, whereas, in May, IAA (50 ppm) was found to be more effective. The nature and amount of nutrient level naturally depend on the season and hence the differences in the response occurred (Gupta *et al.*, 1989).

NAA (2000 ppm) was found to give better results than IBA in *Gliricidia maculata* air layers by Kempanna *et al.* (1961). They also suggested that higher concentrations of hormonal solutions are better for inducing roots in woody species. Air-layering using 1000 ppm NAA was tried for rooting Indian butter tree (*Aisandra butyracea*) by Tewari and Dhar (1997).

The optimum concentration for exogenous application of auxins varies in different plants. Lower levels of NAA and IBA were found favorable in inducing roots while higher concentrations were less effective in layers of *Althea rosea* (Lingaraj, 1960). In *Anthocephalus chinensis,* 5000 ppm *IBA* enhanced callusing and rooting in layers while higher concentrations gave better secondary root formation (Misra and Jaiswal, 1993).

Ficus elastica, known as India rubber, could be successfully propagated by air-layering with the application of IBA (Rajagopal, 1993).

As mentioned earlier sugar and endogenous rooting factors like auxins and phenolics control the rooting in layers and stem cuttings.

Conversion of starch stored in the tissue to soluble carbohydrates and mobilization and accumulation of this sugar at the treated region occurred by the application of auxins. The auxin induced effect on rooting was attributed to these changes in different plant species (Audus, 1953; Nanda *et al.,* 1968a; Nanda and Anand, 1970; Altman and Wareing, 1975 and Singh, 1985). Auxin treatment allows expression of a latent rooting potential in cuttings inherent in the genotype or the physiological status of the shoot (Howard, 1986). Moreover, application of auxin in proper concentrations had initiated root meristems in different mature non-meristematic tissues as reported by Audus (1953). Palanisamy and Kumar (1997) from their studies on seasonal variation on adventitious rooting in branch cuttings of *Pongamia pinnatta* suggested that auxins (IBA) activate the cambium in the active period of cambium resulting in enhanced root formation.

IBA in combination with some phenolic compounds like ferulic acid, chlorogenic acid or catechol showed beneficial effects on rooting in air-layers of jackfruit, custard apple, litchi, etc. (Basu *et al.,* 1969; Dhua and Sen, 1984; Bose *et al.,* 1985; Pathak *et al.,* 1991; Pal *et al.,* 1996). Percentage of rooting was enhanced to 90-95% by the application of phenolics in *Syzygium javanica* L. (Dutta *et al.,* 1982).

Effect of IBA in combination with vitamins like ascorbic acid on rooting of cuttings was reported by many scientists (Basu *et al.,* 1992). The enhanced effect of such combination was reported in *Ficus elastica* by Rajagopal (1993).

Several commercial formulations of IBA in powder form are successfully employed for enhancing rooting in various plant species (Khosla *et al.*, 1979). Among these seradix, rootone, rootex, etc. are widely used. Some of these commercial formulations are available in different strengths suitable for different types of plant materials. Woody and hard-to-root species require formulations with higher concentrations whereas tender plants need only mild preparations (Hartman and Kester, 1976). These commercial formulations are more convenient because they are readily available and easy to use unlike the other usual methods of hormone applications.

1.2.1.4 Budgrafting

Vegetative propagation by budgrafting is an inevitable nursery practice for supplying considerably large quantities of planting materials to be used for commercial plantings, estate trials, etc. Moreover, in cases where easy rooting is not possible by cuttings and layers and the root systems of the plants raised by these techniques are not suitable for that particular species, budgrafting technique is adopted. By this technique two plants are joined together to form and function as a new plant. The upper portion or the top of the plant is known as 'scion' and the lower portion or the root is termed as 'stock' or 'rootstock'.

Besides its application as an important method of vegetative propagation combining the desired characteristics of rootstocks and / or scion into a single plant, overcoming prolonged juvenile period, etc. are some of the

specific advantages of this technique (Hartman and Kester, 1976). Hence, nowadays, this technique is exploited as a horticultural manipulation especially in fruit crops.

Generally grafting is more successful in dicotyledonous plants than in monocots mainly because of their vascular bundle arrangement and presence of continuous cambium. Since the rootstocks and the scion have different genetic make up, their union may sometimes be incompatible. Successful graft combination is essential for growth and further establishment of the budgrafted plants. It was suggested by many scientists that graft compatibility is genetically controlled by multiple genes with additive effects (Copes, 1970 and 1978; Salesses and Alkai, 1985). This two-part-plant acting as a single unit has common metabolic activities controlled by two genetic systems. In other words, stock-scion interaction exists in these plants.

Seedlings raised from unselected seeds were used as the main source of planting materials in *Hevea* during the early periods of its cultivation (Dijkman, 1951). Later, seeds from selected high yielding trees were preferred as the planting materials for improving yield. But due to considerable tree to tree variations recorded in yield among the seedling population attempts were made to propagate *Hevea* vegetatively. Vegetative propagation in *Hevea* to produce clonal materials began with the introduction of brown budding by Van Helten in 1916 (Dijkman, 1951). This technique was later modified and green budding also became popular as a

propagation method in *Hevea*. At present, *Hevea* is propagated commercially by budgrafting.

Young seedlings, both polyclonal and monoclonal are used as rootstocks and desired clones are budded on them. Forket method of patch budding is being adopted for budgrafting in *Hevea* (Marattukalam and Saraswathy Amma, 1992). Budding in four to eight months old seedlings with buds from young green shoots (green budding) or in seedlings above ten months old with brown buds from mature shoots (brown budding) are being generally practiced nowadays. After confirming the success of budgrafting, the plant is uprooted and the roots and the stock stem will be pruned as per the required specifications. The planting materials thus produced are known as 'budded stumps' (Plate 1). This can be planted directly in the field or in polybags in a nursery. Growing budded plants in polybags for five to nine months before planting in the field is adopted widely among the planters. These plants after attaining two to three or six to seven whorls, depending on the requirements, will be transplanted to the field (Plate 2). These plants are referred as advanced planting materials since the plants are in an advanced stage of growth at the time of planting. In addition, maintenance and upkeep of these planting materials in the early stages will be easier in the nursery than in the field. The immaturity period in the field can also be reduced by planting these advanced planting materials (Sivanadhyan *et al.*, 1975).

Plate 1.Budded stumps of *Hevea brasiliensis*

Plate 2. Polybag plant of *Hevea brasiliensis*

PLATE 1

PLATE 2

1.4 STOCK-SCION INTERACTION

As mentioned earlier, though budgrafting is considered as an effective, easy and economic means of vegetative propagation, stock-scion interaction exhibited among the budgrated plants results in considerable variations despite the genetic homogeneity of the scion. The extent of these variations depends on the differences in the stock / scion used. In some combinations the rootstock exerts a profound effect on one or more of the growth characteristics of the scion variety while in some the scion modify certain characteristics of the stock. However, the rootstock, scion, interstock and the budunion itself, all interact with each other affecting the overall performance of the plant. In some cases, a particular character irrespective of its occurrence in the stock, scion or interstock will exert its influence on the entire plant.

One of the most easily recognised effects of the combination of the two genetic constitutions is found in the scion vigour (Rom, 1987). There are other physiological consequences also that are more subtle like mineral uptake, water relations, photosynthesis, yield efficiency, etc. (Rom, 1987). Substantial amount of work on the above aspects of stock-scion interaction was carried out in various plant species (Hartman and Kester, 1976; Poessel, 1989; Pilone, 1992). Though not many, reports are also available in literature on the isozyme/ genetic level variations in plants due to budgrafting (Degani *et al.*,

1990; Yagishita *et al.*, 1986 and 1990; Krishnakumar *et al.*, 1992).

Anatomical and biochemical studies conducted by Shklarman *et al.* (1992) in compatible and incompatible combinations of citrus species showed symptoms of poor graft union, callose development and irregularities in water translocation. Accumulation of tannins and catechols was also detected at the graft union. Anatomical studies in grapevine grafts showed that vascular connections were necessary but not sufficient for graft success. Abnormal starch distribution allowed incompatibility characterisation (D'khili *et al.*, 1995). Differences in trunk xylem anatomy was observed in healthy and blighted grapefruit trees due to rootstocks was reported by Vasconcellos and Castle (1994). Bark thickness of peach scions was affected by rootstocks (Yadava and Doud, 1978).

The genetic differences in certain characteristics like dwarfism, anchorage and extensive rootgrowth, cold hardiness, adaptation to pests and different climate, etc, noticed among rootstocks of various plant species are being beneficially utilised by combining with the scion through budding.

Different rootstocks of citrus grafted with a particular cultivar exhibited different root density while root base diameter, length of secondary roots and spread of the rootsystem roughly followed the same pattern as the primary root lengths (Tayde, 1985).

Comparative studies of the rootsystem of rootstocks of orange by Alluwar and Parihar (1992) showed that those having greater tap root length, greater number and length of lateral roots and higher dry weight of tap and feeder roots could prove good substitutes in times of water scarcity because of their deeper root penetration.

The distribution and efficiency of roots of kinnow mandarin and acid lime (*Citrus aurantifolia*) on Karna Khatta rootstock (*Citrus karna*) was recorded using P^{32} as a tracer by Kurian *et al.* (1994). The influence of the scion on mean specific activity of P measured in leaves differed among cultivars. Root distribution pattern of nine apple rootstocks in two contrasting soil types were studied by Fernandez *et al.* (1995). Regression analysis of their results demonstrated positive correlation between number of roots counted and scion vigour and yield.

1.4.1 Influence of stock / scion on growth and yield

1.4.1.1 Fruit crops

The marked effect of rootstock on scion growth is a well studied aspect especially in fruit trees (Rom, 1987). The dwarfing mechanism induced by dwarfing rootstocks onto the scion in fruit crops like apple, peaches, plum, apricot, cherry, vitis, etc. is beneficially utilised commercially in recent years because of the convenience and less expense associated

with spraying, pruning, thinning and harvesting of these small trees (Layne *et al.*, 1987).

The rootstock influence varies with the variety of scion used (Singh,1980). Rootstock relationship to tree size in apple was reviewed by Ferree and Carlson (1987) and Ferree *et al.* (1992). According to their reports the rootstock effect on tree size generally becomes evident as the trees begin to harvest. Some of the very dwarfing rootstock varieties resulted in cropping of the scion at a very young age, thereby decreasing the rate of vegetative growth. In the review by Lockard and Schneider (1981), the dwarfing mechanism in apple was attributed to the influence of auxin translocated down the phloem to the roots and cytokinins synthesized and translocated to the shoot.

Bergamini and Angelini (1986) reported that leaf parameters were related to branch type, rootstock and cultivar in apple.

Ten years observations by Ugolik *et al.* (1993) on the effect of rootstock on growth, yield and mineral element contents in apple leaves showed that trunk-cross sectional area was determined by the rootstock and scion interaction. Rootstock influenced the yield of the cultivar also. Similar reports on the influence of rootstock on growth in apple was reported by many scientists (Schneider *et al.*, 1978; Costante *et al.*, 1983; Ystaas *et al.*, 1995).

Hirst and Ferree (1995) studied the effect of rootstock and cultivar on the growth and precocity of young apple trees. It was observed that rootstocks exerted more influence than cultivar on total growth. Though rootstock affected branching by influencing tree size, branch density (number of branches per meter of tree height) however, was primarily under scion cultivar control.

Growth, survivability and foliar nutrient content are significantly affected by rootstocks in plum production (Boyhan *et al.*, 1995). In cherry also rootstock influence on tree size and yield was reported (Facteau *et al.*, 1996). The effect of combinations of three scions and three rootstocks on yield and quality of pistachio nuts was reported by Panahi *et al.* (1996) and they found that rootstocks had large and significant effect on all the characters.

Rootstocks of peach induce size control of scion cultivars and the range varies from 50% to 25% vigour induction when compared with standard peach seedling rootstocks (Layne *et al.*, 1987).

In apricots, the influence of rootstock on scion tree vigour is well established in several trials (Crossa-Raynand and Audergon, 1987). Scion cultivars of *Vitis* on different rootstocks reported to yield differently with varying levels of fruit quality (Howell, 1987). Similarly rootstock effect on cold hardiness of scion cultivars of *Vitis* was also observed. Increased yield of varieties like *Vitis labrusca* grapes were obtained when grafted on

vigorous rootstocks.

Singh (1980) also reported the influence of rootstocks on the fruit characteristics of the scion variety in different plant species. This is well demonstrated in different species and varieties of citrus.

The mango cultivar, Alphonsa grafted onto eight different rootstocks were compared and found that tree vigour, height and yield were influenced by the rootstock (Reddy *et al.*, 1989). Kurien *et al.* (1996) also reported the influence of eight rootstocks on the same cultivar 'Alphonsa' mango.

In nectarines, canopy volume differed in different rootstocks at the lowest planting density and the yield at earlier stages. But the differences were reported to be reduced during later years (Loreti *et al.*, 1993).

The rootstock influence on cold-hardiness in apple is advantageous for commercial use in some areas, where rootstock proved to be sufficiently winter hardy. In some species, cold-hardiness of the scion variety exerts its influence on rootstocks. Cold-hardiness of citrus roots was reported to be affected by the scion variety (Hartman and Kester, 1976). Rootstocks also have a role in influencing the sensitivity of the scion cultivars to pests (Ferree and Carlson, 1987).

In peaches, some of the changes in the scion performance induced by the rootstock are subtle and difficult to detect while some are more pronounced

and easily detectable. The influence of rootstock on scion performance was suggested as due to the impedance in the flow of water, nutrients, photosynthates, growth regulators, etc. at the graft union (Layne *et al.*, 1977). Rootstock influence on several aspects of tree growth was also reported by these authors, viz, tree height, spread, volume, trunk cross-sectional area, etc. Rootstock effects on growth and fruiting in peaches was reported by Bussi *et al.* (1995). Wutscher and Hill (1995) reported the performance of *Citrus sinensis* on sixteen rootstocks and found that both quantity as well as quality of fruits were influenced by the rootstocks.

1.4.1.2 Hevea

Earlier attempts to study the influence of stock on scion and *vice versa* in *Hevea brasiliensis* employed twin plants raised from split clonal seedlings (Dijkman, 1951). One of the twin plants was budded with a clone and other was kept as control (unbudded). Analysis of the results on yield in these two types of plants showed a high correlation indicating the influence of stock on the productivity of the scion. It was also reported from these studies that the growth vigour of stock influenced the productivity of the scion. The growth vigour of the stock and growth vigour of the scion also exhibited considerable correlation.

Observations of seven long-term investigations on stock-scion relationship using clonal scions and seedling rootstocks in *Hevea* were reported by

Buttery (1961). It was shown that scion effects are more pronounced than the rootstock effects particularly when yield and trunk girth were considered. Certain stocks were found to be favourable / unfavourable for certain scions indicating the occurrence of stock-scion interaction. Influence of stock on yield of the budded trees was independent of the effect on growth.

Growth of *Hevea* buddings was observed with particular reference to the vigour of various clones by Templeton (1960). It was reported that large stocks promoted a more vigorous scion growth during the early stages. Seneviratne *et al.* (1996) also observed that growth vigour of the stock influenced the scion growth in *Hevea*. They further demonstrated a positive correlation between the diameter and height of the plants. Jayasekhara and Senanayake (1971) showed that the stem diameter exhibits a positive correlation with stem height and leaf area of the plants and hence stem diameter can be considered as a suitable parameter to measure growth vigour. Previous reports by Templeton (1960) also showed that in most of the studies, girth was taken as the main criteria for assessing the growth vigour of various clones of *Hevea*. Moreover, girth is an important criteria taken into consideration for tappability.

Combe and Gener (1977) studied the effect of stock family on growth and production in budded plants of *Hevea* by grafting three clones on four families of stock. The various stock - scion combinations differed in the time of opening

of the trees for tapping and production.

Influence of six rootstocks on growth and yield of six clones of *Hevea* was reported by Ng *et al.* (1981). The results indicated that rootstock influenced the growth and yield of the scion significantly, but there was no stock-scion interaction. As observed in other trials on stock-scion interaction conducted at Rubber Research Institute of Malaysia, PB 5/51 rootstock seemed to be better than the other rootstocks. The results also showed that rootstock influence on yield is independent of its influence on growth as observed earlier by Dijkman (1951) and Buttery (1961).

Goncalves *et al.* (1994) evaluated the vigour of six clones of *Hevea brasiliensis*, viz., GT1, RRIM 701, PB 235, IAN 873, RRIM 600 and unselected seedlings as rootstocks at fourteen months age. Correlations between stem diameter, height, number of whorls and average distance between foliar whorls observed in their studies indicated that stem diameter and number of whorls were positive and highly significant and found significant genotypic differences among the clones for stem diameter, height and number of whorls. They further reported that the above six clones when used as scions and budgrafted onto these six rootstocks in all possible combinations, resulted in variable performance of the budgrafted plants. IAN 873 showed the best performance as the rootstock and this clone increased the average girth of all the scions and when compared with RRIM 600

rootstocks the girth and height of all the scions increased by 10-12% respectively.

Evaluation of the performance of monoclonal and polyclonal rootstocks in *Hevea* by Yeang *et al.* (1995) showed a clear evidence of the rootstock effect both in the stock as well as the scion portions. Seedlings from the clone RRIM 623 seeds self or cross pollinated with PR 107 were used as the rootstocks and these were high budded to evaluate the performance of the stock as well as the scion portions. The results indicated that both girth and yield were high in the scions budded on polyclonal seedlings. They also reported that choice of the rootstock can affect scion latex yield performance by as much as 23%.

Long-term trials on stock-scion relationship in *Hevea* conducted at Rubber Research Institute of Malaysia showed that scion growth at eighteen years after planting was continuously affected by rootstocks significantly. The results showed that yield at the twelfth year of tapping was also influenced by the rootstocks - the highest yielding rootstock was found to be PB 5/51 (Rubber Research Institute of Malaysia, 1982). The results of the studies by Tiong (1989) in *Hevea* showed that rootstock seedling vigour influences scion growth and yield.

Variations in clonal combinations of rootstocks and scion among five clones of *Hevea brasiliensis* were evaluated by Sagy and Omokhafe (1996). They

found significant variation in rootstock effects and general combining ability (GCA), specific combining ability (SCA) and reciprocal effects of clonal combinations of rootstock and scion. Two clones viz, RRIM 600 and GT1 when used as rootstocks gave high budding success in combination with a wide range of clones.

1.4.1.3 Other plants

In a crop like tea, vegetative growth is the most important criteria determining yield. The vigour of the grafted plants, stock and scion was assessed in terms of weight of centered branches, area of plucking surface and yield by Haridas *et al.* (1992) and observed that the rootstocks influenced the leaf yielding capacity of the scion but it did not affect the quality of the made tea. Similar observations were reported in tea earlier by Nyirenda and Karyanga (1984).

Field performance of tea grafts using three high yielding clones as scions and four drought tolerant clones as rootstocks revealed that drought tolerance, yield and production of phytomass of each clone used as scion varied with the rootstock used, indicating varying compatibility between graft partners (Satyanarayana *et al.,* 1992).

Though the photoperiodic control of elongation was primarily dependent on the ecotype of the scion on Salix, the response was significantly modified by

the rootstock (Juntilla, 1988).

Growth and survival of 'Whitespire' Japanese birch was reported to be influenced by five species of birch rootstocks (Ranney and Whitman,1995).

In egg plant, scion dry matter and leaf area were influenced by the rootstock. But root weight did not differ significantly between rootstocks (Shishido *et al.,* 1995).

Just as described above of the influence of rootstock on growth of scion, scion also exerts a major influence on the growth of the rootstock. The growth of a weak rootstock will be stimulated, when a vigorously growing scion variety is grafted on it. Likewise a weak scion variety when grafted onto a vigorously growing rootstock its growth will be reduced.

In *Hevea*, Studies with four clones viz, Tjir 1, GT 1, Gl 1 and RRIM 600 as scions and monoclonal seedlings of Tjir 1 as rootstock revealed that the scion cultivar had significant influence on both the fresh and dry weights of the main and lateral roots (Sobhana *et al.,* 1980).

The rootsystem of the seedling rootstocks in apple was reported to be influenced by the scion variety (Hartman and Kester, 1976). But the morphological characters of the root system of vegetatively propagated clonal rootstocks seemed to be not affected by the scion varieties except the quantity of the roots. The root growth was shown to be independent of the top growth

and top: root ratio was relatively large on all rootstocks with citrus scions (Kojima *et al.*, 1995). Rootgrowth and distribution were reported to be influenced by scion inclination in *Malus domestica* and *Prunus* spp. (Bargioni and Baroni, 1995).

1.4.2 Influence of stock / scion on mineral nutrition

Besides affecting growth and yield of scion cultivar, the rootstock also regulates the uptake of nutrients. The tremendous influence of rootstocks on mineral uptake is well documented in literature (Tukey *et al.*, 1962; Bould and Campbell, 1970; Saric *et al.*, 1977; Oberly and Poling, 1978; Poling and Oberly, 1979; Gowda, 1983; Ahmed and Al-Shurb, 1984; Holevas *et al.*, 1985; Alvino *et al.*, 1989). A suitable combination of rootstock, scion cultivar and tree shape will ultimately ensure a balanced uptake of nutrients from the soil and their further utilization (Sharma and Chauhan, 1991).

Influence of stock / scion on mineral uptake in *Hevea* is not much studied and only meagre information is available in literature regarding this aspect. Pot culture experiments by Teng and Pushparajah (1974) gave an indication that rootstock affected nutrient uptake of certain scions. Studies with different stock/ scion combinations in mature rubber also showed different levels of particular nutrients.

Cation exchange capacity of roots which has a major influence on mineral

uptake in plants was estimated in budded plants of four clones of *Hevea* on monoclonal seedling rootstock by Sobhana *et al.* (1980). It was observed that the cation exchange capacity of lateral roots was found to be significantly influenced by the scion. However, there was no significant difference in the cation exchange capacity of main roots.

Fallahi *et al.* (1984) reported the influence of apple rootstocks on leaf mineral composition and yield in a high density orchard. Ca, Mg and B contents in the leaves showed variations depending on the rootstocks.

The K absorption ability was significantly different in various rootstocks of grapevines during the vegetative period (Brancadoro *et al.,* 1994). Hirst and Ferree (1995) demonstrated rootstock effects on flowering in apple and observed that spur leaf P concentration was unaffected by rootstocks. But leaf mineral nutrient content was reported to be varied between rootstocks, scion and years in apple (Ugolik and Kantorowicz-Bak, 1993). The macronutrient contents of apple leaves indicated a pronounced stionic influence in the studies conducted by Om and Pathak (1983).

Influence of the rootstock on nutrient acquisition in Pistachio was studied by Brown *et al.* (1994). They observed that choice of rootstock was sufficient to overcome visible deficiencies in Cu and Zn. In soils, low in a particular element rootstock selection may be advantageous with regard to the need for fertilizer supplementation.

In mango, grafted on different rootstocks, leaf N, P, K, Ca, Mg and S contents differed significantly between rootstocks (Reddy *et al.*, 1989).

Several rootstocks of peach, nectarines and plum were compared for mineral nutrient absorption and found that leaf P, Ca and Mg contents of rootstocks propagated by cuttings were highest in *Pyrus persica.* K and Mn content of leaves also showed variations in different graft combinations (Ogata *et al.*, 1989).

Knowles *et al.* (1984) reported peach rootstock influence on foliar and dormant stem nutrient content. Rootstock had more important effect on K content than on N, P, Mg or Mn content of the scion leaves. The rootstock influence on foliar nutrient concentrations of peach trees was also shown by Brown and Cummins (1989). But they reported that differences among the rootstocks were most evident in the foliar levels of Ca, Mg, Fe, Mn, B and Cu.

But Chaplin and Westwood (1980) showed that variability of leaf element content of pear growing on fifteen different *Pyrus* species and related genera rootstocks was not great and did not vary appreciably from that expected from intraspecific rootstocks. Their findings indicated that scion has the major influence on leaf mineral content which are contradictory to the finding reported above.

In young and adult trees of lemon also the same effect was observed by El-Shazly *et al.* (1992). They reported scion differences in mineral uptake. Influence of citrus rootstocks on mineral nutrition was shown by several workers (Castle and Krezdorn, 1975; Kunwar and Singh, 1983; Sylversten and Graham, 1985).

Cobianchi *et al.* (1988) showed that macroelement concentration of peaches were affected by the different plum and seedling peach rootstocks. Leaf N, P, K, Ca, Fe and Mn concentrations were reported to be affected more by the cultivars than by the rootstocks in two apple cultivars (Kruezy'nska *et al.*, 1990). According to Jadczuk *et al.* (1995) rootstock was an important contributor to tree nutrition in non-bearing apple trees.

In grapevine, Delas and Pouget (1989) tried the reciprocal grafting technique to determine the influence of rootstock and scion cultivars on K, Ca and Mg content of leaf. They found that the mineral content of the scion is considered to be the result of the ability of the rootstock rootsystem to absorb nutrients and ability of the scion to translocate and accumulate them.

1.4.3 Physiological and biochemical effects

The results of three year studies on photosynthesis of some apple rootstocks with and without a scion cultivar showed that photosynthesis was always greater in the leaves of dwarfing, compared to vigorous rootstocks. The

photosynthetic rate in grafted trees depended on tree age, season, type of rootstocks, etc. (Titova and Shishkaner, 1976).

Studies on carbon partitioning in apple leaves by measuring leaf expansion, Carbon dioxide exchange rate (CER), mass carbon transfer (MCT) and carbohydrate pools in one year old apple trees by Brown *et al.* (1985) showed that CER was influenced by the rootstock. They further showed that the above and below ground dry weight depends on rootstock. But Barden and Ferree (1979) reported that rootstock had no effect on net photosynthesis, shoot growth, transpiration or dark respiration of one year old apple trees. Influence of rootstock on net photosynthesis in apple was also reported by Baugher *et al.* (1994).

Leaf photosynthetic capacity of *Prunus domestica* cultivars on different rootstocks in orchards and growth chamber showed significant differences between cultivars. Levels of soluble sugars and starch in the leaves also vary between cultivars. Although tree growth in the orchard was clearly affected by rootstock, neither photosynthetic capacity nor leaf sugar concentration was affected by the vigour induced by the rootstock. The rootstock appears to modify assimilate partitioning either between shoot and root growth or between storage and growth (Gaudillere *et al.*, 1989).

Photosynthetic rate, assimilate movement and accumulation and phenolic compound concentrations were compared in twelve year old grapefruit

trees on two rootstocks and found that photosynthetic values taken during the fruit growth in the dwarf trees were significantly high, compensating for the reduced leaf area. The total phenol content in the roots was also high (Walt *et al.*, 1995).

CER and photorespiration rates during three year studies with lemon cultivars on nine rootstocks and rooted cuttings of the scion cultivar indicated the influence of rootstock on photosynthesis (Sharma and Singh, 1989).

Observations on the influence of different rootstocks on the stomatal resistance and leaf water potential of apple under different irrigation regimes showed that transpiration rate, leaf water potential and coefficient of correlation between these two parameters were influenced by the rootstock type(Bergamini *et al.*, 1988). Interaction of rootstock and soil temperature was reported to play an important role in regulation of scion water status in peaches (Young and Houser, 1980). Rootstock effects on apple tree water relations was reported by Higgs and Jones (1990). The same authors in 1991 reported water relations and cropping of apple cultivars using dwarfing rootstocks in response to imposed drought.

Studies by Lloyd *et al.* (1990) on photosynthesis and water relations for different rootstocks-scion combinations between *Citrus* species in response to salinisation showed that all scions seemed to be more sensitive to salinity when

budded on a particular cultivar. Scion differences in sensitivity of leaf gas exchange to solute concentration were independent of rootstocks.

Effects of several seedling rootstocks, growing conditions, etc, on the accumulation of total sugars in the leaves of different cultivars of apple were studied by Kul'tabaev (1985). The accumulation of total sugar in the cultivars depends on rootstocks. Soluble carbohydrate content in the shoots was seemed to be influenced by the rootstocks in grapevine also (Abramidze *et al.,* 1984).

Brown *et al.* (1985) reported rootstock and scion influence on the seasonal distribution of dry weight and carbohydrates in young apple trees. Above and below ground dry weights varied depending on the rootstock. Carbohydrates contents also followed a similar pattern.

Different rootstock-scion combinations of *Citrus* sp. were examined in different seasons for their carbohydrate contents and found that starch content varied in different combinatoins and plants on its own roots (Kaplankiran *et al.*, 1985)

The phenolic composition of *Jasminum grandiflorum* was studied chromatographically before and after budding onto *Jasminum officinale.* Grafting produced morphological changes and changes in phenolic composition in the scion (Tahrouch-skouri *et al.*, 1993). Polyphenol oxidase in the leaves of pear

and apple scions was reported to be related to the vigour of the stock (Gliemeroth, 1962). Trees on vigorous rootstocks showed high enzyme activity and vice versa. The enzyme activity value can also be used in incompatibility studies. Incompatibility symptoms were reflected through proteins, enzymes and polyphenols in plants of *Prunus avium/ Prunus cerasus* graft combinations (Schmid and Feucht, 1983). Relationship between leaf chlorophyll content and stock-scion compatibility in apple was reported by Korovin (1971). Compatible rootstock-scion combinations had higher chlorophyll contents than those of incompatible combinations.

1.4.4 Influence of interstock

In addition to the common practice of combining a rootstock and a scion, a third section can also be inserted in between them. This additional section is termed as 'interstock' or 'intermediate stock'. Such three-part-plants are reported to have many advantages (Hartman and Kester, 1976). One is that it can circumvent the incompatibility existing between a particular rootstock and scion. Incorporating the desirable characteristics of the interstock such as disease resistance, cold hardiness, etc. where these characters are not present on the stock or in the scion, is another application of this technique. Interstock itself can modify the growth as reported in fruit trees (Hartman and Kester, 1976).

In *Hevea,* budding using an interstock is referred as crown budding.

Disease resistance in the crown and at the same time high yield in the trunk, can be achieved by this technique. As a result these three-part-parts have the root system of a stock plant, trunk of a high yielding clone and crown of a disease resistant one (Yoon, 1972). Studies on interstock by Leong and Yoon (1978) in *Hevea* indicated that growth of scion could be modified by suitable clonal interstock. Dwarf clone and *Hevea spruceana* interstocks affected scion diameter. Similarly interstock of dwarf clones grow slower than the other interstocks. Length of the interstock used also influenced its effect. Results of studies on such three-part-tree combinations by Hoang (1985) showed that the variations due to the combinations with regard to vigour and yield, were significant. The primary interactions, ie, stock × trunk, trunk × crown were also significant. Lam and Hai (1993) reported that both positive and negative influences of crown on the trunk clone were observed in *Hevea brasiliensis*. Vigour, yield, diseases and latex characteristics were modified by the crown clones.

Koike and Tsukahara (1993) studied the growth and root system of apple trees on dwarfing interstocks. They observed that the top weight and root system of the trees are influenced by the rootstocks.

Roberts and Blaney (1967) reported that when a stem piece of dwarfing rootstock is inserted in between a vigorous scion variety, flowering will be enhanced with subsequent growth reduction.

1.4.5 Isozyme studies

Isozymes are widely used in studies of almost all areas of plant science viz, plant breeding, population genetics, systematics, evolutionary genetics, reproductive biology,etc. Studies of various isoenzymes will provide information on the heritable variations, organisation of gene pool, as genetic markers, cultivar identification, etc. in a large number of species (Scandalios, 1964 &1968; Bhatia *et al.*, 1967; Andrew and Brent, 1980; Bringhurst *et al.*, 1981; Gottlieb, 1982; Hicks *et al.*, 1982; Arulsekar *et al.*, 1985; 1986; Nielson, 1985; Parfit and Arulsekar, 1985; Sujatha *et al.*, 1991; Barone *et al.*, 1996). Intraspecific variability can also be studied by isozyme analysis (Kephart, 1990).

The influence of stock-scion interaction on isoenzymic pattern was reported in a few plant species. In *Hevea,* Krishnakumar *et al.* (1992) demonstrated polymorphic isozyme expression of five enzymes viz, aspartate aminotransferase, leucine amino peptidase, acid phosphatase, alkaline phosphatase and phosphogluco isomerase in the clone RRII 105 due to stock-scion interaction.

Yeet et al. (1977) studied protein and enzyme variation in some cultivars of *Hevea.* Chevallier (1988) studied the genetic variability of *Hevea brasiliensis* germplasm using isozyme markers. The polyclonal rootstocks used for the evaluation of stock influence in *Hevea* by Yeang *et al.* (1995).

Enzyme polymorphism was used as genetic markers in Avocado (Torres *et al.*, 1978), datepalm (Torres and Tisserat, 1980), *Camelia japonica* (Wendel and Parks, 1983), sugarbeet (Van Geyt and Smed, 1984), apple cultivars (Weeden and Lamb, 1985). Isoelectric focusing of peroxidase and acid phosphatase isozymes were used for characterising *Diascoria* food yams by Twyford *et al.* (1990).

Isoenzymic composition of peroxidases and acid phosphatases in citrus to assess the influence of rootstock was carried out by Protopapadakis (1988). Peroxidase bands differed in their position depending upon the scion / rootstock genomic diversity and degree of compatibility. A relationship between these bands and rootstock vigour was reported in the study but patterns of acid phosphatase were seemed to be unaffected by the rootstocks. Similarly, Deloire and Hebaut (1982) reported the peroxidase activity and lignification in compatible and incompatible grafts of *Capsicum* on *Lycopersicum*.

Variations in isozyme patterns of the enzymes aconitase, isocitrate dehydrogenase, leucine-amino peptidase and glucose phosphate isomerase were reported by Degani *et al.* (1990) in mango cultivars due to genetic differences in the rootstocks.

Isoenzymes of peroxidase, acid phosphatase and ATPase in the phloem near the bud union were correlated with the degree of incompatibility in *Prunus*

avium / Prunus cerasus grafts (Schmid and Feucht, 1985). Cambial peroxidase enzymes were related to graft incompatibility in red oak (Santamour, 1988).

The affinity between the scion and different rootstocks can be predicted by the relative electrophoretic mobilities of the total proteins in *Vitis vinifera* (Masa, 1989).

Graft-transmissible influence on fatty acid composition of soybean seeds was reported by Carver *et al.* (1987). The rootstock genotype significantly influenced the seed fatty acid composition.

Genome level changes due to stock-scion interaction was reported in *Capsicum annuum* by Yagishita and Hirata (1987); Yagishita *et al.* (1986 and 1990). Capsicin biosynthesis which is genetically controlled was modified by grafting and this modified trait was reported to be stable.

1.5 Mechanism of stock-scion interaction

Stock-scion interaction is a complex phenomenon. Different theories to explain the mechanism involved in stock-scion relationship was documented by several workers (Hartman and Kester, 1976; Singh, 1980). Predominating influence of the stem portion, root system and the physiological factors are suggested as the three possible causes for stock-scion interaction. According to the theory of predominating influence of the stem portion by Robert and Swarbrick (Hartman and Kester, 1976), effects of the rootstocks are localized

more in the stem portion of the rootstock rather than in the absorbing rootsystem and the rootstock influence is controlled by the translocation effect and not by the absorbing ability of the roots. Evidence to support this theory is the strong influence of scion on the growth characters of rootstock when the scion variety is grafted directly on a root piece of the rootstock, with no rootstock stem.

Another theory postulated by Vyvyan (1930) and Beakane and Rogers (1956) emphasised that rootsystem itself controls the rootstock effect on scion, although presence of a piece of rootstock stem enhances the stock influence.

Physiological factors are suggested as controlling the rootstock influence on scion and vice versa by Chandler, 1925 and Gardner *et al.*1939 (Hartman and Kester, 1976). They suggested that changes in vigour of the plant which in turn result in variations in the carbohydrate supply to the roots will influence the branching habit of the roots.

Movement of endogenous growth factors from one graft partner to another was reported to be one of the reasons for the reciprocal stock-scion influence. Juvenile characters in ivy (*Hedera helix)* was induced in the adult form of the scion by grafting onto juvenile plants. This may be due to the passage of certain hormones between the graft partners.

The effect of rootstock on tree growth and survival can result from

differences in rootstock adaptability to edaphic, climatic and biotic components of the rhizosphere as well as physiological interactions between the rootstocks and scion (Cummins and Aldwinckle, 1983).

1.6 Performance of own-rooted plants

Own-rooted plants of tree crops especially fruit trees are gaining much interest now-adays. Uniform growth and yield, by reducing the heterogeneity induced by the rootstock is expected from these plants. But the performance of these own-rooted plants has to be evaluated before planting them commercially. In *Hevea,* own-rooted plants raised from stem cuttings were not successful for commercial plantings (Yoon and Leong, 1975). However, growth of these plants has been found to be less variable than buddings (Rubber Research Institute of Malaysia, 1962).

Studies with own-rooted plants of apple indicated that these trees in the first year or two grow more slowly than on different rootstocks. These trees seemed to grow quite rapidly after the slow start in the early phase (Ferree and Carlson, 1987).

Micropropagated trees on their own roots were equal in vigour to budded trees in apple (Zimmerman and Miller, 1991). It was reported by Zimmerman and Steffens (1996) that since these plants are growing on their own roots, trees (14 years old) did not show any intraclonal variations in vegetative vigour,

photosynthesis, stomatal conductance, etc.

Kiwi plants obtained by micropropagation, hardwood cuttings or graftings were assessed for root growth and conformation in the first two growing seasons by Piccotino *et al.*(1992). It was found that root density showed little difference between treatments and remained constant for two years.

Okie (1987) in the review on plum rootstocks suggested that increased use of own-rooted trees eliminates the compatibility problem and trees of few cultivars on their own roots were doing well and comparable in size with that of grafted plants of those cultivars.

In *Annona* species variability in seedling rootstock performance due to their genetic diversity is a major cause of scion yield and fruit quality reductions. Clonal propagation of cultivars or rootstocks would eliminate most of this variability (George and Nilssen, 1987).

1.7 Conclusion and objectives

As is evident from the above review, studies on stock-scion interaction show variable results in different species. In *Hevea*, earlier studies were concentrated mainly on growth and yield in different stock / scion combinations. But information is lacking on physiological and biochemical changes due to stock-scion interaction which are vitally related to yield

variations. Though vegetative propagation by stem cuttings were tried, only meagre information was available on rooting by air-layering. No information seems to be available on the physiology of rooting in different clones of *Hevea*. Biochemical factors are also important in view of their role in regulating the development of roots. The performance of own-rooted clonal plants of *Hevea* raised through air-layering was also not reported so far.

In view of these, experiments were conducted in budded and own-rooted plants of different clones of *Hevea brasiliensis* to standardize air-layering technique, find out the influence of stock on scion and vice versa, performance of own-rooted plants in comparison with budded plants, etc. The specific objectives are given below.

1. To standardize air-layering technique for obtaining maximum number of own-rooted plants.

2. To evaluate clonal variability in rooting capacity of different clones.

3. To find out the effect of age on rooting

4. To study the influence of hormones on rooting in layers

5. To characterise easy-to-root and difficult-to-root clones of *Hevea* on the basis of biochemical analysis

6. To examine the influence of stock / scion on growth characteristics viz,

height, diameter, number of leaves, leaf area. biomass, etc., physiological parameters viz, Carbon dioxide exchange rate, stomatal conductance, transpiration rate, etc. before and after budding and biochemical characteristics viz, total sugars, reducing sugars, phenols, amino acids, total chlorophyll, etc. of the plants at eighteen months after budding.

7. To study the effect of rootstock on foliar mineral elements composition viz, N, P, K, Ca, Mg, Fe and Mn

8. To evaluate the polymorphism in three enzymes viz, peroxidase, catalase and esterase induced by rootstocks in different clones.

9. To study the performance of own-rooted and budded plants, viz, rootgrowth, cation exchange capacity of roots, foliar mineral nutrient composition, etc.

Chapter 2

MATERIALS AND METHODS

2.1 Introduction

The plant materials utilized for the studies included polyclonal seedlings, own-rooted plants and budded plants of *Hevea brasiliensis*. These plants were grown in the research gardens of the Rubber Research Institute of India, Head Quarters, Kottayam, Kerala (76° 36′ E and 9° 32′ N at an altitude of 73m MSL) and all the experiments described here were carried out at this nursery and the Plant Physiology laboratory of RRII. The materials and methods employed in these studies are briefly given below.

2.2 Plant materials

For the various experiments the following clones of *Hevea brasiliensis* were grown in cement pots and polybags. The clones selected for the different experiments are given below.

1. RRII 105
2. RRII 118
3. RRII 203
4. RRII 208
5. RRIM 600
6. RRIM 701
7. GT1
8. GL1
9. PB 5/51
10. PB 235
11. PB 260
12. PCK1
13. PCK2

2.2.1 Methods of raising plant materials

2.2.1.1 Polyclonal seedlings

Seeds were collected from the polyclonal seed gardens situated in Kanya Kumari District of Tamil Nadu. Since only a limited number of seedlings were required aluminium trays filled with river sand were used for germinating the seeds instead of the conventional germination beds in the nursery. The seeds were sown in a single layer, close to each other by pressing into the sand. The trays were covered with pieces of wet gunny bags and kept in shade. Water was sprinkled daily to keep the sand in moist condition. Seeds were germinated within six to seven days and the germinated seeds were planted in cement pots (65cm×35cm) filled with approximately 40 Kg. of soil. These seedlings were grown in these pots by following standard cultural practices.

2.2.1.2 Budded plants

Budgrafting was carried out on polyclonal seedlings maintained in cement pots / polybags (polybags of height 55cm , width 25cm and 400 gauges thickness). Buds were taken from budwood plants of selected clones, maintained in the budwood nursery of Rubber Research Institute of India, Kottayam. Brown budding was adopted and the plants were budgrafted at the age of 18 months. The stock stem was cut off above the budpatch, in successful

buddings and the plants were grown in pots by following standard cultural practices. N, P, K, Mg mixture 10:10:4:1.5 was applied once in three months in equal doses to all these plants. The plants were maintained in well watered condition.

2.2.1.3 Own-rooted plants

(i) Standardization of air-layering technique

a) ***Plant material***

Three year old potted plants of three popular clones of *Hevea brasiliensis* viz, RRII 105, RRIM 600 and GT1 were used for the experiment to find out the best medium for rooting in air layers.

b) ***Rooting media***

The rooting media tried were sphagnum moss, soil, coconut husk and coconut husk with soil. Fifteen branches were layered per clone in each treatment.

c) ***Procedure of air layering***

Mature branches of 45-60 cm in length, having 1.5-2.0 cm diameter with two whorls of leaves, were girdled by completely removing a ring of 2.0-2.5 cm bark with a sharp budding knife (Plate 3a). The latex from the girdled

portion was carefully removed using moist cotton without damaging the cut ends. The girdled portion was then immediately covered with moist rooting medium and completely wrapped with a polythene film 25-30 cm in length and 15cm width. The polythene film was tied firmly on the stem with jute twine to prevent moisture loss and water seepage (Plate 3b). Established layer put forth roots which were visible through the polythene cover within 45 days after girdling.

d) *Planting of rooted air layers*

Sixty days after layering, when the roots were fully developed the branches were severed 2-3 cm below the girdled portion and planted in polybags filled with garden soil.

2.2.1.4 Plants with double-root system

Young budded plants maintained in polybags and having two to three whorls of leaves were used for the study. Air-layering was done on scion portion about 3-4 cm above the stock-scion union using sphagnum moss. After 45-60 days when the roots were well developed the polythene film around the layer was removed and the plants having both stock and scion roots were planted in cement pots after cutting the polythene bags carefully so that the soil core was not disturbed. The cement pots were filled with soil so as to keep both the scion and stock roots completely under the soil.

Plate 3 (a). Air layered branch

(b). Air layer

PLATE 3

2.3 Growth hormones and rooting

Three year old budded plants of three clones viz, RRII 105, RRIM 600 and GT1 were used for the experiment. Two growth hormones IBA (Indole-butyric acid) and NAA (Naphthalene acetic acid) and Rootex (a formulation of IBA in powder form) were used. (IBA & NAA were procured from M/s Sisco Research Laboratories, Mumbai and Rootex is a product of Rootex laboratories, Mumbai). IBA and NAA were applied at four levels viz, 100, 250, 500 and 1000ppm.

2.3.1 Preparation of IBA and NAA paste

IBA / NAA was mixed with lanolin to form a paste. 10, 25, 50 and 100mg each of IBA / NAA was dissolved in few drops of ethanol. 100g melted lanolin was added to this and mixed thoroughly. The paste was kept open for sometime to get the ethanol evaporated.

2.3.2 Application of the hormones

The hormone paste / Rootex powder was applied at the distal edge of the girdled portion of the layers and then covered with moist sphagnum moss as described earlier.

2.4 Parameters studied

2.4.1 Growth parameters

Growth characteristics viz, height, stem diameter, number of leaves, mean area of the leaf, total leaf area of the plant, fresh and dry weight of the plant (fresh and dry weight of the roots and shoot separately), root-shoot ratio, etc.

2.4.2 Physiological parameters

Gas exchange measurements viz, Carbon dioxide exchange rate(CER), stomatal conductance (gs), transpiration rate, etc., foliar nutrient levels viz, N, P, K, Ca, Mg, Fe and Mn, cation exchange capacity of roots (CEC), etc.

2.4.3 Biochemical parameters

The leaf/ bark samples were analysed for soluble carbohydrates, starch, amino acids, total phenols, total chlorophyll, total soluble proteins, etc.

2.4.4 Isozyme analysis

Isozymes viz, Peroxidase, Catalase and Esterase

2.4.1 Growth parameters

2.4.1.1 Plant height

Plant height (cm) was measured from the soil surface to the tip of the main stem in seedlings. In the case of budded plants the height was measured from the rootstock-scion union to the tip of the main stem.

2.4.1.2 Diameter

Diameter of the main stem (cm) was measured at 5 cm above the soil surface in seedlings and just above the rootstock-scion union in budded plants.

2.4.1.3 Leaf area

Leaves were detached from the plant just before uprooting the plants for determining the biomass and leaf area (cm^2)was measured using leaf area meter (LI 3000, LI COR, USA).

2.4.1.4 Specific leaf weight (SLW)

It is the ratio of the leaf dry weight to leaf area.

$$\text{SLW (mg cm}^{-2}) = \frac{\text{Leaf dry weight}}{\text{Leaf area}}$$

2.4.1.5 Root weight

The pots / polybags were soaked well with water in order to loosen the soil and get the entire rootsystem intact without damage to the lateral roots. The roots were washed thoroughly in running water to remove the adhering soil particles after uprooting and was separated from the stem. The tap roots and lateral roots were separated and fresh weights were taken after carefully removing all the sticking water. These root samples were oven dried at 80°C.

2.4.1.6 Shoot weight

The shoot portion was weighed just after uprooting and then oven dried at 80°C to get the dry weight.

2.4.1.7 Total biomass

The total weight of the different parts after oven drying was taken as the biomass and expressed as g dry matter / plant.

2.4.1.8 Root-shoot ratio

It was calculated as the ratio of root dry weight to shoot dry weight of the plant.

2.4.2 Physiological parameters

2.4.2.1 Gas exchange measurements

These parameters were measured by a Portable Photosynthetic System (LI 6200, LI COR, USA).

Fully expanded mature leaf was inserted into the chamber as identical to natural position and exposed to sunlight for gas exchange measurements. All measurements were taken between 08.30 and 09.30 hrs. The ambient temperature was 27±2°C, relative humidity approximately 60% and light intensity 1000± 200 μ mol $m^{-2}s^{-1}$.

2.4.2.2 Cation exchange capacity of roots

Root cation exchange capacity was measured by the method of Crooke (1964).

(i) Reagents

a) 1N KCl (pH 7.0): 74.5g KCl was dissolved in distilled water and made upto 1000 ml with distilled water.

b) 0.01N KOH : 0.56g of KOH was dissolved in distilled water and made upto 1000 ml. This was standardised using a primary standard of Potassium hydrogen phthalate.

c) 0.01N HCl : 0.83 ml concentrated HCl was diluted to 1000 ml with distilled water.

(ii) Estimation of root cation exchange capacity

Fresh lateral roots were taken and washed thoroughly to remove the adhering soil particles. These roots were dried in an oven at 80°C for 72 hrs. It was ground to pass through 1mm sieve. 0.5g of powdered root sample was taken in a 500ml beaker and moistened with few drops of water. 200ml of 0.01N HCl was added and shaken intermittently for 5 min. Excess HCl was decanted off using Whatman No.1 filterpaper. The root samples were then washed repeatedly with distilled water till the leachates became chloride free. (Approximately 300ml of distilled water was required for washing each sample). The bottom of the filter paper was pierced and the roots were transferred to a beaker using 200ml 1N KCl (pH 7.0) solution. The roots were stirred in this solution for 5 min. The pH of the solution was decreased due to H^+ ions released from the root. The pH of the solution was again brought to 7.0 using 0.01N KOH solution adding drop by drop from a burette. The volume of 0.01N KOH used as recorded for calculating the CEC.

$$\text{CEC of root} = \frac{\text{Volume of KOH} \times \text{Normality of KOH} \times 100}{\text{Weight of roots}}$$

CEC is expressed as meq/100g dry roots.

2.4.2.3 Estimation of foliar N, P, K, Ca, Mg, Fe and Mn

Leaf samples were dried in an oven for 72 hrs. at 80°C. The dried leaves were used for the estimation of mineral elements. N, P, K, Ca and Mg were estimated as described in Plant and Soil analysis (Karthikakutty Amma, 1976). Fe and Mn were estimated by the method as described by Piper (1950).

2.4.3 Biochemical parameters

2.4.3.1 Extraction and estimation of carbohydrates, sugars, starch, phenols and free amino acids in the bark and leaves.

Mature leaves of physiologically same stage of development and bark of the girdled portion at the time of air-layering were used for the estimations. 500 mg of bark or leaf sample after removing the main veins was homogenised in a mortar with 80 percent ethanol. The sample was transferred to centrifuge tubes and heated in a boiling water bath for 10-15 minutes. After cooling these samples were centrifuged at 10000 rpm for 20 minutes. The supernatant was collected. The residue was again extracted with 80 percent ethanol in boiling water bath and centrifuged. The supernatant was made upto 10 ml with 80 percent ethanol. The residue in the tube was dried in the oven at 80°C for 48 hours.

(i) Estimation of reducing sugars

Reducing sugars were estimated by the method of Nelson (1944).

Reagents

a) Reagent A

25 g anhydrous Na_2CO_3, 25 g Sodium Potassium tartrate (Rochelle salt), 20 g $NaHCO_3$ and 200 g anhydrous Na_2SO_4 were dissolved in 800 ml H_2O and made upto 1000 ml. Filtered through Whatman No.1 filterpaper.

b) Reagent B

15 percent $CuSO_4.5H_2O$ containing 1-2 drops of concentrated H_2SO_4 per litre.

The Copper reagent was prepared on the day of use by mixing Reagent A and Reagent B in the proportion 25:1 by volume.

c) Arsenomolybdate solution

25 g of Ammonium molybdate was dissolved in 450 ml of H_2O. 21 ml of concentrated H_2SO_4 was added with stirring. The solution was mixed well. 3 g of $Na_2HAsO_4.7H_2O$ was dissolved in 25 ml of water and then added to the above. The solution was mixed well and filtered. The solution was kept at 37°C for 24-28 h. and stored in amber colored reagent bottles.

0.1ml of the extract was pipetted out in boiling tubes. The tubes were kept in boiling water bath for evaporating the ethanol. 1 ml of distilled water and 1 ml of copper reagent were added to the tubes. The tubes were heated in a boiling water bath for 20 min. After cooling, 1 ml of Arsenomolybdate reagent was added to each tube and the tubes were kept for 15 min. for colour development. The samples were made upto 25ml with distilled water and mixed thoroughly. A blank containing 1 ml water, 1 ml copper reagent and 1 ml Arsenomolybdate reagent and the volume made upto 25 ml was taken. The optical density was measured for estimating reducing sugar at 520nm in a UV spectrophotometer.

$$\text{Reducing sugar (mg / gm)} = \frac{\text{Concentration} \times \text{Total volume of the extract}}{\text{Weight of tissue} \times \text{Aliquot} \times 100}$$

(ii) Estimation of non-reducing sugars

0.1ml of ethanol extract was taken in a boiling tube and the contents were evaporated to dryness in a boiling water bath. 1 ml of distilled water and 1 ml of 1N H_2SO_4 were added to each tube and the tubes were heated in a water bath at 60-62°C for 30 min for hydrolysing the samples. After cooling 1 ml of 1N NaOH and 1 ml Copper reagent were added. The tubes were heated in a boiling water bath for 30 min. 1 ml of Arsenomolybdate reagent was added to each tube after cooling. The tubes were kept for 15 min and then the volume

was made up to 25 ml with distilled water. The optical density was measured at 520 nm as in reducing sugar.

$$\text{Non reducing sugar (mg / gm)} = \frac{\text{Concentration} \times \text{Total volume of the extract}}{\text{Weight of tissue} \times \text{Aliquot} \times 100}$$

(iii) Estimation of total soluble sugars

Total soluble sugars was estimated by the method of Scott and Melvin (1953).

Reagents

2 g anthrone was dissolved in 1000ml of concentrated H_2SO_4 under ıder icecold conditions and kept in icebath.

0.1ml ethanol extract was pipetted out into boiling tube and the contents were evaporated to dryness in a boiling water bath. To each tube 1 ml distilled water was added. The tubes were kept in icebath. 4 ml of icecold anthrone reagent was added to the samples through the sides of the tubes. The tubes were kept in the ice bath for 10 min and shake well. The tubes were heated in a boiling water bath for 10 min and then cooled. A blank containing 1 ml distilled water and 4 ml anthrone reagent was also taken. Optical density was measured at 625 nm in a UV spectrophotometer.

$$\text{Total sugar (mg / gm)} = \frac{\text{Concentration} \times \text{Total volume of the extract}}{\text{Weight of tissue} \times \text{Aliquot} \times 100}$$

(iv) Estimation of chlorophyll

Total chlorophyll was estimated by the method of Ozerol and Titus (1965). Fresh leaves of physiologically same stage of development were collected for the estimation of total chlorophyll. 20 discs were punched from leaves of each plant and their total area was recorded. The discs were put in 10 ml of methanol in small vials and were kept in dark for 24 hrs. Optical density of the methanol extract was measured at 651 and 664 nm in a UV spectrophotometer. Pure methanol was taken as blank. Substituting O.D values at 651 and 664 by the following equations total chlorophyll, chlorophyll a and chlorophyll b were calculated.

C (mg/cm^2) = 25.5 D651 + 4.0 D664 mg/litre of chlorophyll in methanol

Ca = 0.0165 D664 - 0.0083 D651

where,

C = Total chlorophyll (mg/cm^2)

D651 = Optical density of the extract at 651 nm

D664 = Optical density of the extract at 664 nm

Ca = Chlorophyll a

(v) Estimation of total phenols

Total phenols was estimated by the method of Swain and Hillis (1959).

Reagents

1N Folins reagent

Saturated Na_2CO_3

To 0.1ml alcoholic extract 0.5 ml distilled water, 0.5 ml Folin reagent and 1 ml saturated Na_2CO_3 solution were added. The mixture was incubated for an hour after making the final volume to 10 ml with distilled water. A blank containing water, Folins reagent and saturated Na_2CO_3 solution and volume made upto 10 ml with water was also taken. The optical density was measured at 725 nm in a UV spectrophotometer.

$$\text{Total sugar (mg / gm)} = \frac{\text{Concentration} \times \text{Total volume of the extract}}{\text{Weight of tissue} \times \text{Aliquot} \times 100}$$

(vi) Estimation of free amino acids

Free amino acids were estimated by the method of Moore and Stein (1948).

Reagents

a) Ninhydrin solution: 0.2 g of reagent grade Stannous chloride was dissolved in 125 ml citrate buffer. This solution was added to 5 g of ninhydrin dissolved in 125 ml methyl cellosolve.

b) 0.2 M Citrate buffer pH 5.0 :10.5 g of reagent grade citric acid monohydrate was dissolved in 100 ml of 1N NaOH and diluted to 250 ml.

c) Diluent solvent: Equal volumes of water and reagent grade n-propanol were mixed.

0.2ml of the ethanol extract was taken in test tubes and evaporated to dryness. 1 ml of ninhydrin reagent was added, mixed and heated for 20 min in a boiling water bath. 5 ml of diluent was added to each tube and the contents were mixed. The optical density was read at 570 nm after 15 min. A blank was also prepared with the above reagents except the extract.

$$\text{Total sugar (mg / gm)} = \frac{\text{Concentration} \times \text{Total volume of the extract}}{\text{Weight of tissue} \times \text{Aliquot} \times 100}$$

(vii) Estimation of starch

Starch was estimated by the method of McCready *et al.* (1950). The dried

residue obtained after centrifuging the samples with 80 percent ethanol for the estimations of sugars, phenols, amino acids, etc. was used for the determination of starch. The residue was solubilised with 52 percent perchloric acid for 30 min. This was filtered through glass wool and the volume was made upto 10 ml with distilled water.

Reagents

0.2 g anthrone was dissolved in 100ml concentrated H_2SO_4 and kept in icebath.

0.1 ml of the extract was taken in test tube and 1.9 ml of distilled water was added. The tubes were kept in icebath. 5 ml of freshly prepared icecold anthrone was added to the samples through the sides of the tubes. The tubes were shaken and then heated in boiling water bath for 7 min. The tubes were cooled and measured the colour intensity at 630 nm in the UV spectrophotometer. A blank containing 2 ml distilled water and 5 ml anthrone reagent was also taken. Standard curves were prepared with known amounts of glucose and the starch content was calculated by multiplying the glucose equivalent present in the sample with 0.9.

(viii) Estimation of total soluble proteins

Method described by Lowry *et al.* (1951) was followed for the estimation of total soluble proteins.

Reagents

a) Reagent A: 2g Na_2CO_3 was dissolved in 100ml of 0.1N NaOH.

b) Reagent B: 500mg $CuSO_4.5H_2O$ was dissolved in 100ml of 1% Sodium potassium tartrate.

Copper reagent was prepared on the day of use by mixing 50ml of reagent A with 1ml of reagent B.

c) 1N Folins reagent

d) 10% TCA

e) BSA: 10mg Bovine serum albumin in 20ml of 0.1N NaOH

Total soluble protein was estimated using the extract prepared for isozyme analysis. 0.1ml extract was taken in test tube and 0.5ml 10% TCA was added to each tube. The tubes were kept in refrigerator overnight. The tubes were centrifuged at 5000 rpm for 20 min. The supernatant was decanted and the precipitate was dissolved in 1ml of 0.1N NaOH. A blank containing 1ml of 0.1N NaOH was also taken. 5ml of alkaline Copper reagent was added to each tube and kept for 10 min. 0.5ml of 1N Folin's reagent was added and mixed well. The tubes were kept for 30 min. The optical density was measured at 670nm in a UV spectrophotometer. BSA was used as standard.

2.4.4 Isozyme analysis using PAGE

Leaf samples from eighteen months old budded plants of five clones of *Hevea brasiliensis*, viz, RRII 105, RRII 208, RRIM 600, GT1 and GL1 were used for the study. Isozymes of the following three enzymes were studied.

1. Peroxidase (PER, E.C.1.11:1.7)
2. Catalase(CAT, E.C.1.11:1.6)
3. Esterase(EST, E.C.3.1:1.1)

(i) Extraction buffer

The extraction buffer had the following composition

0.1M Tris-HCl pH 8.0

0.2% 2-mercaptoethanol

0.001M EDTA

0.01M $MgCl2.6H_2O$

15% Insoluble Polyvinyl pyrrolidone

(ii) Extraction

Fresh leaf samples at identical stage of development (Lebrun and

Chevallier, 1988) were collected for extraction. 500mg of leaf sample was crushed in liquid N_2 and homogenised with 4ml of the extraction buffer (Arulsekhar and Parfitt, 1986). The homogenates were centrifuged at 20,000rpm for 20 min at 0°C. The clear supernatant was collected in eppendorf tubes and stored in deep freeze.

Composition of various solutions and buffers used in electrophoresis

Running buffer

Stock solution

Composition	Quantity (for 100ml)
Tris	6.0g
Glycine	15.02g

pH 8.9(adjusted with 1M Tris)

500ml from the stock solution was diluted to 1000ml.

(iii) Composition of the gel

Reagents	Composition	Quantity(g)	pH
1.Stock A (100ml)	1.5M Tris	18.171	8.9
2.Stock B (100ml)	1.0M Tris	12.14	6.7 (adjusted with 1N HCl)
3.Stock C (100ml)	4.2M Acrylamide	29.853	
	0.065M bisacryl-amide	1.00	
4.Stock D (2ml)	10% APS	0.20 (prepared at the time of mixing)	
5.TEMED			

The composition of the different solutions for the preparation of one gel slab is as follows:

Stock solution	Spacer gel(4%)	Separation gel(10%)
C	2.5ml	13.3ml
A	--	10.0ml
B	5.0ml	--
Distilled water	5.6ml	14.3ml
D	0.2ml	0.4ml
TEMED	0.01ml	0.02ml

(iv) Preparation of the gel

The polyacrylamide gel used for resolving the isozymes consists of a 4% spacer gel poured over a 10% separation gel. Two gel plates were adhered together with 3 grease coated Teflon spacers of 1mm thickness. The components of the separation gel were mixed and poured in between the glass plates within 2.5 cm from the top. After the separation gel was polymerised the mixture for spacer gel was poured over this and immediately inserted a comb firmly into the spacer gel for making wells. Care was taken to avoid bubbles in the gel while pouring the gel mixture. The glass plates were kept undisturbed until the gel was polymerised and after polymerisation the comb was taken out.

(v) Electrophoresis

The polymerised gel was fixed in an electrophoretic tank after removing the basal spacer. 30 ul each of the samples was loaded in the wells. The marker dye Bromophenol blue was also added to each well and the upper and lower tanks were filled with the running buffer. The gels were run initially at 50V and 20 mA for 1 hr. Then it was increased to 100 V and 20 mA. 6-7 hrs. was taken for completing the electrophoresis and the gel was kept inside the refrigerator during the entire period of electrophoresis. After completion of running, the gel was separated from the glass plates and put in different isozyme specific stain solutions.

(vi) Staining of the gels

Staining solutions and procedure were different for different enzymes and are mentioned below.

a. Peroxidase

The staining procedure of Vallejos (1983) was followed.

Stain solution

Reagents

1. Benzidine hydrochloride
2. Acetic acid
3. H_2O_2 (freshly prepared)

0.1g of Benzidine hydrochloride was dissolved in 0.4ml of acetic acid and diluted with distilled water to 100ml. The solution was filtered and 0.2ml H_2O_2 was added.

The gel was stained in this solution for 10 min. After the bands became clear drained off the staining solution and the reaction was stopped using 3% acetic acid. The gel was stored in 3% acetic acid.

b. Catalase

Staining procedure of Conkle *et al.* (1982) was followed with minor modifications.

Reagents	Quantity	Formulation
1. Phosphate buffer	100ml	a) Sodium phosphate monobasic 18.5g b) Sodium phosphate dibasic 17.9g c) Distilled water 1000ml (pH 6.5)
2. 2% KI solution	100ml	
3. 3% H_2O_2	100ml	
4. Guaiacol*	3-4 drops	

* Components modified.

The gels were put in phosphate buffer and kept in the refrigerator for 40 min. The buffer was drained off and rinsed with water. 2% KI solution was poured over the gel and kept for 5 min. The KI solution was poured off and again rinsed the gel with water thoroughly. Then 3% H_2O_2 solution mixed with 3-4 drops of guaiacol was added. The bands appeared immediately and the solution was drained off and after washing with distilled water the gels were kept in 3% acetic acid.

c. Esterase

The staining procedure by Lebrun and Chevallier (1988) was followed.

Staining solution

Reagents	Quantity	Formulation
1. Phosphate buffer	40ml	a) Sodium phosphate 18.5g monobasic b) Sodium phosphate 17.9g dibasic c) Distilled water 1000ml (pH 6.5)
2. ∝ -Naphthyl acetate	30mg	
Acetone*	10ml	
Phosphate buffer	40ml	
3. Fast Blue RR Salt	50mg	
Distilled water	100ml	

* Amount varies with opacity of the solution

The gel was incubated in phosphate buffer for 10 min. The buffer was then drained off and ∝-naphthyl acetate dissolved in acetone and phosphate buffer was added and incubated for 10 min. at 40°C. After incubation , Fast blue RR salt solution was added. The bands became clear after few minutes . The gels were washed and kept in 3% acetic acid.

Chapter 3

EXPERIMENTS ON PHYSIOLOGY OF ROOTING IN *HEVEA*

3.1 Introduction

As mentioned in Chapter 1, the method adopted for propagation of *Hevea brasiliensis* throughout the NR growing countries is grafting of buds of known superior genotypes (scions) on rootstocks of unknown genetic constitution. This method generates appreciable amount of variability due to stock-scion interaction. Lack of sufficient homogeneous clonal rootstocks remains a constraint for carrying out stock-scion interaction studies. Rooting by stem cuttings and air-layering is an accepted practice of vegetative propagation to obtain homogeneous plant population of desired genetic traits within a short period. Budgrafting on such vegetatively propagated homogeneous clonal rootstocks will enable us to get a

clear understanding of the stock-scion interaction. In order to obtain sufficient numbers of homogeneous clonal rootstock material, I have tried to induce rooting in stem cuttings and air-layers. In this context, an evaluation of the factors affecting rooting in *Hevea* was done.

Recently micropropagation through shoot tip culture also ensures the production of genetically identical plant population.

Vegetative propagation of *Hevea* clones by stem cuttings has been reported earlier (Tinley, 1960; Yoon and Leong, 1975). One of the constraints for the production of rooted cuttings, is the requirement of a good mist propagation system, the installation of which is very expensive. There is only meagre information available on vegetative propagation in *Hevea* by air-layering. Hence an experiment was carried out to standardize the technique of air-layering to propagate *Hevea* clones vegetatively. The rooting medium seemed to influence rooting in air layers (see section 1.3.3. for a review). There are no reports available on the effect of different rooting media on rooting success of air layers in *Hevea brasiliensis*. The rooting ability of different clones of *Hevea* shows variability. To obtain optimum production of homogeneous rootstock materials, it will be desirable to assess different clones of *Hevea* for their rooting ability. Biochemical characterisation of easy-to-root and difficult-to-root plant species have been reported by many scientists. So far no reports are available on this aspect in *Hevea.*

It is well established that growth regulators especially rooting hormones like IBA and NAA enhance root growth (see section 1.3.5 for a review). Effect of these hormones on root growth in budded stumps and stem cuttings was reported in *Hevea* earlier. However, no information is available on the effect of these hormones on rooting in air layers of *Hevea.*

In view of these objectives the following experiments were carried out to standardise air-layering technique using different rooting media, effect of growth hormones and age of the plants on rooting success, clonal response and biochemical basis of rooting, etc.

3.2 Materials and methods

The trial for standardisation of air-layering was undertaken in three year old potted plants. Three popular clones of *Hevea brasiliensis* (RRII 105, RRIM 600 and GT1) were selected for the study. The procedure and different rooting media used were described in details in Chapter 2. Fifteen branches were layered per clone under each treatment.

One, two and three year old budded plants of 12 clones of *Hevea* were used for the studies on clonal variability in rooting, effect of age on rooting and biochemical characterisation of rooting ability. The clones selected were RRII 208, PCK 2, GT1, PB 260, RRIM 600, RRII 118, PB 235, RRII 105, PB 5/51, PCK 1, RRII 203 and RRIM 701. Air-layering was carried out as

described in Chapter 2 with moist sphagnum moss as the rooting medium.

The biochemical factors estimated were reducing sugars, non reducing sugars, starch, phenols and amino acids. The methodology used for the estimations are described in Chapter 2.

The plants used for the experiment on the effect of growth hormones on rooting in air layers were three year old potted plants belonging to three clones, viz, RRII 105, RRIM 600 and GT1. The hormones used and method of application are given in Chapter 2.

3.3 Results and Discussion

3.3.1 Standardisation of air-layering technique

Root formation was observed in the air-layers after about 45 days of layering (Plate 4a). This could be detected through the transparent film of polythene. The roots were fully developed in about 60 days after layering (Plate 4b). Among the four rooting media tried, sphagnum moss soaked in water was found to be the best (Table 1). The clones differed in response to rooting, RRIM 600 giving a success of 87 percent and RRII 105 only 33 percent. While no rooting of any clone was observed in soil and coconut husk, RRIM 600 gave 7 percent success in the medium consisting of coconut husk and soil (Table 1).

PLATE 4

Table 1: Effect of various rooting media on rooting percentage of air-layers in three clones of *Hevea brasiliensis*

Clones	Rooting media			
	Sphagnum moss	Soil	Coconut husk	Coconut husk and soil
RRIM 600	87	0	0	7
RRII 105	33	0	0	0
GT1	67	0	0	0
Mean	62.20	0	0	2.22

Since the stem is not severed in air layers, the xylem remains intact, so that water and mineral supply to the girdled shoot is not affected unlike in propagation by cuttings. Earlier reports showed that rooting in stem cuttings of *Hevea brasiliensis* was successful only in a mist propagation system (Leong *et al.*, 1976). It is essential to maintain the turgidity of the stem cutting throughout the period of root induction by mist system unlike in layers and hence the failure of the mist system even for few hours will adversely affect the rooting. Girdling causes an interruption in the downward translocation of organic materials, carbohydrates, auxin and other growth factors from the leaves and growing shoot tips. These materials accumulate near the distal edge of the girdled portion and rooting occurs in this area. This is one of the important

reasons for layering being more successful with many plants than propagation by cuttings. The air-layered plants have an advantage over other propagation methods since the food reserves of the parent branch induces the formation of a balanced and well developed root system (Chauhan and Dua, 1982).

The importance of sphagnum moss as a rooting medium is also well established (Hartman and Kester, 1976). Sphagnum moss soaked in water retains moisture for a long time and provides better aeration. Wrapping with polythene film further prevents moisture loss during the entire phase of the root development in the layers. Moreover, this technique is simple to perform and requires less skill, effort and equipment than is necessary for other methods for raising homogeneous population of *Hevea brasiliensis.*

3.3.2 Clonal response and influence of age on rooting

Wide clonal variation was observed in rooting success and survival of rooted air-layers in 12 clones of *Hevea* at three ages of growth. (Table 2, Fig.1). Variability of rooting in these clones ranged from 0-95%. Highest rooting success of 95% was observed in one year old budded plants of RRII 118 followed by GT1, RRII 208, PCK2, PB 260 and PCK 1.

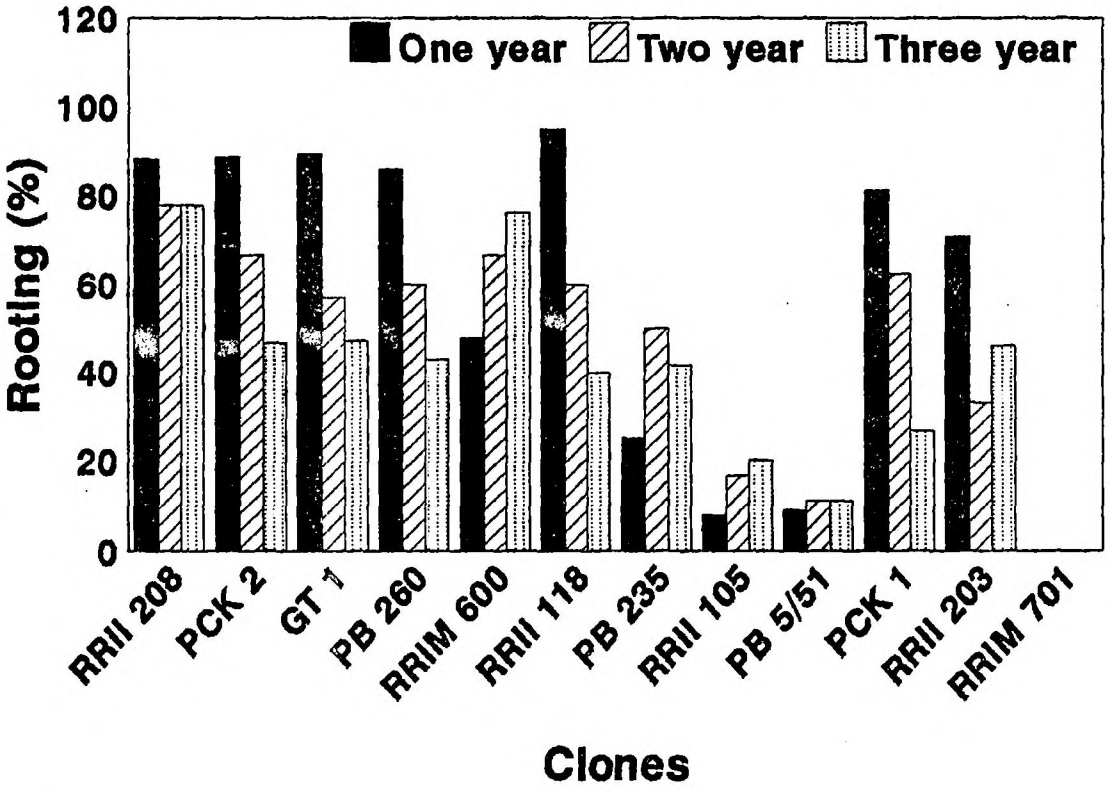

Fig: 1. Variability in rooting percent in twelve clones of *Hevea brasiliensis* at three ages of growth

Table 2. Variability in rooting (%) and survival (%) of air-layers in twelve clones of *Hevea brasiliensis*

Clones	Age of the mother plant								
	One year			Two year			Three year		
	Branches layered	Rooting (%)	Survival (%)	Branches layered	Rooting (%)	Survival (%)	Branches layered	Rooting (%)	Survival (%)
RRII 208	43	88	74	18	78	57	18	78	50
PCK 2	45	89	75	15	67	70	15	47	57
GT 1	38	90	74	14	57	63	36	47	53
PB 260	36	86	68	15	60	44	14	43	50
RRIM600	23	48	55	15	68	50	21	76	44
RRII 118	20	95	63	15	60	44	15	40	50
PB 235	16	25	50	16	50	50	12	42	40
RRII 105	25	8	50	18	17	33	15	20	33
PB 5/51	33	9	33	18	11	0	18	11	0
PCK 1	16	81	46	16	63	40	14	29	50
RRII 203	24	71	53	15	33	40	13	46	33
RRIM701	17	0	-	16	0	-	18	0	-
Mean	-	57.5	58.3	-	47	44.6	-	39.9	41.9
S.D	-	37.2	13.6	-	25.4	18.3	-	23.1	15.9
CV(%)	-	64.6	23.3	-	54.1	41	-	57.9	37.9

Survival of transplanted air-layers also showed considerable variability. Based on rooting success in one year old plants (Fig.2), six clones were grouped as easy-to-root and four as hard-to-root types (Table 3).

TABLE 3: Classification of 12 clones of *Hevea brasiliensis* based on percent of rooting success

Rooting (%)	Type	Clones
Above 75%	Easy-to-root	RRII 118, PCK 2, GT 1, RRII 208, PB 260, PCK 1
40-75%	Medium-to-root	RRIM 600 & RRII 203
Below 40%	Hard-to-root	RRII 105, PB 5/51, PB 235, RRIM 701

It is interesting to note that no rooting could be induced in layers of RRIM 701 at any age. It is well documented in literature that the rooting ability varies in different cultivars even within a species. The observations in the present study are in agreement with earlier findings in *Hevea* stem cuttings, that clonal variability existed in rooting response and also rooting success was influenced by the age of the plant (Tinley, 1960; Yoon and Leong, 1975 and Leong *et al.*, 1976). The first visible change observed in the air-layers was the formation of callus at the distal edge of

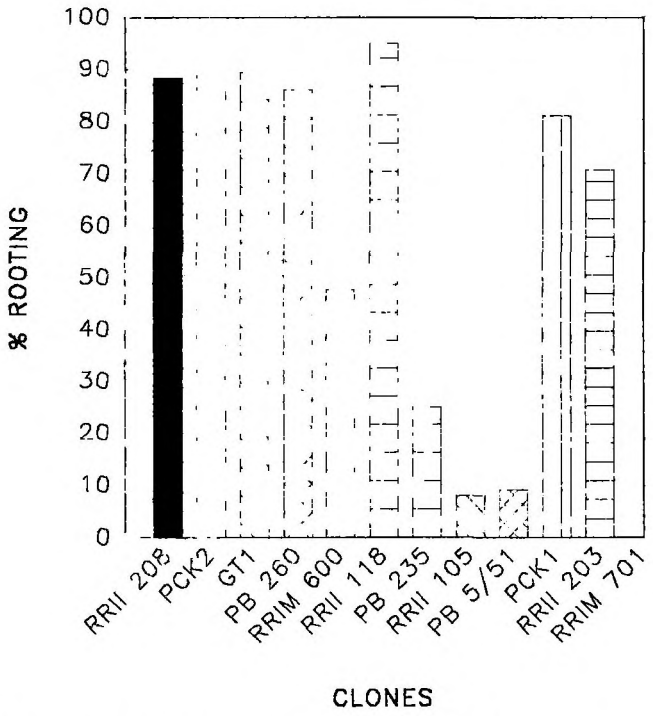

Fig.2. Clonal response in percent of rooting in one year old budded plants of twelve clones of Hevea

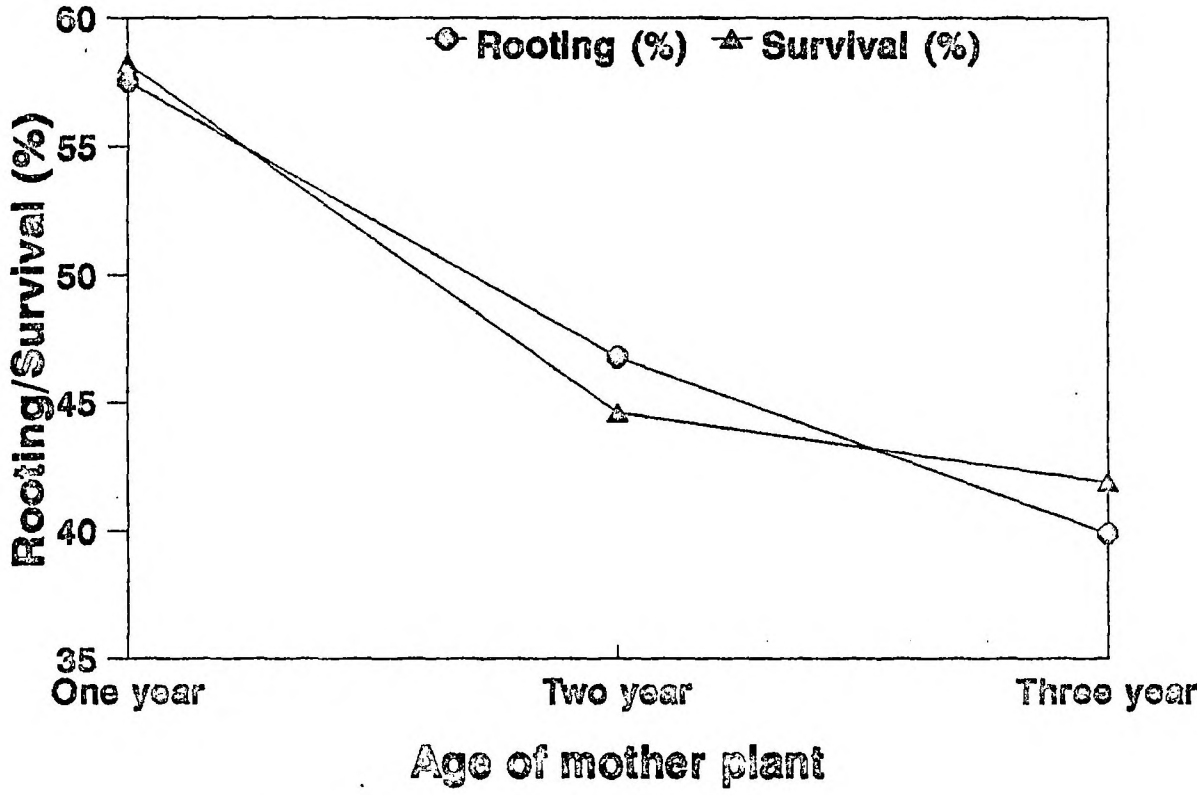

Fig: 3. Effect of age of mother plant on rooting / survival (%) of air-layers in *Hevea brasiliensis*

the girdle at about 20-25 days of the treatment. Even in the layers having no root development, callus formation was observed and in few, even rudimentary structures similar to root primordia emerged from the callused portion. Similar observations were reported in stem cuttings also (Uniyal *et al.*, 1993). It is assumed that the non-differentiation of callus into roots may be due to the lack of sufficient food reserve or some internal factors and/or age of the plants. The levels of endogenous auxins may also play a vital role in the differentiation and growth of roots. Rooting is a complex physiological process and is controlled and regulated by a number of genetical, physiological, biochemical and environmental factors. Variability in rooting among the different clones may be attributed to the differences in biochemical characters and their inherent capacity to produce roots in layers.

There was a definite effect of the age on the percent of rooting in the layers and survival of rooted layers. In general, first and second year old plants gave better rooting percent and survival (Figs.3&4). But there was clonal variations in rooting among the plants of different ages. RRII 105, RRIM 600 and PB 235 responded to rooting in a reverse manner, ie, one year old plants gave the least rooting success in the present study. The observation of the influence of juvenility on rooting success is in conformity with similar results reported earlier by other workers (Couvillon, 1985; Desai and Patil, 1984; Kumar and Chauhan, 1972; Rao *et al.*, 1988 and Hartman and Kester, 1976).

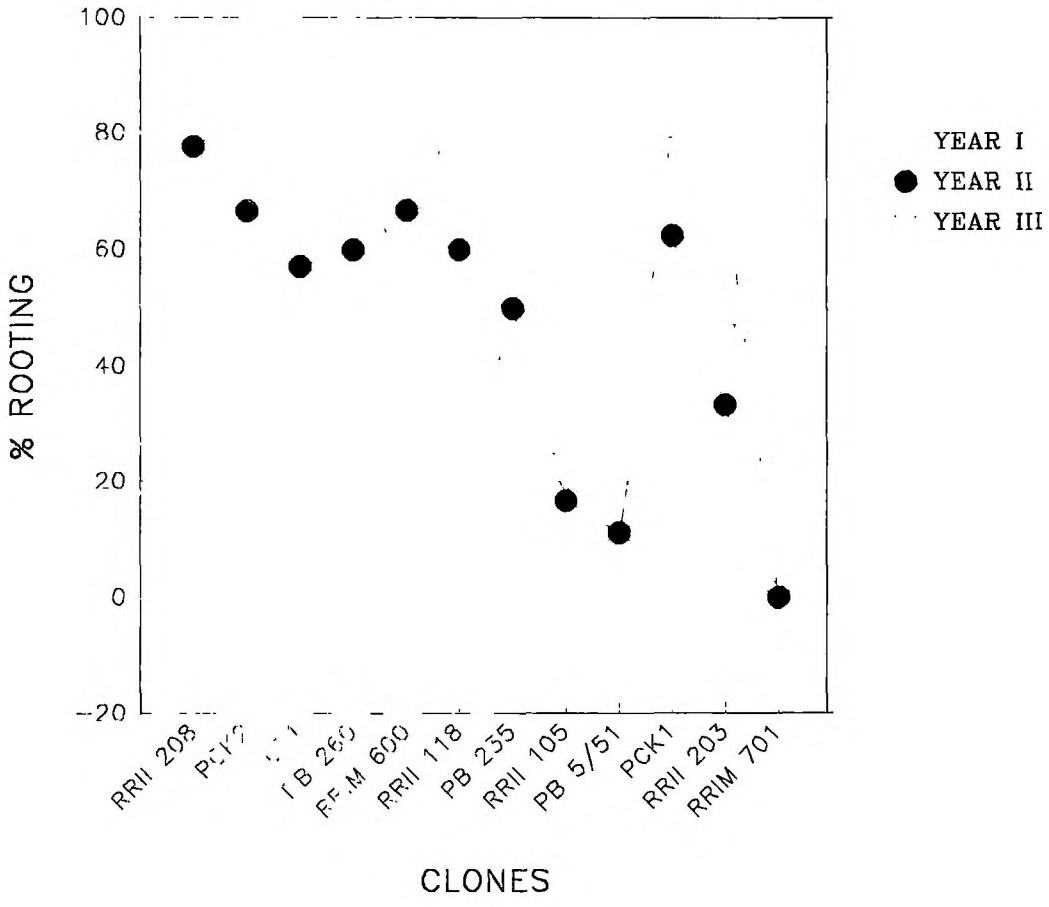

Fig:4. Effect of age on rooting percent of twelve clones of Hevea

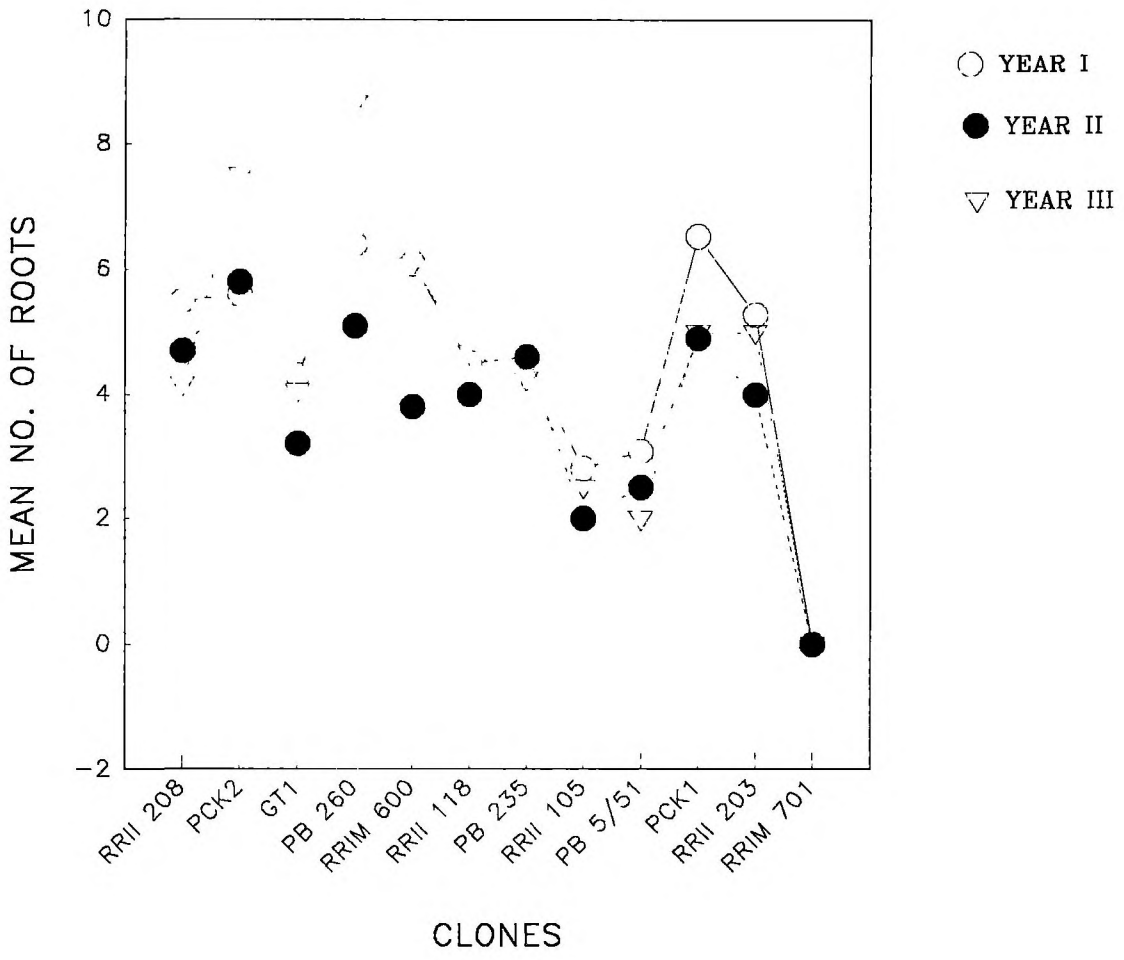

Fig: 5. Effect of age on mean no. of roots per layer in 12 clones of Hevea brasiliensis.

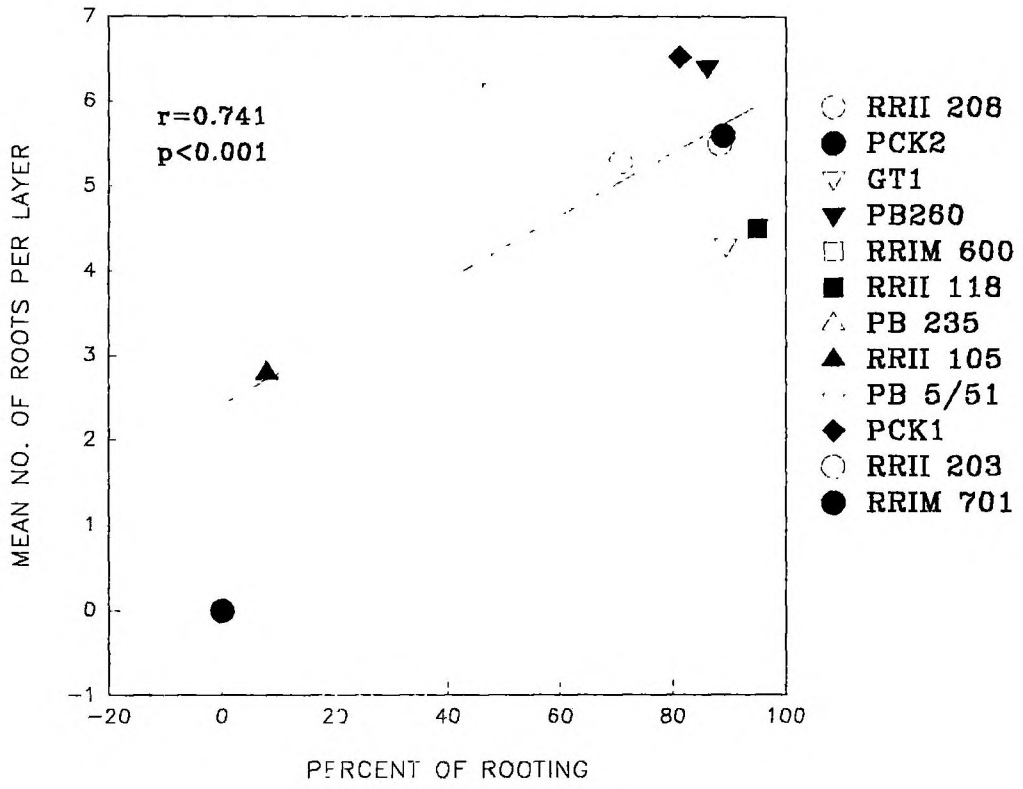

Fig: 6: Relationship between rooting percent and mean no. of roots per layer in twelve clones of Hevea

However, the mean number of roots produced per layer did not depend much on the age of the plant (Fig.5).

It is assumed that one of the reasons associated with variations in rootability in stems of different ages may be the differences in the quantity of endogenous phenolic compounds. The biochemical analysis of root regeneration in ringed shoot cuttings of cashew at different ages showed that rooting was governed not only by hydrolysable carbohydrates and nitrogen content but also by endogenous auxin and inhibitor levels (Rao and Satyanarayana, 1989). Davies (1984) reported that maximum rooting in juvenile materials of *Ficus pumila* was associated with the occurrence of higher vascular cambial activity and shoot RNA levels.

The percent rooting and mean number of roots per layer were compared in these clones. There was a strong positive correlation between percent of rooting and mean number of roots per layer in these clones (Fig.6). This indicates that favorable factors for root induction in these clones enhanced further root growth also. Moreover, the data on percent of rooting and survival of the rooted layers of these clones at all the three ages indicated a strong positive relationship between these two parameters (Fig.7). Likewise, a close relationship was also observed between survival of rooted layers and mean number of roots per layer (Fig.8).These findings further confirm the role of endogenous root inducing factors in root growth and establishment of rooted layers.

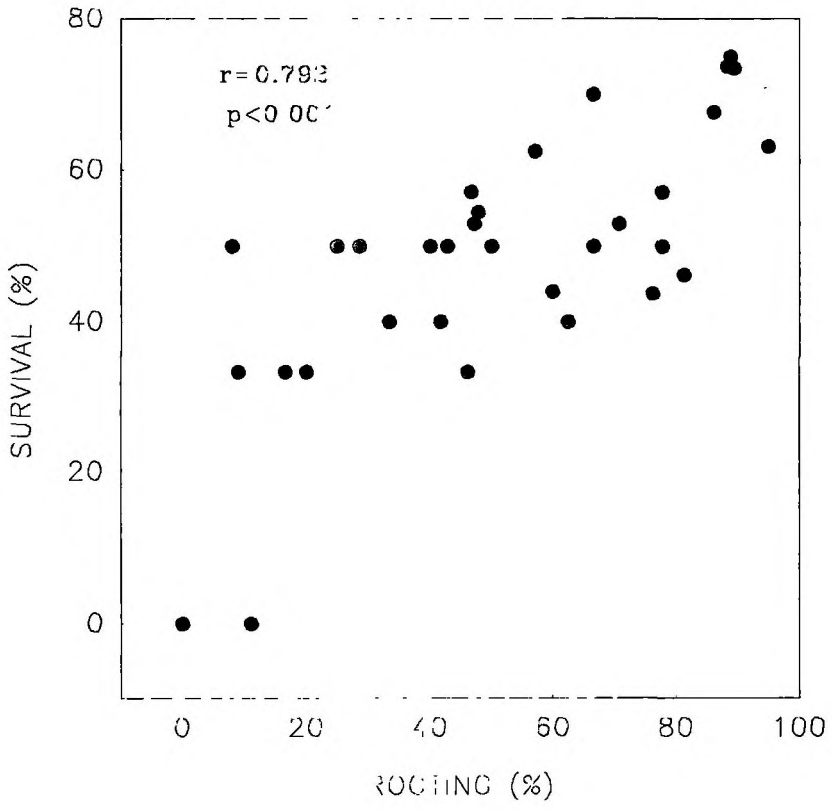

Fig: 7. Relationship between rooting percent and survival percent in twelve clones of Hevea

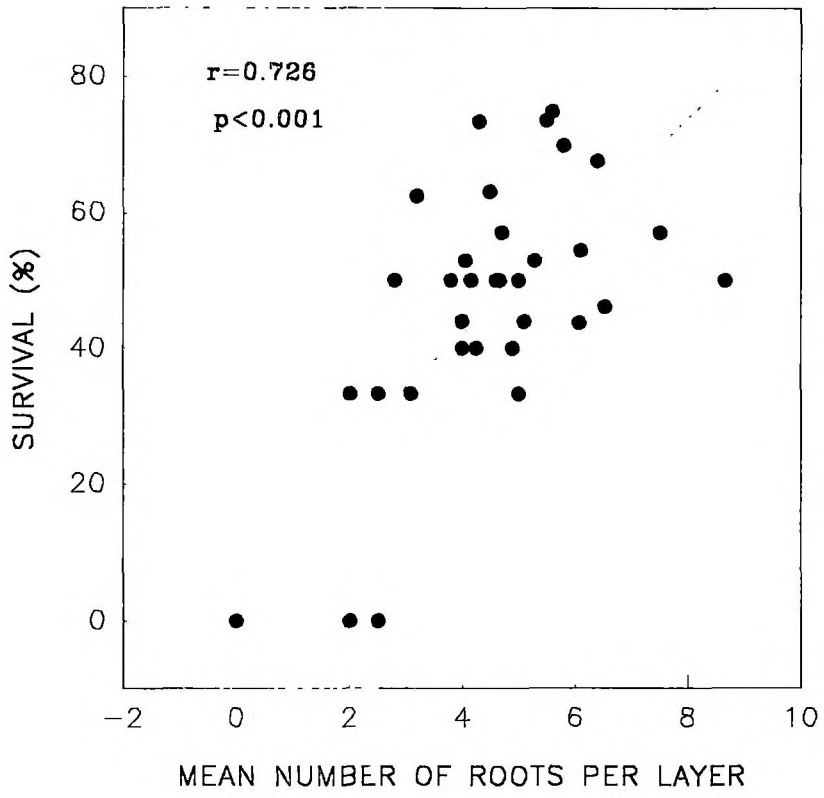

Fig :8.Relationship between mean number of roots per layer and survival percent in twelve clones of Hevea

Also it indicates that higher the number of roots the chance of survival was high. Hence profuse root development is important for success in propagation by layers in *Hevea.*

3.3.3 Biochemical studies

It is evident from the results of the biochemical analysis of bark samples showed that there is clonal difference in the biochemical composition of the bark. Mean percent of rooting of a clone and mean number of roots per layer were positively correlated with the concentration of reducing sugar and phenols in the bark (Figs.9&10). The levels of non-reducing sugars, starch and amino acids in the bark did not show any strong correlation either with the percent of rooting or mean number of roots (Figs.9&10).

The role of soluble carbohydrates, starch, phenols, amino acids, etc., on rooting was studied in many plant species (Stolz, 1968; Nanda and Anand, 1970). Considerable experimental evidences suggested a direct relationship between sugar content and root initiation. Biochemical characterisation of easy-to-root and difficult-to-root cultivars were tried in many species based on such relationships. In *Ficus elastica,* one of the earliest sources of natural rubber, the easy-to-root cultivar had more total, reducing and non-reducing sugars than difficult-to-root cultivar but starch content was higher in difficult-to-root cultivar (Kumar *et al.*,1985). Clones of *Hevea* belonging to easy-to-root and difficult-to-root categories used in the present study also exhibited the same trend.

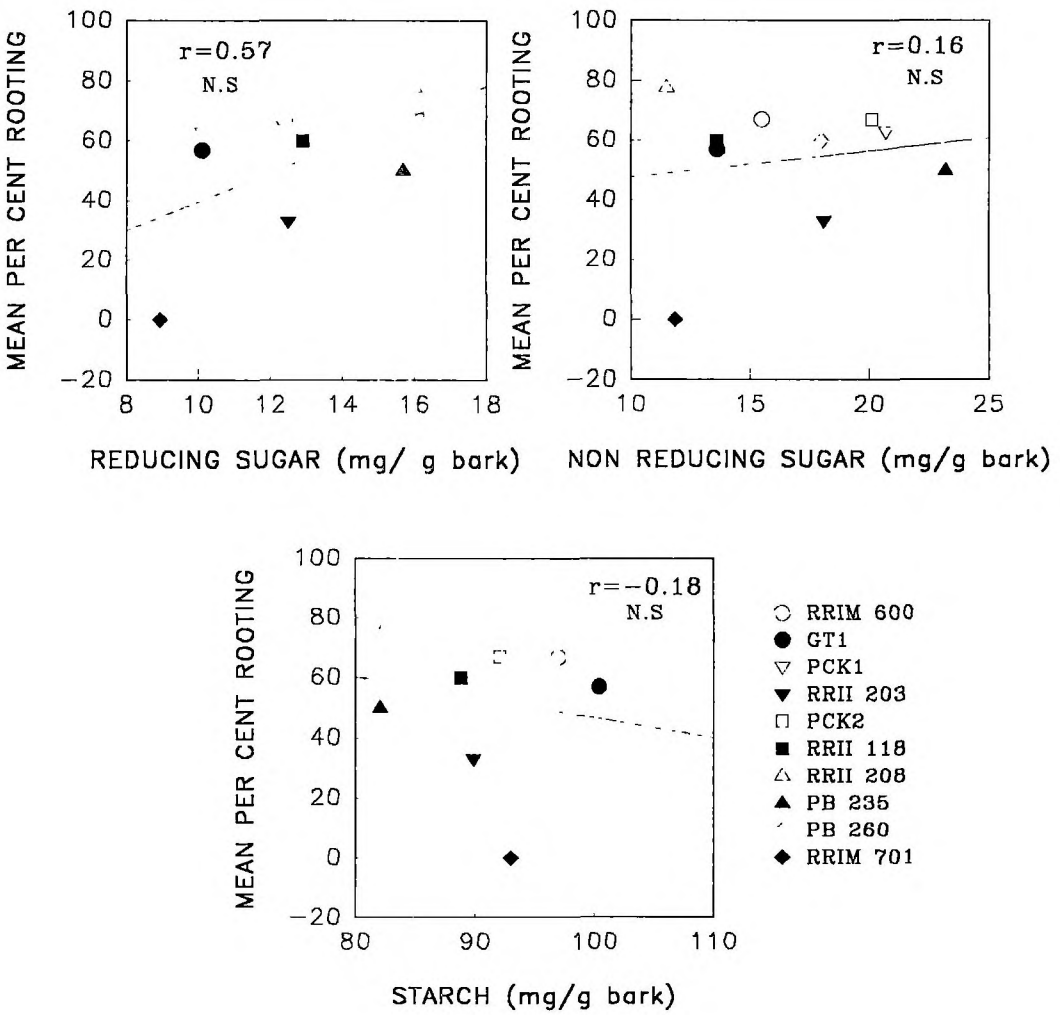

Fig 9. Relationship between reducing sugar, non-reducing sugar and starch with rooting percent in ten clones of Hevea

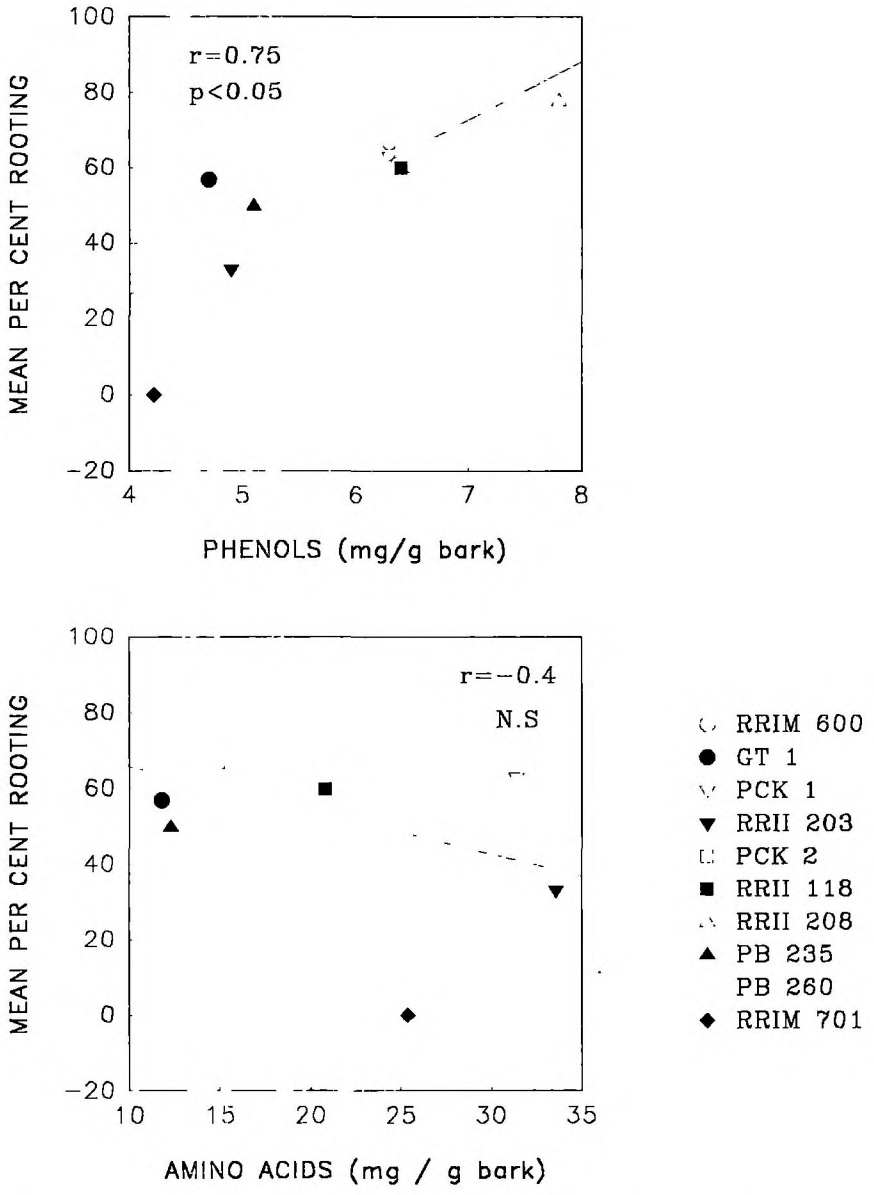

Fig:10. Relationship between phenols and amino acids with rooting percent of ten clones of Hevea

Though correlation between starch content and rooting percent was not strong there is an indication that it had a negative influence on rooting. Stolz (1968) reported higher levels of carbohydrates in easy-to-root cultivars of *Chrysanthemum* than in difficult-to-root cultivars and produced more number of roots. A close relationship existed between rooting and mobilization of reserve food materials. In *Populus nigra* the starch content in the cortical cells of the stem showed seasonal variations and low rooting corresponded with higher starch content and vice versa (Nanda and Anand, 1970). The availability and mobilization of carbohydrates towards the base of the cuttings appeared to be the major factor related to rooting (Rio *et al.*, 1991). On the contrary, high starch content was positively correlated with rooting in cuttings of *Rosa multiflora* (Hambrick *et al.,* 1991). The photosynthetic rates of cuttings seemed to reach near zero as soon as the cuttings are planted in rooting bed and remained at that level until roots appear (Couvillon, 1988). Hence it can be concluded that the carbohydrate level at the time of cutting was very important for rooting. No previous reports appear to be available in this aspect in girdled shoots. Since there is no interruption in the translocation of water and minerals to the shoot by girdling there may not be any drastic depletion in the production of carbohydrates. Moreover, the downward translocation of photosynthates and auxins was restricted in the girdled portion, there will be accumulation of auxins and carbohydrates at the distal edges. This in turn enhances rooting by providing the optimal conditions.

The total phenolic content and rooting percent showed a strong positive correlation in different clones of *Hevea* studied (Fig.10). Similar relationship was established in ringed shoot cuttings of cashew by Rao *et al.* (1989). Differences in rooting ability of different plant species may be due to the differences in the quantity of these phenolic compounds (Hartman and Kester, 1976). Phenolic compounds are known to act synergistically with auxins in promoting rooting and even the requirement of IBA was reduced to 80-90% by the application of phenolic compounds (Basu *et al.*, 1969; Pal *et al.*, 1996). This indicates that phenolic compounds play an important role in the process of rooting and adequate endogenous phenol content is essential for root induction. Higher amounts of phenols in the tissues combined with both endogenous and exogenous auxins resulted in the formation of a root forming substance (rhizocaline) inducing profuse rooting (Balakrishnamurthy and Rao, 1988).

Though the relationship between amino acids and rooting was not significant in the present study, a negative trend was observed as shown in (Fig.10). A negative correlation of amino acids with rooting was reported by Hambrick *et al.* (1991). A reduction in amino acids level during root induction was observed in many plant species. This depletion in various amino acids concentration implies a preferential utilization of the same in the rooting process (Suzuki and Kohno, 1983; Madhusudhanan, 1987).

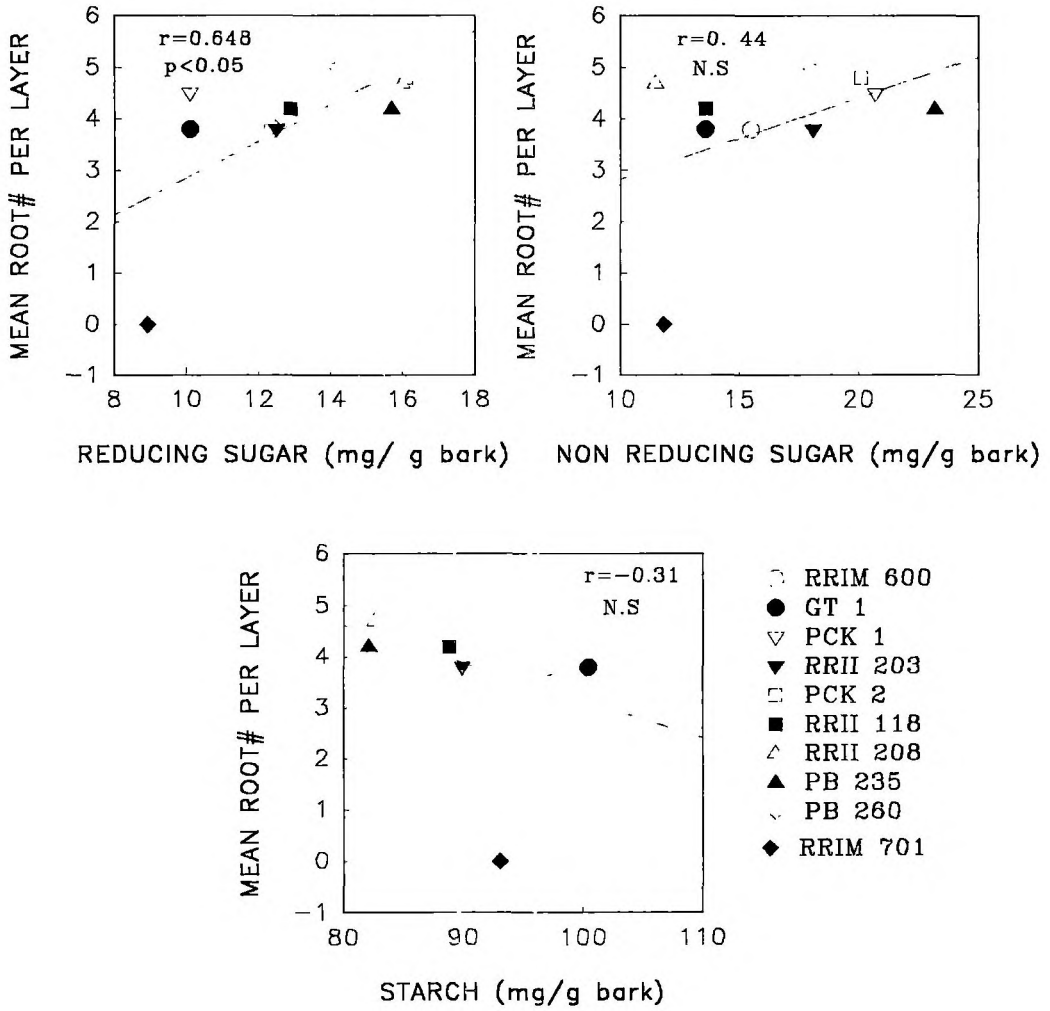

Fig:11. Relationship between reducing sugar, non–reducing sugar and starch with mean number of roots per layer in Hevea

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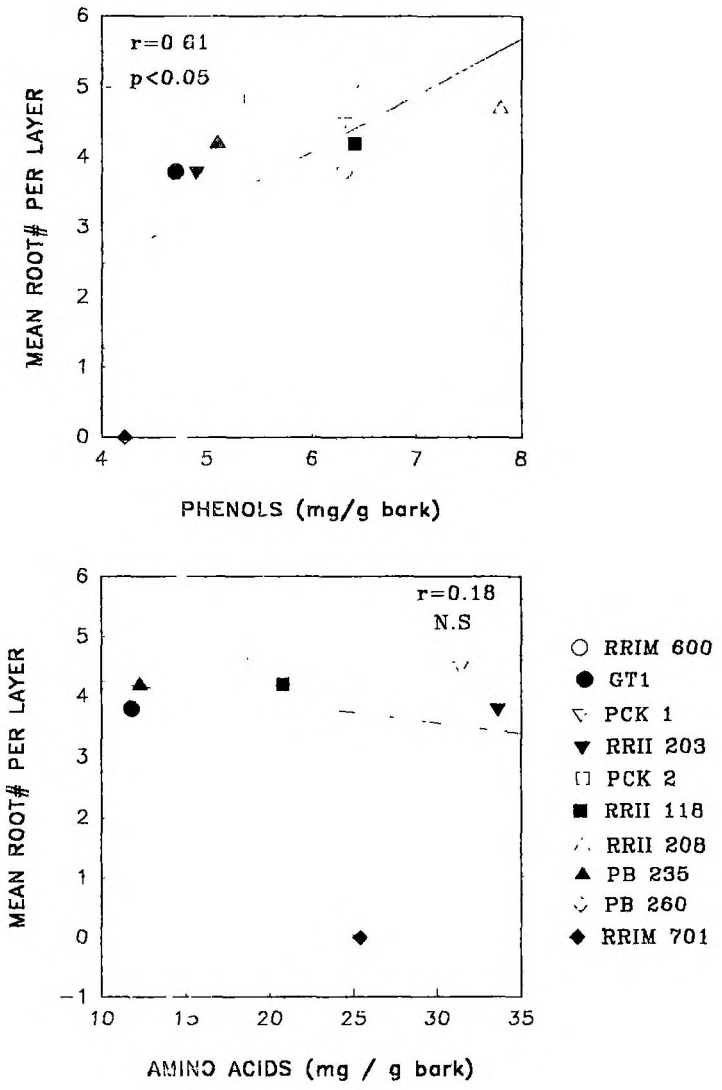


Fig 12. Relationship between phenols and amino acids with mean number of roots per layer in Hevea

Mean number of roots per layer and the above recorded biochemical factors in these clones, also showed the same relationship as that of the percent of rooting (Figs.11&12). This suggested that these factors inhibit or enhance not only the root induction but also influenced further root growth.

3.3.4 Effect of growth regulators on rooting

The results obtained were compared for the relative effects of different concentrations of IBA and NAA and Rootex on percent rooting and mean number of roots per layer. There was an appreciable increase in the percent rooting as well as the mean number of roots per layer with the application of rootex as compared with water (Figs.13&14).

Percent of rooting as well as mean number of roots per layer were depended on the concentration of IBA and NAA and the optimum concentration was different in different clones studied. Maximum rooting was observed in RRIM 600 and GT1 treated with 250ppm IBA (Fig. 15). A decline in the percent of rooting was recorded in 500 and 1000ppm of IBA in both these clones. In RRII 105, on the other hand, an increase in the rooting percent was noted in 500ppm IBA. Different concentrations of NAA except 1000ppm had no influence on rooting percent in GT1. Similarly, in RRIM 600 also there was not much variations in rooting percent with respect to differences in the concentrations of NAA. RRII 105, which was observed as a difficult-to-root clone , responded only with 250ppm NAA (Fig.17).

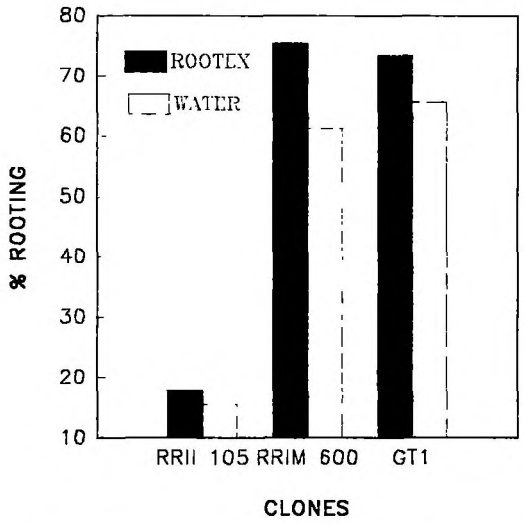


Fig:13. Effect of Rootex on rooting in three clones of Hevea brasiliensis

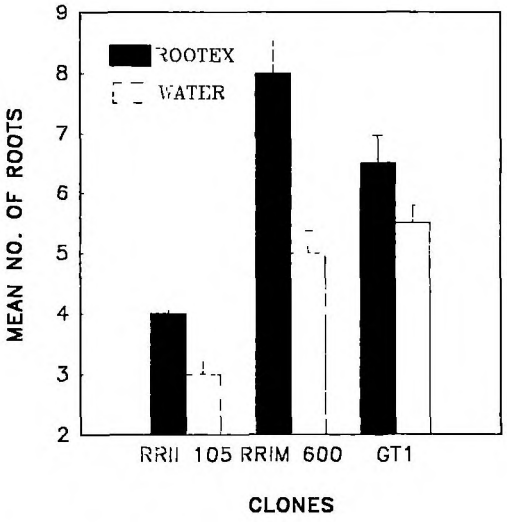


Fig 14. Effect of Rootex on mean number of roots per layer in three clones of Hevea

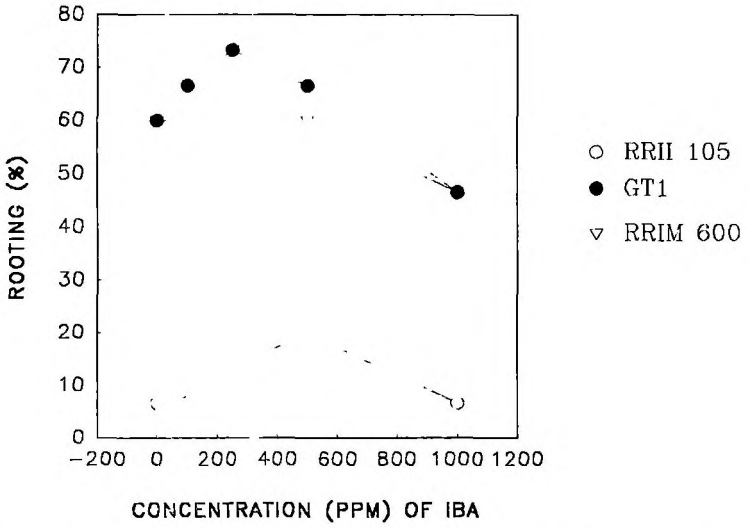


Fig :15 Effect of different concentrations of IBA on rooting percent of three clones of Hevea

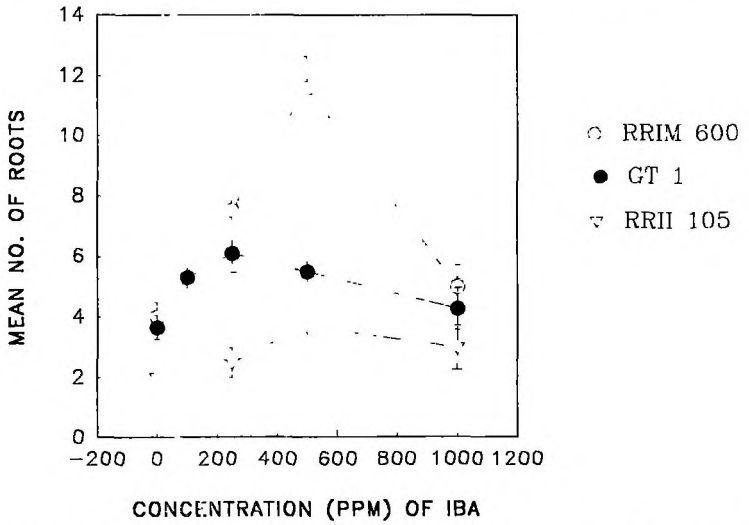


Fig 16. Effect of diffrent concentrations of IBA on mean number of roots per layer in three clones of Hevea

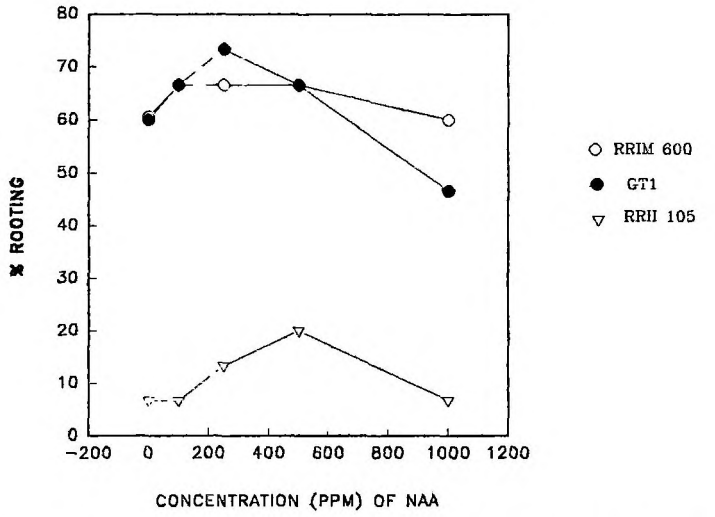


Fig 17. Effect of different concentrations of NAA on percent of rooting in three clones of Hevea

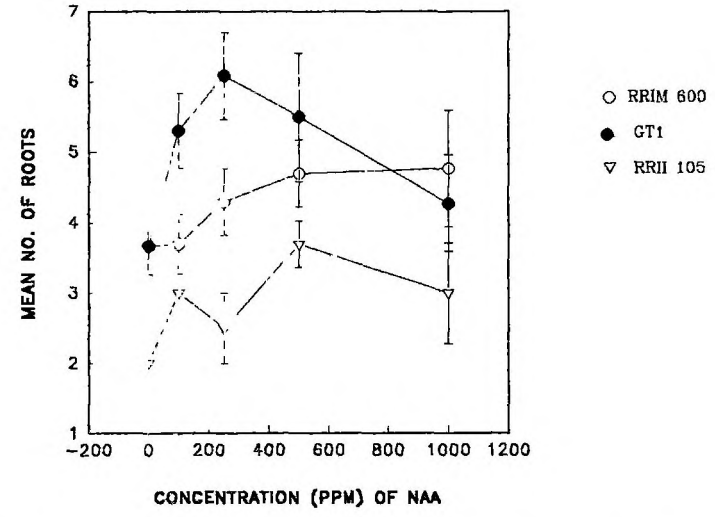


Fig 18. Effect of different concentrations of NAA on mean number of roots per layer in three clones of Hevea

The mean number of roots per layer also showed variations with respect to different concentrations of IBA and NAA. Maximum number of roots per layer was recorded in IBA treated layers of RRIM 600 (Fig.16). Effect of NAA on mean number of roots was also more pronounced in the clone RRIM 600 (Fig.18). In all these treatments both percent of rooting and mean number of roots per layer were lowest in RRII 105. Irrespective of the clones studied, highest concentration tried in the present study (1000ppm) was found to be inhibiting the percent of rooting as well as mean number of roots per layer.

Though all the three clones studied responded positively to the application of rootex, various levels of IBA and NAA the relative effectiveness varied in different clones. Tinley (1960) reported marked toxic effects of relatively low concentrations of IBA, NAA, 2,4-D and other growth substances in stem cuttings of *Hevea* and varying tolerance to these chemicals by different clones. Air-layering studies in other laticiferous plants like *Ficus elastica,* also showed enhanced rooting by IBA treatment (Rajagopal, 1993). Similar observations were reported by Sundaram and Rangasamy (1994) in *Ficus auriculata.* Couvillon (1988) reported that the response of the rooting hormones and the optimum concentrations in rooting of stem cuttings are species dependent. Application of auxins seems to elevate the level of endogenous auxins and expressed the full ability of rooting (Uniyal *et al.,* 1993). The inadequate endogenous level of auxins in RRII 105 may be one of the reasons for the higher requirement of exogenous auxins for optimum rooting than RRIM 600 and GT1. Treatments

with IBA and NAA above 500 ppm reduced the rooting percent in all the three clones which may be due to the supra optimal level of these chemicals . The auxin induced effect on rooting of cuttings was suggested as mediated through its effect in mobilising the food reserves by enhancing the activity of hydrolytic enzymes (Nanda *et al.*, 1968a).

Available literature indicate that starch stored in the tissues was rapidly converted to simpler transportable carbohydrates such as sugars by the application of auxins. A mobilization of starch reserve takes place and sugars accumulate in the treated region (Audus, 1953). An increased breakdown of starch to soluble sugars with auxin in the leaves, stem and roots of plants and increased accumulation of sugars at the cutting base by auxin were reported by Nanda and Anand (1970). The effect of auxin on sugar accumulation and basipetal transport of C^{14}-labelled assimilates in relation to root formation in *Phaseolus vulgaris* cuttings was studied by Altman and Wareing (1975). Increased sugar availability at the site of root formation was suggested as the major factor for high rooting ability by auxin treatment by many scientists (Singh, 1985).

Application of auxin has also been shown to stimulate cambial activity resulting in mobilization of reserve food materials to the site of root induction (Gurumurti *et al.*, 1984). A bimodal effect of auxin comprising of an initial meristematic locus formation leading to subsequent asymmetric division of the cells to a meristemoid and continued growth of this meristemoid to

root initiation was shown in stem cuttings of *Pinus radiata* (Smith and Thorpe, 1975). Addition of IBA and NAA enhanced initiation of cell division, callus formation and differentiation of root primordia in litchi air-layers (Sharfuddin and Husain, 1973).

One of the reasons for the enhancement of rooting by the application of auxins may be due to its synergistic effects with the endogenous phenolic compounds Basu *et al.* (1969).

Keeping the above facts in view, the differences in the relative effectiveness of these chemicals at various concentrations by different clones can be attributed to the endogenous availability of the factors , viz, auxins, phenols, carbohydrates, etc.

The increase in the mean number of roots per layer in treated layers may be due to the acceleration of the rate of initiation of root meristem and subsequent production of greater number of roots. Sharfuddin and Husain (1973) were of the opinion that application of growth regulators might have induced large areas of differentiated parenchyma, pericycle and endodermis tissue to differentiate into callus and redifferentiate this callus tissue into meristem and subsequently to greater number of roots in the air-layers.

Chapter 4

STOCK-SCION INTERACTION IN *HEVEA*

4.1 Introduction

Stock-scion interaction is a complex phenomenon exhibited among the budgrafted plants of several crop species. Various aspects of this phenomenon are described in details in Chapter 1.

Budgrafting, which is a well accepted practice of vegetative propagation has definite advantages, since this technique is an effective means of rapid multiplication of desired genotypes and is also easy to perform and economic. As mentioned in Chapter 1, by this technique two plants are joined together to form a new 'compound' plant having an upper portion known as

'scion' and a lower portion or root known as 'stock' or 'rootstock'. Scion is the desired genotype to be propagated and the stock is raised from the same species or closely related species / genus. Wider genetic differences between stock and scion could lead to incompatibility symptoms in the budgrafted plants. Moreover, compatibility between the two plants is a prerequisite for successful graft union which is essential for further growth and establishment. The seedling rootstocks raised from open pollinated seeds are heterozygous and hence budgrafting even within the same species may result in variations. The extent of these variations depends on the differences between these rootstock plants and the resultant effect may be due to either partner.

In *Hevea,* unselected seeds were used for raising planting materials in early periods. Later, seedlings from seeds of selected high yielding trees were used. But due to the high variability in yield among these seedling trees, steps were taken to propagate desirable genotypes vegetatively. Budgrafting, which is being employed nowadays for raising planting materials of *Hevea* throughout the rubber producing countries started with the introduction of brown budding by Van Helten in 1916 (Dijkman, 1951) (See section 1.2.1.4 for details).

Earlier studies on stock-scion interaction in *Hevea* are reviewed in Chapter 1. These studies gave emphasis to growth and yield of the budded

plants except a very few reports on mineral nutrition. Physiological and biochemical aspects of stock-scion interaction in *Hevea brasiliensis* were not reported so far and hence the present studies were carried out to evaluate the effect of stock / scion on these aspects in addition to the growth characteristics.

Experiments were conducted with the following objectives.

1. To find out the influence of stock / scion on growth characteristics viz, height, stem diameter, total number of leaves, leaf area, biomass, root:shoot ratio, etc.

2. To evaluate the effect of stock / scion on physiological aspects viz, Carbon dioxide exchange rate, gs, transpiration rate, mineral nutrition, etc.

3. To study the changes induced by stock / scion in some of the biochemical parameters viz, carbohydrates, amino acids, phenols, total chlorophyll, isozymes, proteins, etc.

4.2 Materials and method

Experiments on stock-scion interaction were carried out in five clones of *Hevea brasiliensis.* These clones were budgrafted on heterogeneous seedling rootstocks. Seedlings were raised from polyclonal seeds and these seedlings were grown in cement pots as described in Chapter 2. The five

clones selected for the experiment were RRII 105, RRII 208, RRIM 600, GT1 and Gl1. To avoid any possible plant to plant variation, while selecting buds for budding, buds were taken from a single budwood plant for each clone.

Eighteen months old seedlings, after recording the different growth parameters viz, height, stem diameter, total leaf area, etc., were budgrafted with the above five clones with replications ranging from seven to twelve plants per clone. The method adopted for budgrafting is given in details in Chapter 2. Shoot biomass of the stock plants was recorded by drying the above ground plant parts when the stock portion was cut off in successful graftings. Eighteen months after budgrafting the same parameters were recorded. Physiological parameters viz, carbon dioxide exchange rate, conductance, transpiration rate, etc. were recorded at 18 months after budding as described in Chapter 2. Then the plants were uprooted and fresh and dry weights of different plant parts were recorded. Mineral analysis of leaves before and after budding for estimating N, P, K, Ca, Mg, Fe and Mn was carried out as given in Chapter 2. Isozyme analysis of three enzymes viz, peroxidase, catalase and esterase and other biochemical analyses viz, reducing sugars, total sugars, phenols, amino acids, total chlorophyll, etc. were also done as described in Chapter 2.

4.3 Results and Discussion

4.3.1 Growth characteristics

4.3.1.1 Plant *height*

Plant height was recorded in eighteen months old polyclonal seedlings just before budding (rootstock) and budgrafted plants (scion) of five clones viz, GT1, RRIM 600, RRII 105, RRII 208 and Gl1 eighteen months after budding (five recordings at regular intervals). Highly significant positive correlation was observed between height of the rootstock before budding and height of the scion after budding ($r=0.71$, $p< 0.001$) as shown in Fig 19. Mean, CV and range of height of budgrafted plants of the above five clones after eighteen months of budding and height of polyclonal seedlings (18 months old unbudded) on which these clones were budgrafted are shown in Table 4. Considerable variation was observed among the plants within a clone in all the five clones studied (Table 4). In spite of the homogeneity of scion materials, (ie, each clone being propagated from a single mother plant) the high CV observed among the budgrafted plants gives a clear indication of the occurrence of rootstock influence. Moreover, it is interesting to note that high CV (23.3 and 24.5%) in the height of seedlings on which RRII 105 and Gl1 were budgrafted, showed similar high variations in the scion heights also (CV 25.4 and 24.6% respectively). Other clones also exhibited the same trend of CV in

Table 4. Mean, range and coefficient of variation of height of seedlings (before budding) and budgrafted plants after 18 months of budding in five clones of *Hevea*

Clones	Treatment	Mean (cm)	SE(±)	CV(%)	Range
GT1	Before budding	206	9.8	13.4	142 -226
	After budding	230	11.4	14.1	164 -265
RRIM 600	Before budding	178	8.0	11.9	143 -208
	After budding	205	8.1	10.2	165 -232
RRII 105	Before budding	193	13.6	23.3	122 -259
	After budding	264	20.4	25.4	152 -375
RRII 208	Before budding	201	9.2	12.9	153 -237
	After budding	220	13.4	17.3	178 -283
Gl1	Before budding	200	14.8	24.5	105 -277
	After budding	277	20.6	24.6	153 -387

height of both rootstocks and scions.

Gl1 showed the highest mean height followed by RRII 105 and GT1 and the lowest was RRIM 600. Clonewise comparisons at eighteen months after budgrafting showed that height of the budgrafted plants of RRIM 600 was significantly lower ($p < 0.05$) than RRII 105 and Gl1. RRII 208 also had significantly lower height than Gl1 ($p < 0.05$). No significant differences exist among other clones. There was no significant differences among the mean heights (five groups) of the unbudded plants on which these five clones were budgrafted.

Height at different stages of growth of the scion upto eighteen months of budgrafting in five clones are shown in Fig 24. It was found that clones having higher / lower heights at eighteen months were showing higher / lower values from initial stages onwards and the periodic increase was almost linear in all the five clones.

Height and girth are the two main parameters measured for assessing the growth vigour of plants in various experiments. Generally, during the early stages of growth in *Hevea,* height will be recorded and after about three years, recordings on height are discontinued and only stem diameter (girth) will be measured (Dijkman, 1951). A strong positive correlation between height of the rootstock (before budgrafting) and height of the scion in five clones (Fig.19) observed in the present study indicates the influence of rootstock on the height

observed in the present study indicates the influence of rootstock on the height of the budded plants.

It has been shown from earlier studies in various clones of *Hevea* that growth vigour is a genotypic character (Dijkman, 1951; Goncalves *et al.*, 1994). In the present study also significant differences in height were observed among clones though the mean heights of their corresponding stock plants were not significantly different. The significant differences in height among the clones and the CV exhibited among the plants within clones (Table 4) indicates that height is a clonal character and at the same time it is influenced by the rootstock also.

The different clones showing high / low mean values of height at all stages of growth from three months of sprouting onwards in the present study (Fig.24) further confirmed the genotypic nature of this trait.

It is well documented that dwarfing rootstocks retard the vegetative growth especially in fruit crops like apple, peaches, etc. (Ferree and Carlson, 1987). In *Hevea* also interstocks of dwarfing clones and *Hevea spruceana* affected scion growth (Leong and Yoon, 1978). The influence of auxin translocated down the phloem to the roots and cytokinins synthesised and translocated to the shoot was attributed as the mechanism involved in dwarfing (Lockard and Schneider, 1981).

4.3.1.2 *Stem diameter*

Recordings of stem diameter were also taken in eighteen months old polyclonal seedlings (rootstock) just before budgrafting and budgrafted plants of five clones at the time of height measurements. It was observed that stem diameter of scion after eighteen months of budgrafting showed a significant positive correlation ($r=0.35$, $p< 0.05$) with the stem diameter of seedlings at the time of budgrafting (Fig 20). Mean, CV and range of the stem diameter of seedlings before budgrafting and after eighteen months of budgrafting with five clones are shown in Table 5. Stem diameter also showed somewhat similar CV as that of height among the plants within clones.

Clonewise comparisons of scion diameter in the five clones showed significant differences between clones. RRII 105 had significantly high ($p< 0.05$) stem diameter than GT1, RRIM 600 and RRII 208. Also, stem diameter of Gl1 was significantly high ($p < 0.05$) when compared with RRIM 600 and GT1. Recordings of scion stem diameter at different stages of growth from three months of sprouting after budgrafting upto eighteen months in five clones are shown in Fig.24. It was observed that stem diameter also exhibited the same trend as that of height of the plants at different stages and shows a linear increase.

Table 5. Mean, range and coefficient of variation of stem diameter of seedlings (before budding) and budgrafted plants after 18 months of budding in five clones of *Hevea brasiliensis*

Clone	Treatment	Mean (cm)	SE(±)	CV(%)	Range
GT1	Before budding	3.5	0.19	14.3	2.5 - 4.3
	After budding	3.2	0.13	10.9	2.5 - 3.6
RRIM 600	Before budding	3.1	0.15	12.9	2.5 - 3.5
	After budding	3.1	0.16	12.9	2.8 - 4.0
RRII 105	Before budding	3.7	0.24	21.3	2.3 - 4.5
	After budding	4.0	0.20	16.5	3.0 - 4.9
RRII 208	Before budding	3.9	0.20	14.9	2.8 - 4.5
	After budding	3.5	0.15	12.4	3.1 - 4.2
Gl1	Before budding	3.5	0.16	14.4	2.8 - 4.3
	After budding	4.0	0.16	13.8	3.0 - 4.9

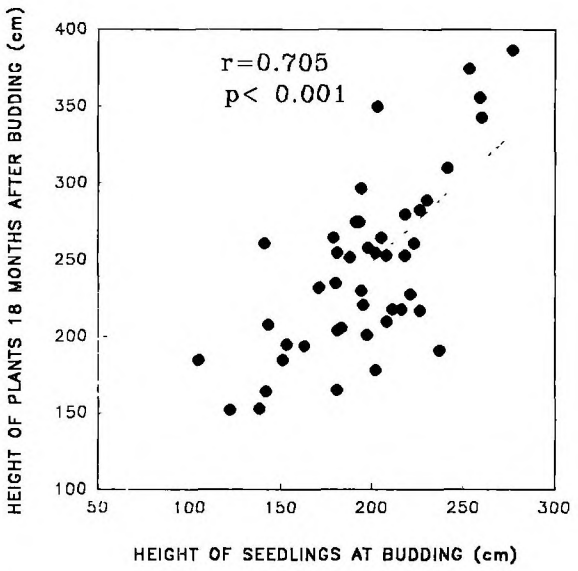


Fig: 19. Relationship between height of the plants before and after budding in five clones of Hevea

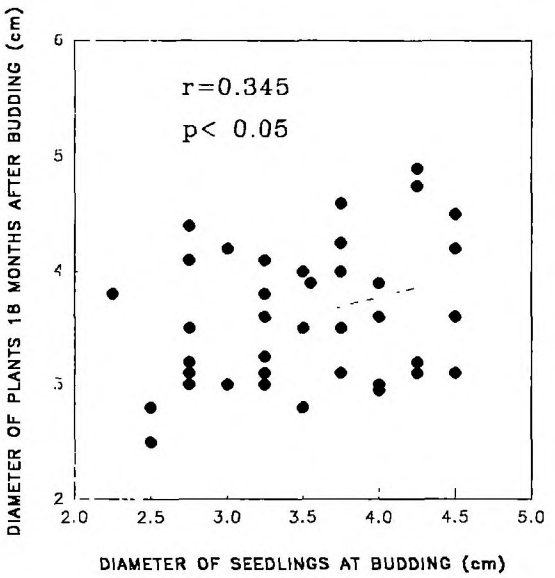


Fig:20. Relationship between stem diameter before and after budding in five clones of Hevea

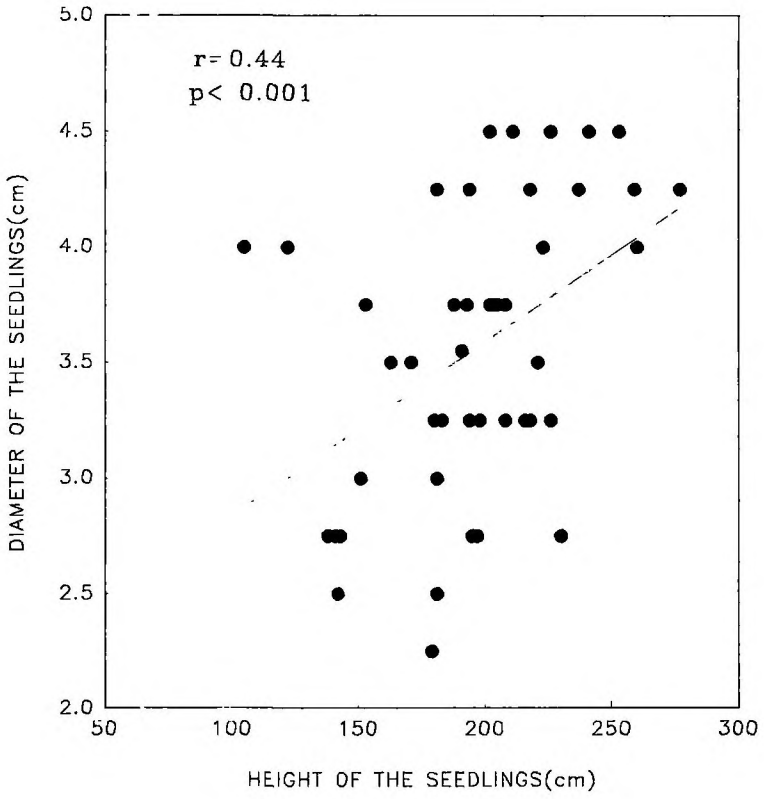


Fig:21.Relatioship between height and diameter in seedlings before budgrafting in Hevea brasiliensis

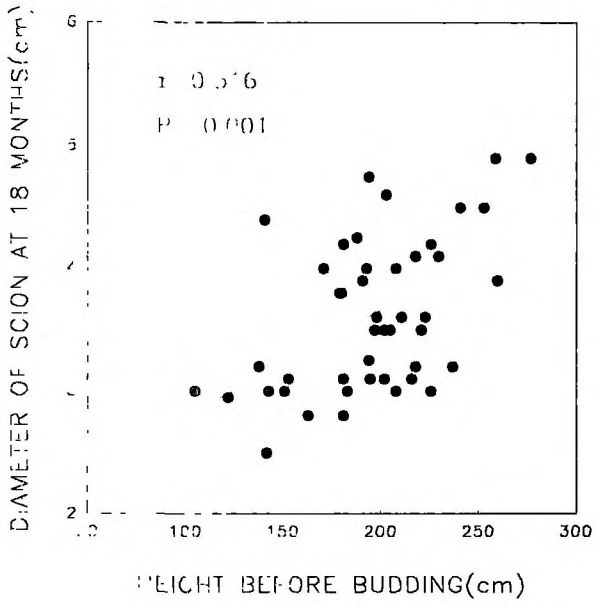


Fig:22. Relationship between height of the seedlings before budding with diameter of scion iin five clones of Hevea

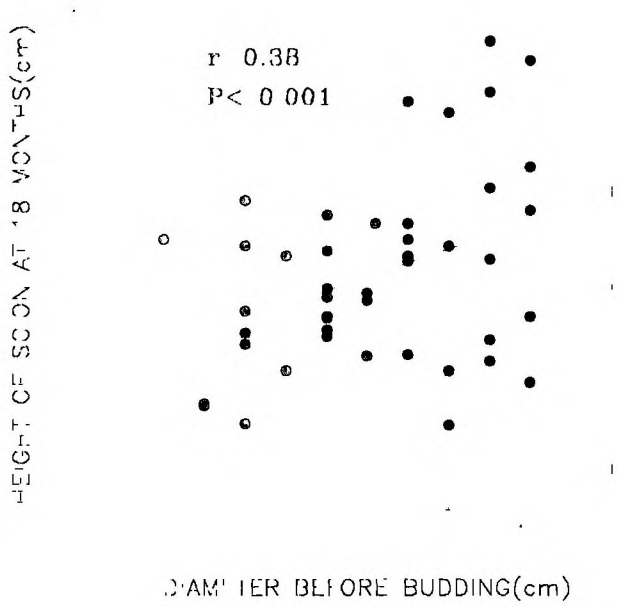


Fig. 23 Relationship between diameter of seedlings before budding with height of scion in five clones of Hevea

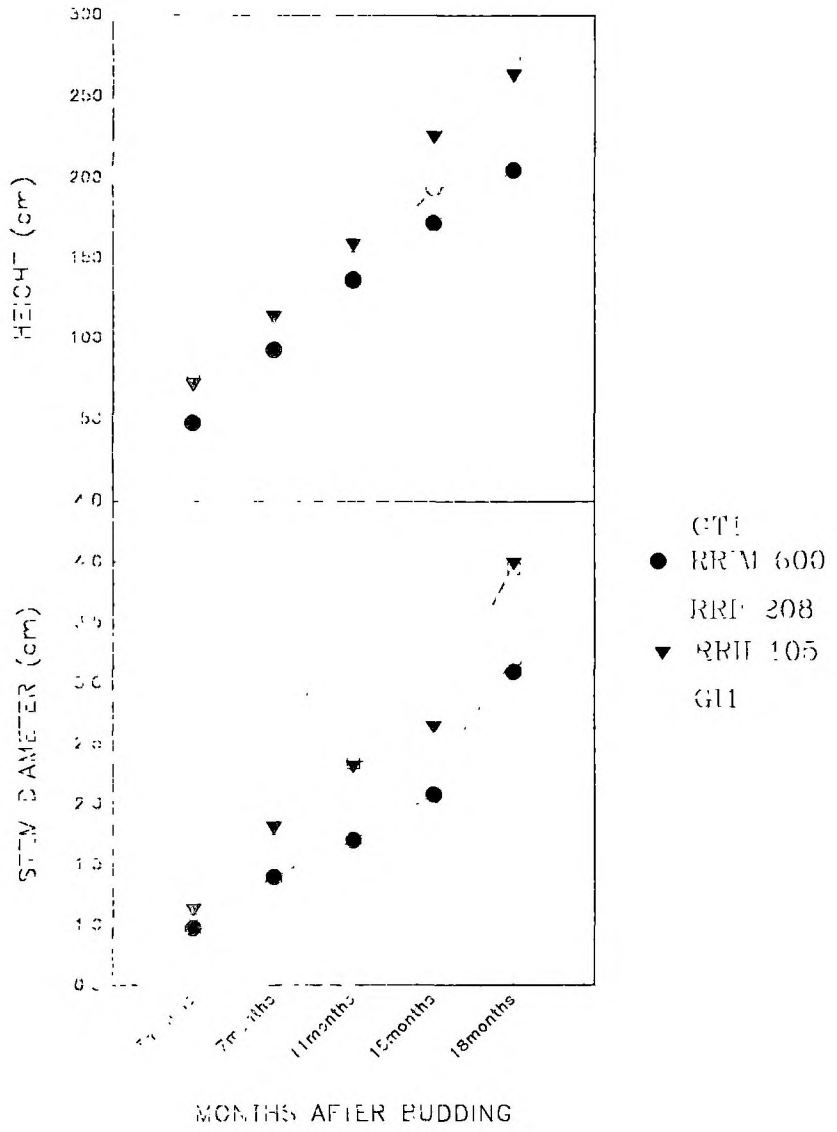


Fig 24: Height and stem diameter at different stages of growth after budding in five clones of Hevea

Significant positive correlation existed between stem diameter of the rootstock at budgrafting and the scion height at eighteen months after budgrafting in the five clones ($r=0.38$, $p < 0.001$) as shown in Fig 23. Height of the rootstock plants at budgrafting also showed a significant positive relationship ($r=0.52$, $p < 0.001$) with the diameter of the scion after eighteen months of budgrafting (Fig 22). Significant positive correlation ($r=0.44$, $p<0.001$) between height and stem diameter of the plants (unbudded seedlings) observed in the present study (Fig 21) is in agreement with earlier reports by Jayasekhara and Senanayake (1971). Hence stem diameter / girth can be considered as a suitable parameter to measure growth vigour as suggested earlier by the above authors.

The significant differences in stem diameter among the different clones observed in the present study indicate that like height this trait also is genetically controlled. The variations noticed among plants within a clone revealed the influence of stock plants.

The relationship between height of the plants before budding and diameter of plants (scion) after budding and diameter before budding and height after budding indicate that growth vigour of the rootstocks (both height and diameter) influence the scion growth vigour (height and diameter). Influence of rootstock on growth vigour of scion in *Hevea* was reported earlier (Buttery, 1961; Ng *et al.*, 1981; Seneviratne *et al.*, 1996). Tiang (1989) showed that there was a definite positive relationship between rootstock diameter and

scion diameter in *Hevea*. Moreover, this influence on scion diameter was reported to be maintained throughout the recordings upto sixty months after planting. This has resulted in early tappability in plants budgrafted on bigger rootstock diameter classes. Hence culling of rootstocks was suggested by him as a useful practice to improve growth and yield of grafted rubber plants. In addition, uniformity among the grafted plants was also expected from this.

4.3.1.3 *Leaf area per plant*

Total and mean leaf area of the plants at budding and 18 months after budding with five clones were compared and the results are shown in Fig 25. Total leaf area of the plants before budding showed a significant positive correlation ($r=0.28$, $p < 0.05$) with that of scion indicating the influence of stock on this parameter. Moreover, clonewise comparisons of total leaf area in these five clones indicated that there were no significant differences among these clones. Rootstock effect on total leaf area of budded plants was reported in other plants. Influence of stock on total leaf area in terms of area of plucking surface and leaf yielding capacity of the budgrafted plants of tea was reported by Haridas *et al.* (1992). In egg plants also, scion leaf area was reported to be influenced by the rootstock (Shishido *et al.*, 1995). Total leaf area also is considered as one of the parameters of growth vigour in plants. The present result further supports the influence of stock plants on scion vigour. However, mean leaf area of the plants before budding is not showing any relationship with that of the scion 18 months after budding in any of the five

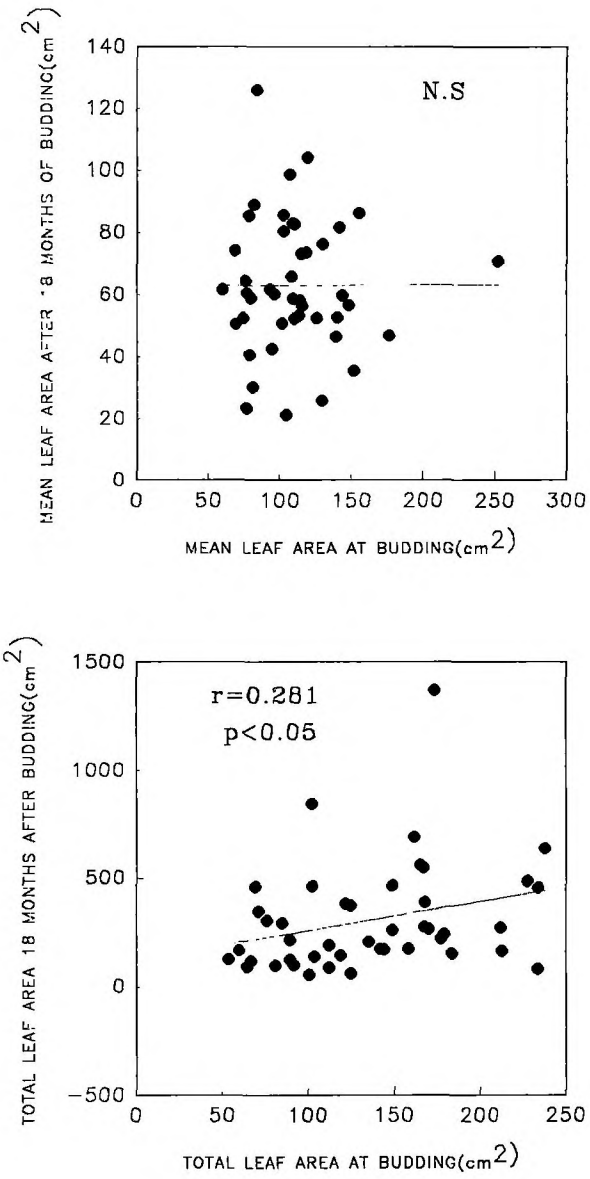


Fig.25: Relationship between total and mean leaf area before and after budding in 5 clones of Hevea

clones studied (Fig 25). Clonewise also no significant differences were observed in mean leaf area among the five clones except between GT1 and RRII 208.

Specific leaf weight of the rootstock and scion showed no significant relationship.

4.3.1.4 *Number of leaves per plant*

No significant relationship existed between total number of leaves before and after budding in the clones studied (Fig 26). This indicates that stock has no effect on total number of leaves of the scion. Moreover, no significant differences were observed on total number of leaves among these five clones in the present study.

4.3.1.5 *Shoot biomass per plant*

Above ground biomass of the plants before budding (18 months old) and after 18 months of budding with five clones are shown in Table 6. The shoot biomass in the budgrafted plants of the five clones showed high variations within clones and among clones. The lowest shoot biomass of 322g / plant was recorded in RRIM 600 and the highest of 1247g / plant in RRII 105 (Table 6). Interclonal comparison showed that shoot biomass of RRIM 600 was significantly lower than that of RRII 105 and Gl1. No significant differences were observed among other clones. The shoot biomass means of the stock of these five clones did not show any significant differences in the present observations. Except RRIM 600 and RRII 208, the coefficient of variation among the budgrafted plants were higher than their corresponding

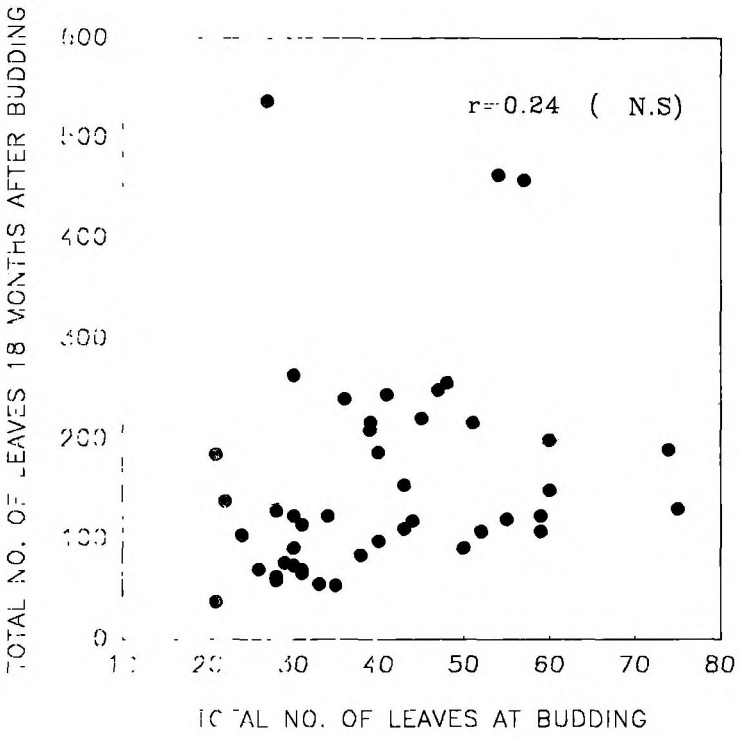


Fig 26 Relationship between total no. of leaves before and after budding in five clones of Hevea

Table 6. Mean and coefficient of variation of above ground biomass in seedlings (unbudded) and budgrafted plants after 18 months of budding with five clones of *Hevea*

Clone	Before budding (Seedlings)		After budding	
	Mean (g)	CV(%)	Mean (g)	CV(%)
GT1	161	35	803	78
RRIM 600	184	50	322	32
RRII 208	170	72	644	65
RRII 105	173	44	1247	71
Gl1	174	44	856	77

unbudded plants. Regression analysis of the above ground biomass of plants before budding (18 months old) and after 18 months of budgrafting with five clones of *Hevea* is shown in Fig 27. The results showed a strong positive relationship ($r=0.53$, $p < 0.001$) indicating the influence of stock plants on scion shoot biomass.

Shoot biomass, in general, is the cumulative effect of the growth vigour of the plants. Hence the positive relationship between shoot biomass of the stock and the scion further confirms the strong influence of stock on scion growth vigour. Clonal differences observed in the shoot biomass and high CV among the plants within a clone showed that this is a clonal character and at the same time being influenced by the rootstocks. Moreover, the higher CV observed in the

shoot biomass of the budgrafted plants of RRII 105, Gl1 and GT1 than their corresponding unbudded plants indicates the existence of stock-scion interaction also in addition to the stock influence.

The occurrence of stock-scion interaction on growth vigour, observed in the present study is in conformity with earlier report by Buttery (1961) in *Hevea* on trunk girth. On the contrary, Ng *et al.* (1981) reported that in *Hevea*, though rootstock influenced the growth and yield of scion significantly, there was no stock-scion interaction.

In temperate tree crops, Rogers and Beakbane (1957) reported a strong influence of rootstock than the scion on the budded plants. But Vyvyan (1955) (Hartman and Kester,1976) showed that in fully compatible combinations the rate of growth of the two-part tree might be the resultant of the growth rates of the two components. In other words, stock-scion interaction exists even in compatible combinations.

4.3.1.6 *Root growth*

Root biomass was determined in 18 months old budgrafted plants of the five clones. Considerable variation in root growth existed among the five clones and the results are shown in Table 7.

Highest tap root weight was noticed in RRII 105 and lowest in RRIM 600. Clonewise comparisons of the growth of tap root in the five clones showed that both fresh and dry weight of the tap root of RRIM 600 were significantly lower than that of GT1 ($p < 0.05$) and RRII 105 and Gl1 ($p<0.001$).

No significant differences existed among other clones. In the case of lateral roots, GT1 had a significantly higher ($p < 0.05$) lateral root growth (both fresh and dry weights) than RRII 208 and Gl1.

Significant differences observed in the root growth of different clones in the present study indicate that root growth is genetically controlled and is a clonal character. No other reports except that of Sobhana *et al.* (1980) was

Table 7. Fresh and dry weights of tap roots and lateral roots in 18 months old budgrafted plants of five clones of *Hevea brasiliensis*

Clones	Tap root		Lateral root	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
GT1	1106	562	153	64
RRIM 600	470	207	101	29
RRII 105	1524	729	101	44
RRII 208	868	404	61	18
Gl1	1212	608	77	28

available on stock / scion effect on root growth of budgrafted plants in *Hevea.* The observations of significantly low root growth in RRIM 600 and higher root growth in GT1 in the present study are in agreement with the earlier studies in four clones. The results of the present study showed that seedlings budded with RRII 105 showed highest rootgrowth in terms of both fresh and dry weight of tap root followed by Gl1 and GT1. Likewise, GT1 showed the highest lateral root growth when compared with other clones. These observations indicate that scion controls the root growth of the budgrafted plants. Scion influence on root

growth was reported earlier in other plants (Hartman and Kester, 1976). They reported that the rootsystem of the seedlings rootstock in apple was influenced by the scion variety. But the morphological characters of the rootsystem of vegetatively propagated clonal rootstocks seemed to be not affected by the scion varieties except the quantity of roots.

4.3.1.7 *Root : shoot ratio*

Root : shoot ratio in 18 months old budgrafted plants of five clones are shown in Table 8. The results indicate that there was no significant differences in the root : shoot ratio of the five clones. The mean values of root : shoot ratio in the five clones ranged from 0.71 to 0.94.

The results of the analysis of root and shoot weights of these clones separately indicated the existence of clonal variations in these two parameters (Tables 6 & 7). But the differences were not reflected in the root : shoot ratio of the same plants. Pattern of root and shoot growth (expressed as dry weights (g)) in the five clones are shown in Fig. 28. Both root and shoot growth show the same trend in all these clones. RRIM 600 recorded the lowest root and shoot growth while RRII 105 showed the highest. In other words, the root : shoot ratio is maintained in all the clones irrespective of the higher or lower root / shoot growth.

However, high CV (ranging from 32 to 50%) was observed among plants of all the five clones studied. In spite of the homogeneity of the scion materials of each clone, high CV observed in the root : shoot ratio in the present

Table 8. Mean, range and coefficient of variation of root : shoot ratio in budded plants of five clones of *Hevea* after 18 months of budgrafting

Clone	Mean	CV(%)	Range
GT1	0.94	39.4	0.5 - 1.5
RRIM 600	0.83	50.3	0.3 - 1.5
RRII 105	0.72	36.1	0.5 - 1.0
RRII 208	0.71	32.4	0.4 - 1.3
Gl1	0.86	40.7	0.4 - 1.5

study may be due to the stock-scion interaction.

Studies on root shoot balance of *Hevea* planting materials by Othman *et al.* (1988) suggested that root : shoot ratios were stable and characteristic for each technique especially after plants had reached 40% of their maximum heights. They concluded that root : shoot and root weight ratios can be used to predict rootstock effects and to select rootstocks in *Hevea.*

The development of the rootsystem in young rubber trees in relation to shoot development was reported by Thaler and Pages (1996). Results of their studies indicated that rootgrowth is related to competition for assimilates and sink strength of different root types and leaf development promotes root branching. This also showed that the growth pattern of shoot and root follows same trend which in turn can maintain root : shoot ratio.

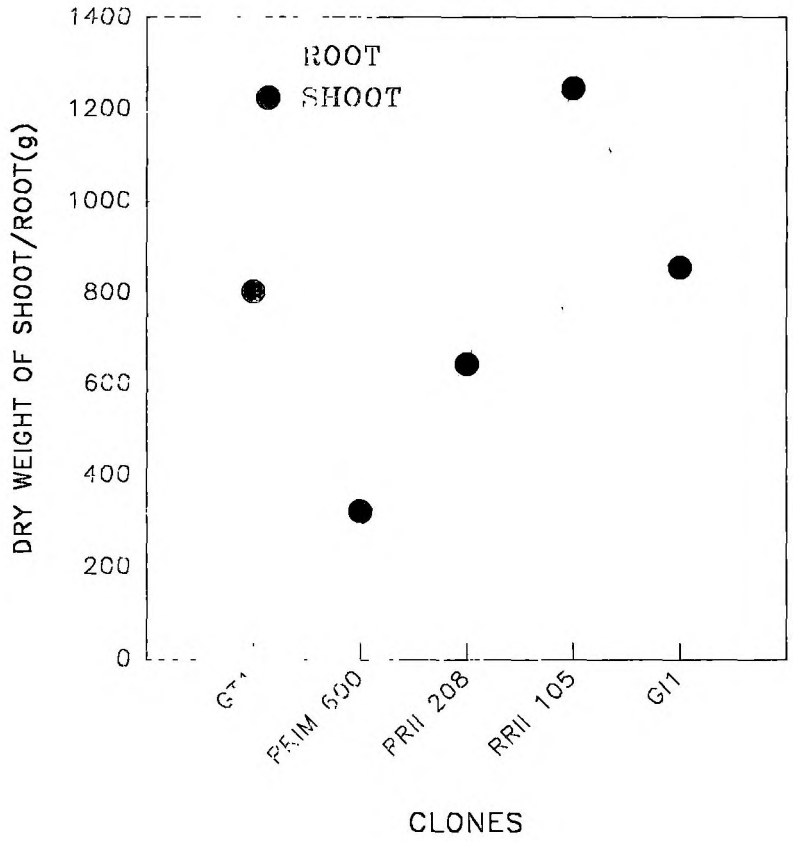


Fig: 28 Dry weight of root and shoot in five clones of Hevea brasiliensis

Studies on root distribution of nine apple rootstocks revealed a positive correlation between number of roots and scion vigour (Fernandez *et al.*, 1995). Shoot biomass can be considered as a measure of growth vigour of plants and hence the above finding supported our observations that when root growth was high shoot biomass will also be high.

A mutual dependency of rootsystem and shootsystem was reported to be involved in the optimal growth requirements of plants by Skene (1975). Roots absorb essential mineral elements, water, etc. and produce hormones like cytokinins and supply them to shoot and shoot in turn will provide the required carbohydrates and hormones like auxins, gibberellins, etc. to the roots. Hence the relative sizes of the root system and shoot system are very crucial for proper growth and functioning of the plants. On the other hand, loss of water from the plants was emphasised as the important function of the root : shoot ratio (Parker, 1949). According to his reports an increase in root : shoot ratio results in increased transpiration rate. Development of more secondary roots in *Sorghum* provides more water to shoot thereby increasing the transpiration rate (Delvin and Witham, 1986). Usually different species possess characteristic root : shoot ratios unless they are disturbed by certain environmental factors or cultural treatments (Kramer and Kozlowsky, 1979).

4.3.2 Physiological parameters

4.3.2.1 *Rate of Carbon dioxide exchange (CER) by leaves*

CER of 18 months old budgrafted plants of five clones and their

corresponding rootstocks just before budding (18 months old) was recorded and summarised in Table 9. Mean and CV of the above recordings as shown in the Table 9, revealed considerable variations among clones and within clones.

RRII 208 showed the lowest CER among the five clones studied and was significantly lower than that of RRIM 600 and RRII 105 ($p < 0.05$). When Gl1 and RRII 105 were compared CER of Gl1 was significantly lower than RRII 105 ($p < 0.05$). No significant differences existed between other clones. The lowest CV was observed among plants of RRII 208 (9.8%) and the highest in RRIM 600 (27.6%). Other clones also exhibited considerable variations. The CV was narrowed considerably in budgrafted plants of RRII 208 than their corresponding stock plants before budding (from 26.3 to 9.8%).

Table 9. Mean and coefficient of variation of CER in seedlings (before budding) and budgrafted plants after 18 months of budding with five clones of *Hevea*

Clones	CER (μ mol $m^{-2} s^{-1}$)			
	Before budding (Seedlings)		After budding	
	Mean	CV(%)	Mean	CV(%)
GT1	8.8	18.2	9.6	27.1
RRIM 600	10.6	31.0	13.4	27.6
RRII 105	9.3	16.8	13.1	24.4
RRII 208	8.3	26.3	8.4	9.8
Gl1	8.8	30.7	10.5	20.7

Regression analysis of CER (Fig 29) before budding and after budding in five clones of *Hevea* studied showed a strong positive relationship (r=0.74, $p < 0.001$). This indicated that rootstock exerts a strong influence onCER of the scion. Moreover, considerably high CV observed among the plants within a clone except in RRII 208, indicates the stock influence. However, significant clonal differences noticed among the five clones suggested that CER is a clonal character. Hence from the above findings CER is seemed to be a clonal character and alsc being influenced by the rootstock.

4.3.2.2 *Stomatal Conductance (gs)*

Mean and covariance of gs in seedlings (before budding) and budgrafted plants after 18 months of budding in five clones are shown in Table 10. Stomatal conductance before budding did not vary much among the five groups

Table 10. Mean and coefficient of variation of gs in seedlings (before budding) and budgrafted plants after 18 monthsof budding with five clones of *Hevea*

Clones	gs			
	Before budding		After budding	
	Mean	CV(%)	Mean	CV(%)
GT1	0.11	27	0.31	77
RRIM 600	0.13	39	0.69	100
RRII 105	0.12	42	0.66	41
RRII 208	0.12	50	0.28	89
Gl1	0.14	37	0.17	54

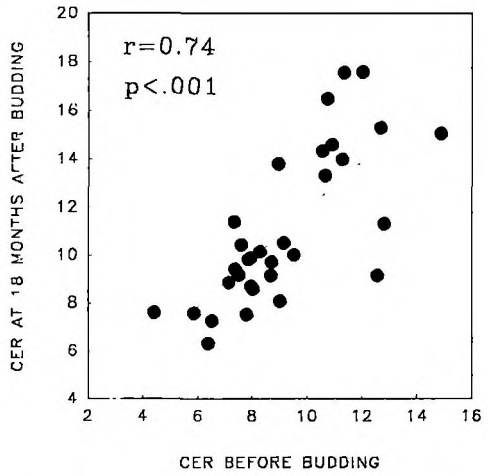


Fig 29. Relationship between CER before and after budding in five clones of Hevea

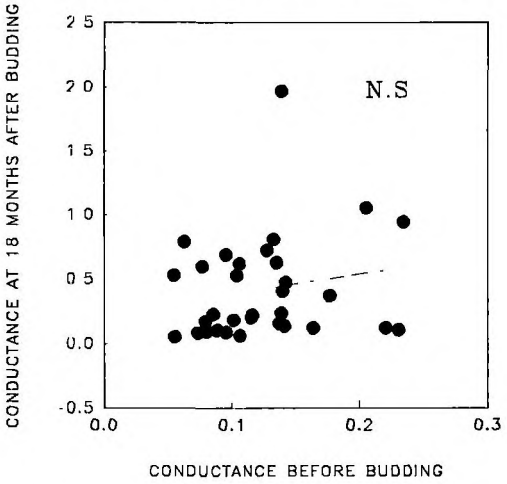


Fig 30. Relationship between gs before and after budding in five clones of Hevea

of seedlings on which the five clones were budgrafted. But significant differences existed among the budgrafted plants of these clones. A higher CV when compared to their corresponding stock plants were also observed. There was no significant relationship between gs before budding and after budding (Fig 30).

The instantaneous water use efficiency defined as the ratio of carbon dioxide exchange rate and stomatal conductance (A/gs) was calculated in the plants before budding and after 18 months of budding with five clones. Table 11 shows the mean and covariance of A/gs in seedlings and budgrafted plants of the above clones.

Table 11. Mean and coefficient of variation of A/gs in seedlings before budding) and budgrafted plants after 18 months of budding with five clones of *Hevea*

Clones	A/gs			
	Before budding (Seedlings)		After budding	
	Mean	CV(%)	Mean	CV(%)
GT1	84.6	24	60.0	80
RRIM 600	86.2	33	41.3	77
RRII 105	88.7	39	25.8	83
RRII 208	75.2	30	53.7	65
Gl1	68.2	30	74.8	35

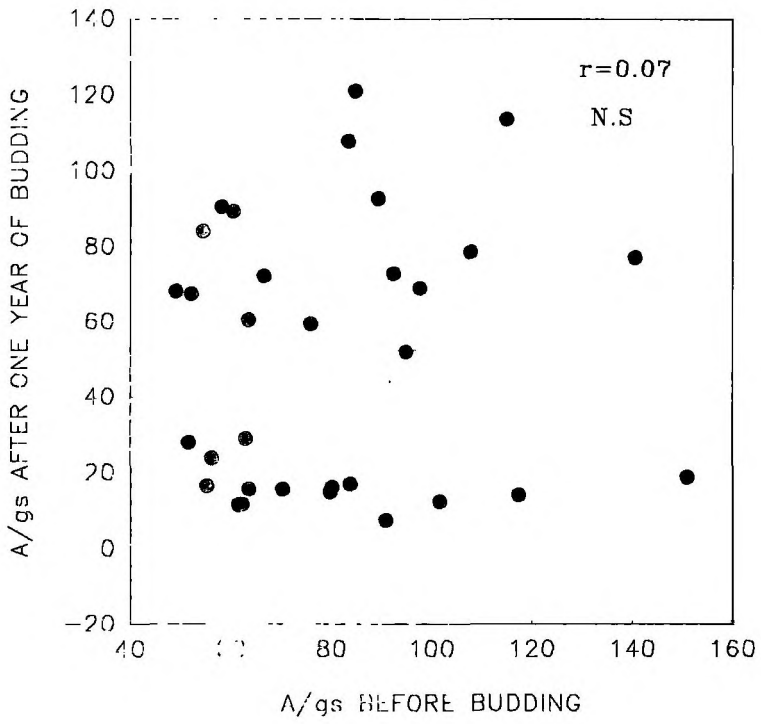


Fig:31: Relationship between A/gs before and after budding in five clones of Hevea

Here also, CV was high among budded plants of different clones than their corresponding seedling rootstocks. The mean values of A/gs were different only between RRII 105 and Gl1 ($p < 0.05$). No significant relationship exists between A/gs before and after budding in these clones. The above results indicate that there was no rootstock influence on gs or A/gs of the scion in all the five clones studied. Very high CV observed among plants within clones in these two parameters may be due to stock-scion interaction.

4.3.2..3 *Mineral nutrition*

Foliar N, P, K, Ca, Mg, Fe and Mn were analysed in 18 months old budgrafted plants of five clones and their rootstocks before budding (at 18 months old). Mean and CV of these mineral elements before and after budding were given in Tables 12 to 18.

(i) Nitrogen

Table 12 showed the foliar N content (%) of plants before budding and budgrafted plants of five clones. Clonewise comparisons indicated that RRII 105 had the highest foliar N content followed by Gl1 and RRIM 600. It was observed that foliar N of GT1 and RRII 208 were significantly lower than that of RRII 105 ($p < 0.05$). The mean values of foliar N of the stock plants were not showing any significant differences. Among the five clones RRII 208 exhibited the lowest CV (6%).

Regression analysis of foliar N before and after budding showed no significant relationship in all the five clones studied (Fig.32).

Table 12. Mean and coefficient of variation of foliar N content in seedlings (before budding) and budgrafted plants after 18 months of budding with five clones of *Hevea*

Clones	Foliar N content (%)			
	Before budding (Seedlings)		After budding	
	Mean	CV(%)	Mean	CV(%)
GT1	3.4	11	2.7	11
RRIM 600	3.4	10	2.8	16
RRII 105	3.5	14	3.1	11
RRII 208	3.5	14	2.7	6
Gl1	3.4	13	2.9	12

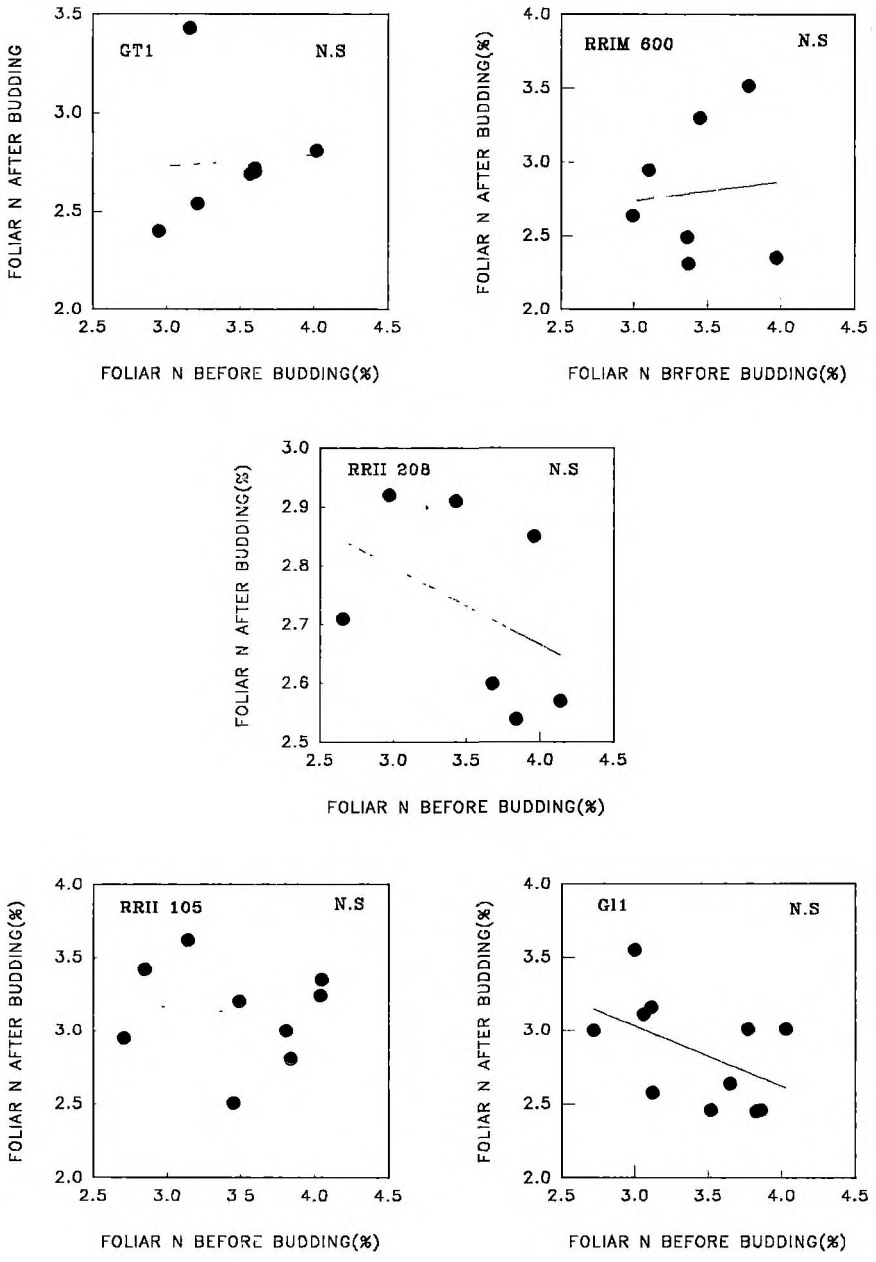


Fig: 32: Relationship between foliar N before and after budding in five clones of Hevea brasiliensis

(ii)Phosphorus

Mean and CV of foliar P in seedlings (before budding) and budgrafted plants are shown in Table 13.Clonal differences were observed in foliar P content. GT1 showed significantly low foliar P than RRII 105 and RRII 208 ($p < 0.05$). Like foliar N, the mean values of foliar P also showed no significant differences among the stock plants before budding. Here also, RRII 208 showed the lowest CV (3%).

Table 13 . Mean and coefficient of variation of foliar P content in seedlings (before budding) and budgrafted plants after 18 months of budding with five clones of *Hevea*

Clones	Foliar P content (%)			
	Before budding (Seedlings)		After budding	
	Mean	CV(%)	Mean	CV(%)
GT1	0.19	16	0.14	14
RRIM 600	0.19	10	0.16	15
RRII 105	0.17	12	0.17	12
RRII 208	0.18	11	0.16	3
Gl1	0.17	17	0.15	11

Regression anlalysis as shown in Fig 33 revealed no significant relationship between foliar P before and after budding in all the five clones.

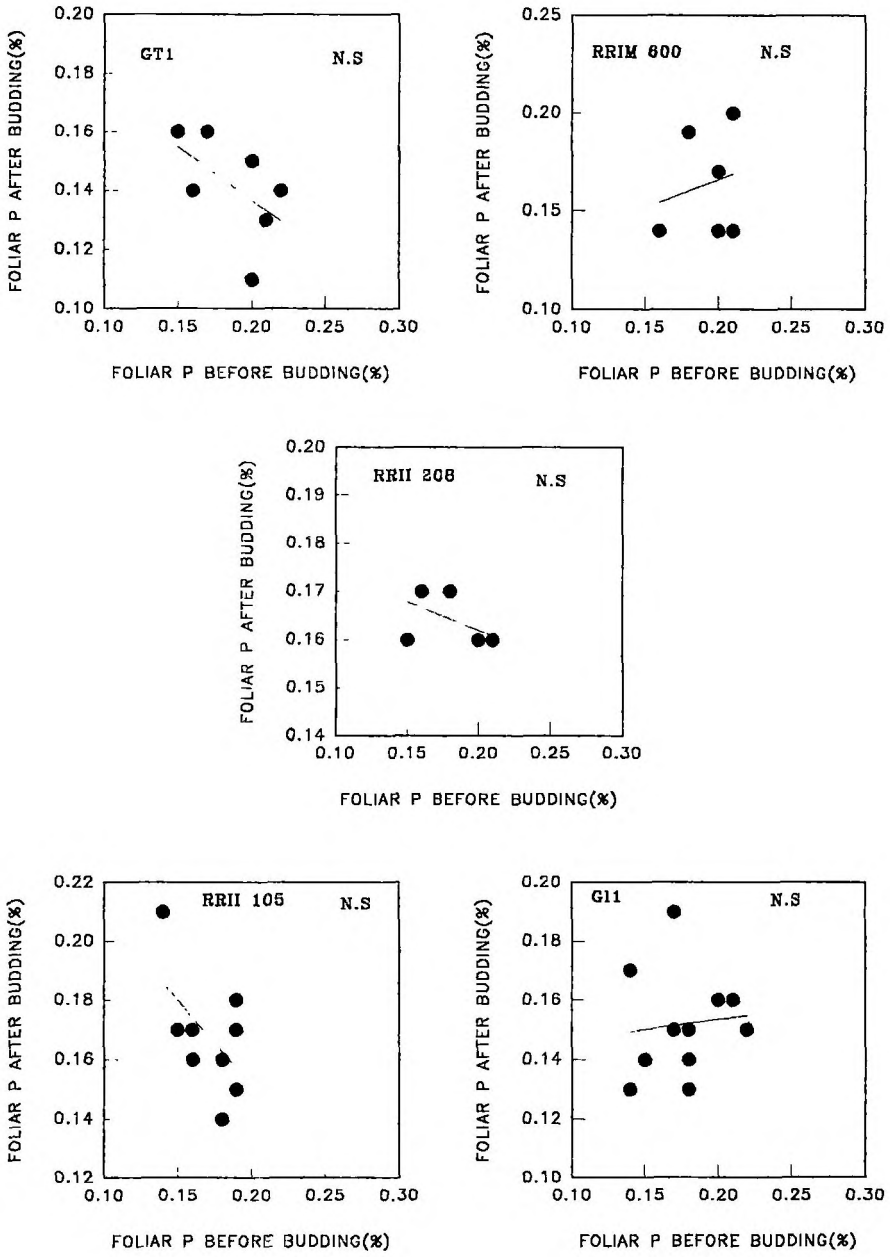


Fig:33: Relationship between foliar P before budding and after budding in five clones of Hevea brasiliensis

(iii) Potassium

Foliar K content of seedlings before budding and after budding are shown in Table 14. Considerable variations exist among plants within a clone and among different clones. Clonewise comparisons indicated that foliar K of GT1 was significantly lower than the other four clones viz, RRIM 600, RRII 105, RRII 208 and Gl1. No significant differences were observed among these four clones. Lowest variation in foliar K also was recorded among plants of RRII 208. Here also, there was no significant relationship between foliar content of K before and after budding in all the five clones (Fig 34).

Table 14. Mean and coefficient of variation of foliar K content in seedlings (before budding) and budgrafted plants after 18 months of budding with five clones of *Hevea*

Clones	Foliar K content (%)			
	Before budding		After budding	
	Mean	CV(%)	Mean	CV(%)
GT1	1.16	10	0.92	22
RRIM 600	1.31	18	1.15	33
RRII 105	1.18	14	1.01	19
RRII 208	1.22	18	1.07	13
Gl1	1.29	13	1.03	15

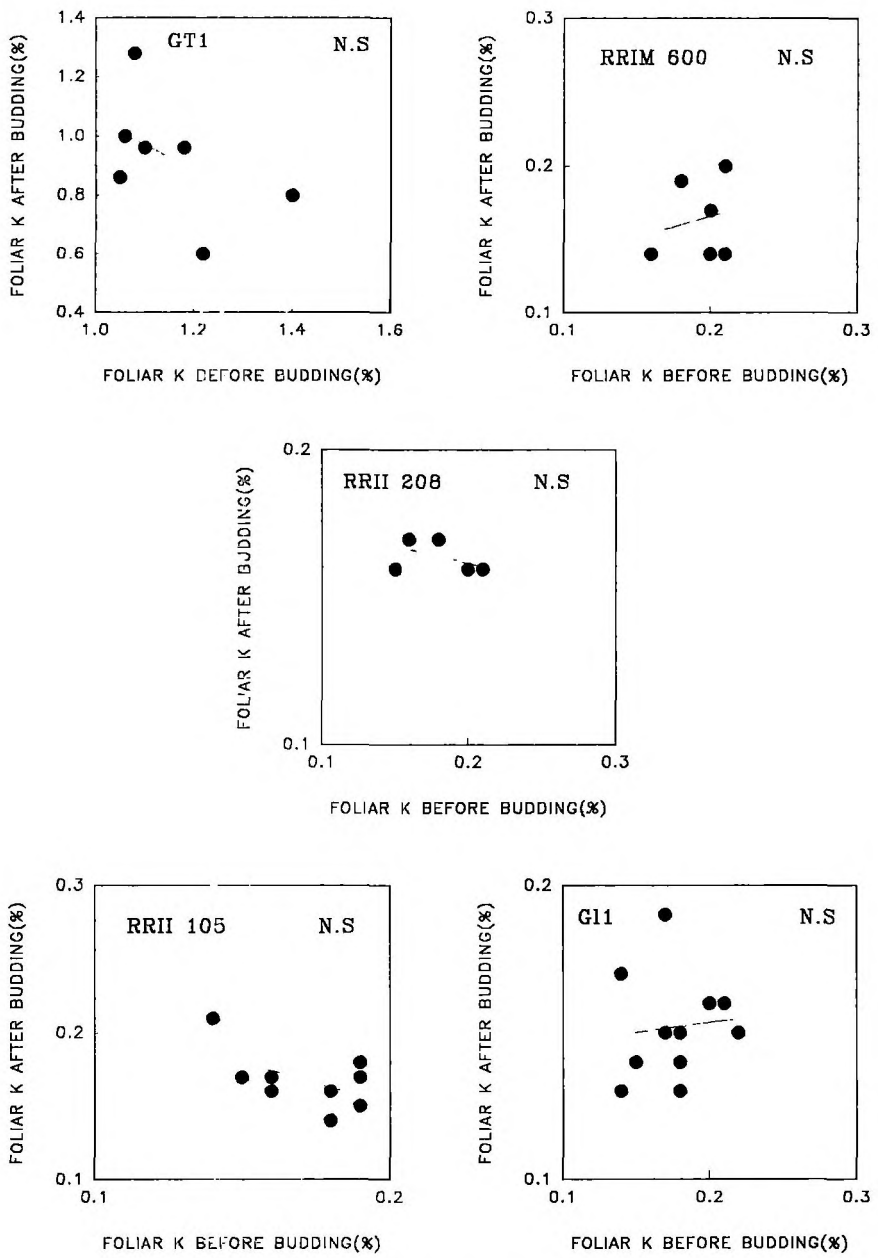


Fig.34: Relationship between foliar K before and after budding in five clones of Hevea brasiliensis

(iv) Calcium

Mean and CV of foliar Ca of seedlings and budgrafted plants of five clones of *Hevea* are given in Table 15. In the present study, no significant differences existed in foliar Ca among the different clones. Budgrafted plants of all the clones showed almost similar range of foliar Ca content as their corresponding stock plants. But CV among the plants within a clone showed considerable variation as in the stock plants.

Table 15. Mean and coefficient of variation of foliar Ca content in seedlings (before budding) and budgrafted plants after 18 months of budding with five clones of *Hevea*

Clones	Foliar Ca content (%)			
	Before budding		After budding	
	Mean	CV(%)	Mean	CV(%)
GT1	0.99	31	1.91	22
RRIM 600	0.95	21	2.17	21
RRII 105	0.84	37	1.94	32
RRII 208	0.82	29	2.03	21
Gl1	0.84	24	2.65	38

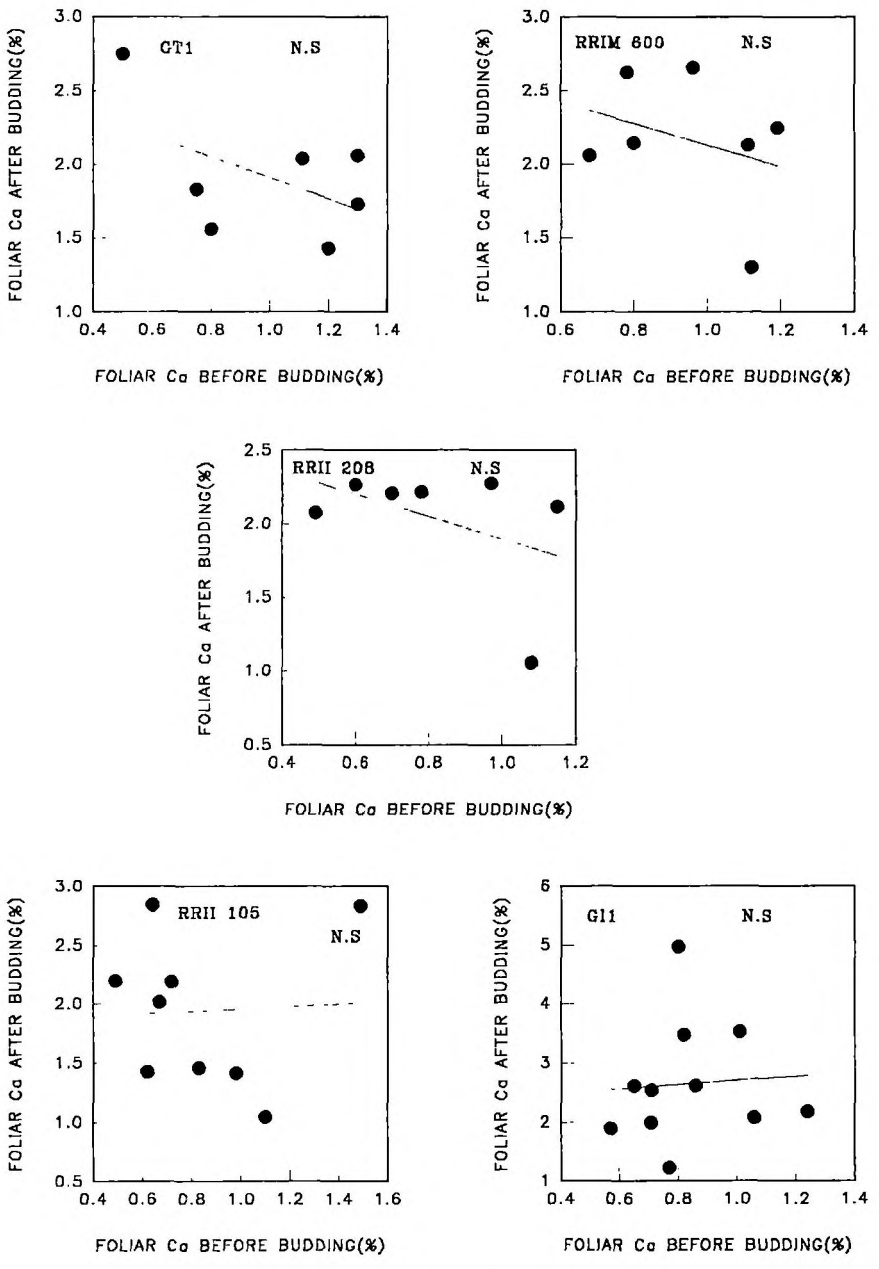


Fig.35: Relationship between foliar Ca before and after budding in five clones of Hevea brasiliensis

Regression analysis as shown in Fig 35 indicated that there was no significant relationship between foliar Ca before and after budding.

(v) Magnesium

Foliar Mg content of seedlings before and after budding was shown in Table 16. Mean values of foliar Mg in the budded plants of five clones and their corresponding rootstocks did not show any significant differences. But considerable variation (17-46%) was observed among the plants within clones.

Table 16. Mean and coefficient of variation of foliar Mg content in seedlings (before budding) and budgrafted plants after 18 months of budding with five clones of *Hevea*

Clones	Foliar Mg content (%)			
	Before budding (Seedlings)		After budding	
	Mean	CV(%)	Mean	CV(%)
GT1	0.20	35	0.20	40
RRIM 600	0.23	22	0.18	17
RRII 105	0.24	62	0.15	20
RRII 208	0.19	42	0.15	46
Gl1	0.21	33	0.20	45

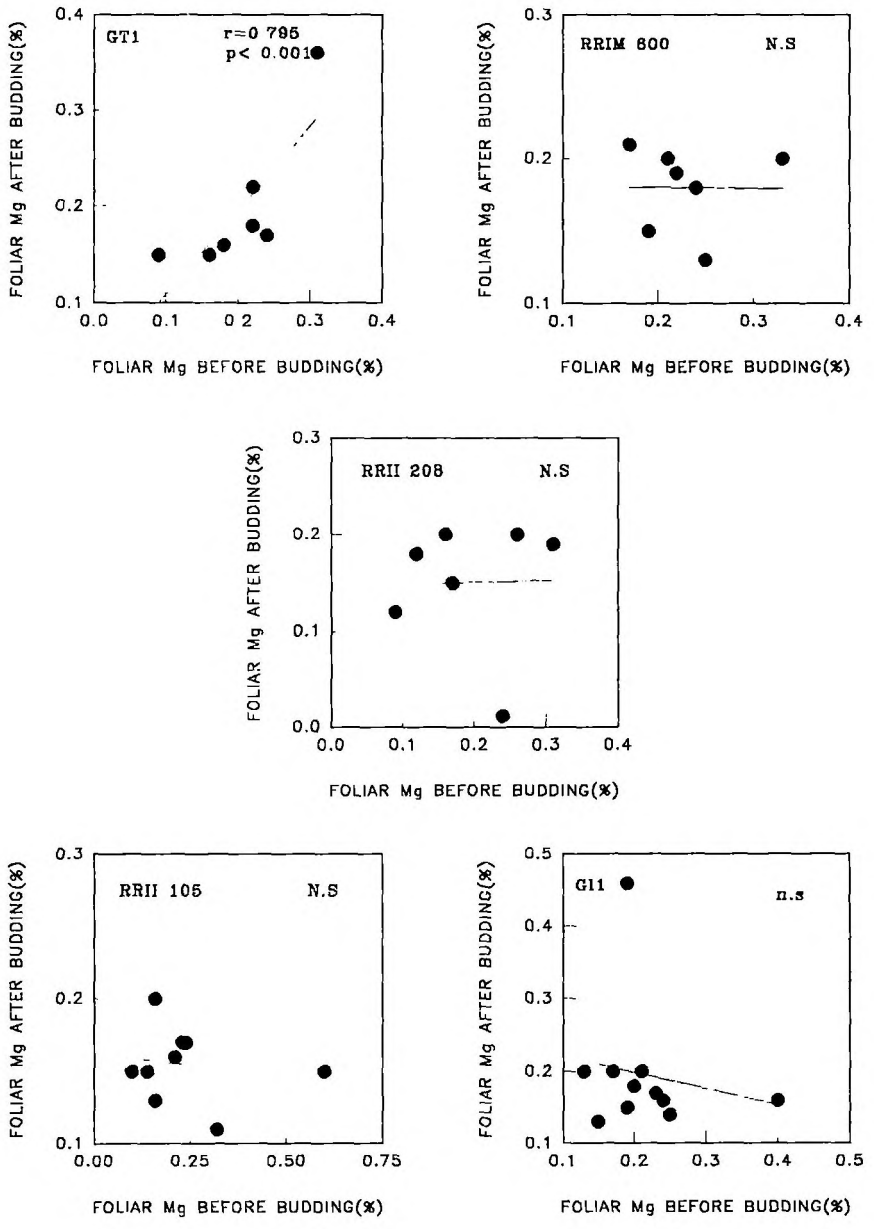


Fig.36: Relationship between foliar Mg before budding and after 18 months of budding in five clones of Hevea brasiliensis

There was no significant relationship between foliar Mg before and after budding in these clones except in GT1 as shown in Fig (36). A highly positive relationship ($r=0.80$, $p < 0.001$) was observed between foliar Mg of stock plants and the budgrafted plants of GT1.

(vi) Micronutrients

Foliar micronutrients viz, Fe and Mn were analysed in seedlings before and after budding in the five clones and shown in Tables 17 and 18.

Table 17. Mean and coefficient of variation of foliar Fe content in seedlings (before budding) and budgrafted plants after 18 months of budding with five clones of *Hevea*

Clones	Foliar Fe content (ppm)			
	Before budding (Seedlings)		After budding	
	Mean	CV(%)	Mean	CV(%)
GT1	677	72	216	15
RRIM 600	605	39	180	11
RRII 105	502	22	417	100
RRII 208	456	15	223	30
G11	381	19	192	16

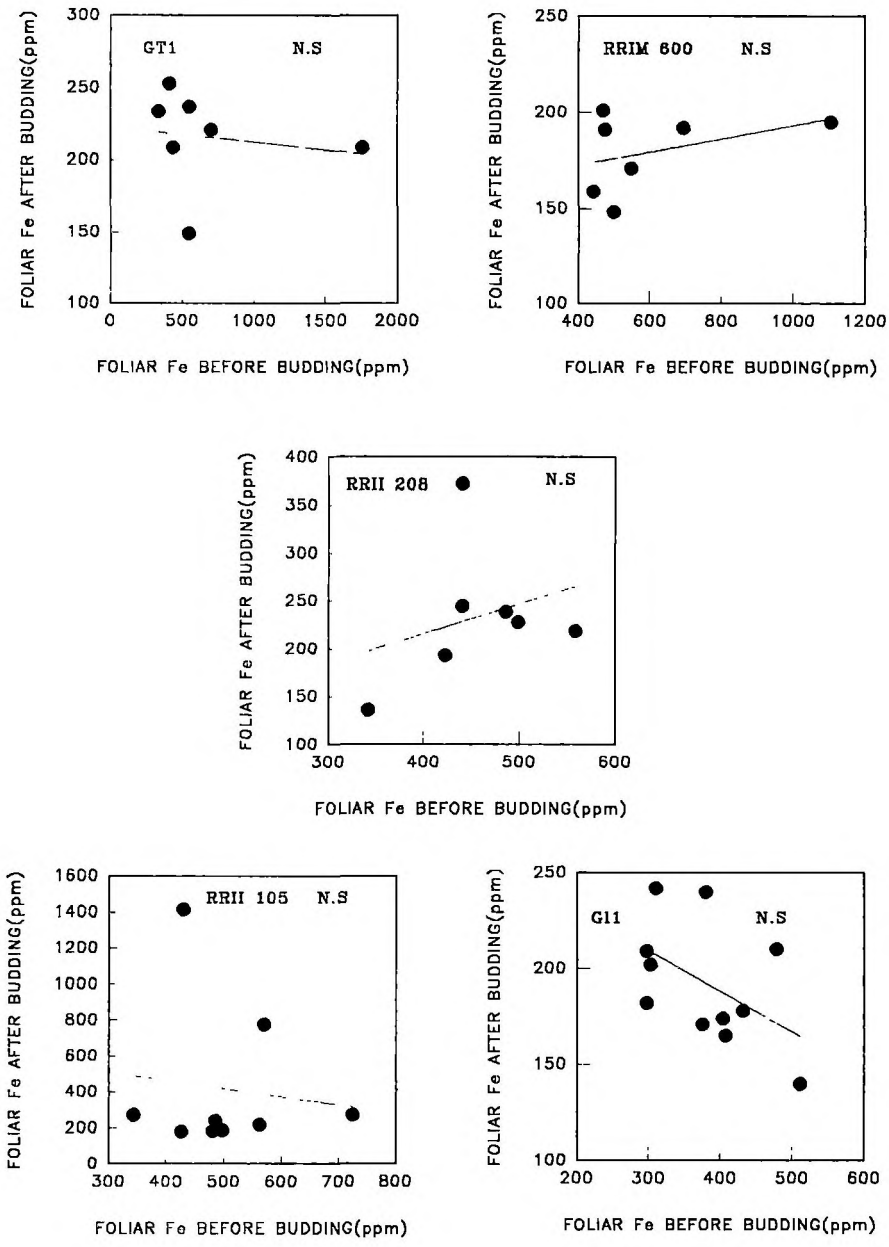


Fig:37: Relationship between foliar Fe before budding and 18 months after budding in five clones of Hevea

Foliar Fe among the different clones did not show any significant differences. But considerable variations were observed among plants within a clone especially very high CV (100%) shown by RRII 105 corresponding to a CV of 22% among their stock plants (See comparison of foliar Fe contents of own-rooted and budded plants described in Chapter 5). No relationship between foliar Fe before and after budding could be observed in any of the clones in the present study (Fig.37).

Foliar Mn as shown in Table 18 showed significant differences among

Table 18. Mean and coefficient of variation of foliar Mn content in seedlings (before budding) and budgrafted plants after 18 months of budding with five clones of *Hevea*

Clones	Foliar Mn content (ppm)			
	Before budding		After budding	
	Mean	CV(%)	Mean	CV(%)
GT1	81	35	147	21
RRIM 600	64	25	154	26
RRII 105	75	25	113	27
RRII 208	59	20	160	37
Gl1	71	20	146	38

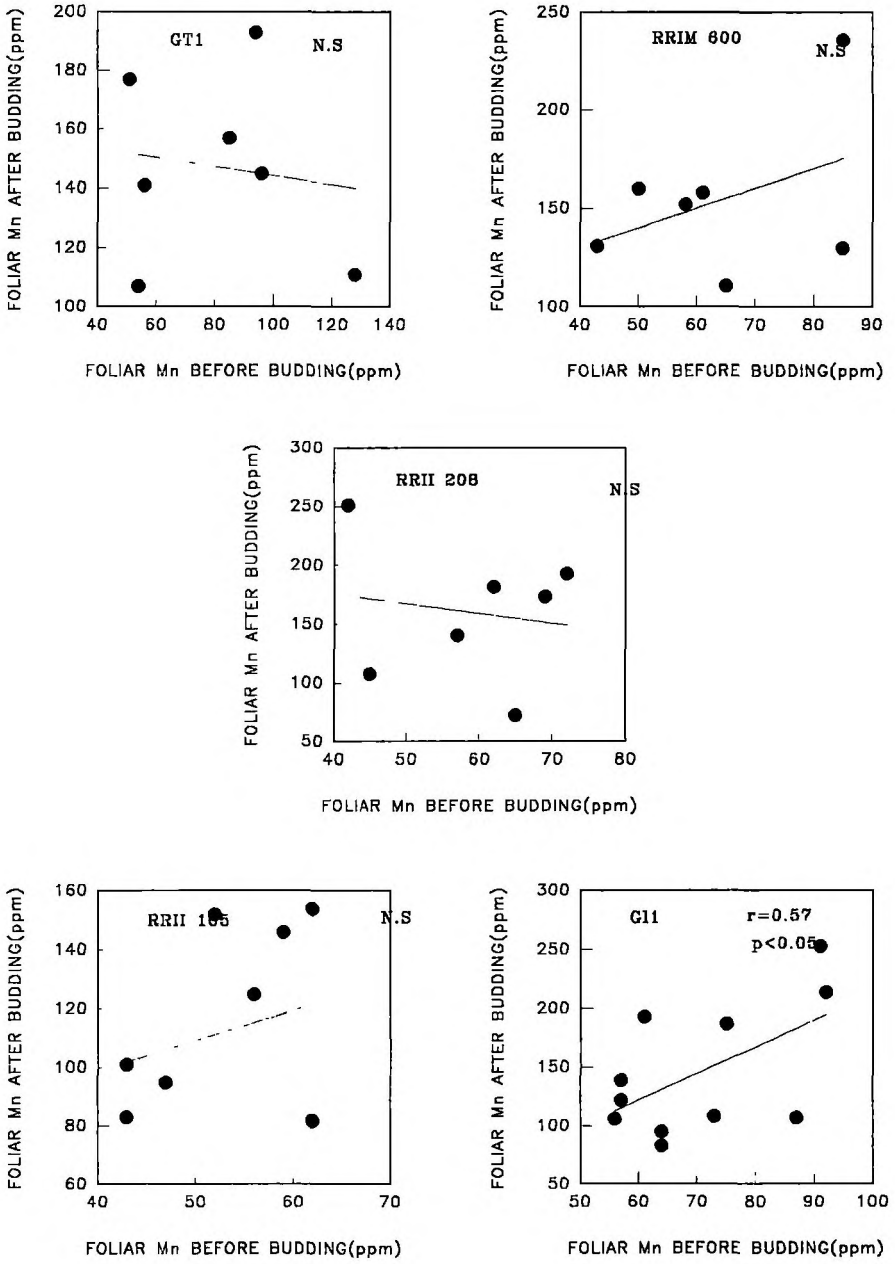


Fig.38: Relationship between foliar Mn before and after budgrafting in five clones of Hevea brasiliensis

clones. RRII 105 had a significantly low foliar Mn ($p < 0.05$) when compared with that of GT1 and RRIM 600. No significant differences existed among other clones. High CV among plants within a clone was also observed as shown in the Table 18. Regression analysis of the data (Fig.38) revealed that no significant relationship exists between foliar Mn before and after budding except in Gl1 which showed a positive relationship ($r=0.57$, $p<0.05$).

The degree of nutrient variability among plants within a clone in different clones was different in various elements. Likewise, clonal differences were also observed for certain elements only. Among the nutrients, foliar N and P showed the minimum range of intraclonal variations (CV 3-16%) followed by K (13-33%) and Ca (21-38%). Their corresponding rootstocks also exhibited almost similar range of variations (Tables 12 to 15). RRII 208 exhibited the lowest variations in foliar N and P contents (6 and 3% respectively) among the plants. In both these the CV was narrowed appreciably from their corresponding rootstock plants. In other clones the same range of CV was exhibited in both budgrafted and their corresponding rootstock plants. Lowest CV of foliar K was also observed in RRII 208 budgrafted plants and was lower than the CV of its corresponding stock plants. But in other clones CV of foliar K among plants within clones showed higher values than their corresponding stock plants.

Significant differences observed among the clones indicated that the

major nutrients N, P and K contents are determined by the scion. Moreover, the observation of no significant differences among the seedling rootstocks on which these five clones were budgrafted and very low CV exhibited by the clone RRII 208 support the fact that nutrient status of these three elements are depended on clones. Foliar N, P and K of all these clones before and after budding did not show any relationship in the present study. This showed that rootstock had no influence on foliar N, P and K contents of budded plants. Our results are in agreement with the observations of Knowles *et al.* (1984) in peaches that there were no differences existed in N and P of the scion leaves due to rootstocks.

Foliar Ca and Mg showed no significant differences among the different clones in the present study. But a high CV was observed among the plants before and after budding. Highest CV among the plants were observed in foliar Mg especially among the unbudded seedling stock plants. The significant positive relationship between foliar Mg of rootstock and scion exhibited by the clone GT1 indicated the rootstock influence on foliar Mg of the scion in that particular clone. Influence of rootstock on scion foliar Mg was reported earlier in other plants like pear (Chaplin and Westwood, 1980; Woodbridge, 1973) and apple (Fallahi *et al.*,1984). But in our present study the stock influence on foliar Mg of the scion is not reflected in all the clones. Hence it can be concluded that clones may be specific towards the stock influence in different parameters.

Foliar micronutrients viz, Fe and Mn of budgrafted plants showed that highest CV (100%) of foliar Fe was exhibited by the plants of RRII 105. In this context, it is worth mentioning that own-rooted plants of RRII 105 had a higher foliar Fe content (see Chapter 5) indicating the higher absorption capacity of Fe of that clone. Eventhough no significant relationship existed between foliar Fe of stock and scion in all the five clones studied, high CV among the plants of RRII 105 and an appreciable reduction in CV than their corresponding stock plants in clones GT1 and RRIM 600 indicated the possibility of stock-scion interaction. Moreover, no significant clonal differences were observed in foliar Fe content in all these five clones. Hence it can be assumed that foliar Fe in budgrafted plants of *Hevea* is determined neither by the scion nor the stock alone but may be due to the resultant of the interaction of both. In other plants like apple also, no significant effect of rootstock on scion foliar Fe was reported (Fallahi *et al.*, 1984). On the other hand, rootstock effect on foliar Fe of scion was reported by Sharma and Chauhan (1991).

Foliar Mn as shown in Table 18 revealed significant clonal differences. High CV was also noticed among plants within clones. Regression analysis indicated that there is no significant effect of stock on scion foliar Mn except in Gl1 which showed a positive relationship. This observation indicated that there may be clonal differences in the response to stock influence.

Pronounced differences in nutrient status depending on the rootstock and scion combinations were reported by many scientists (Tukey *et al.*, 1962; Lockard, 1976; Bould and Campbell, 1970). The variations in nutrient content was attributed to be due to the selective absorption and their further utilization by the stionic combinations (Om and Pathak, 1983). (See Chapter 1, section for more details). Teng and Pushparajah (1974) reported that rootstock affected the nutrient uptake of certain scions in *Hevea*. Also, different rootstock / scion combinations showed different levels of particular nutrients. But in the present study, as evident from the results shown in the tables and figures, scion seemed to be the determinant in the foliar nutrient status of most of the elements of budgrafted plants except few cases. Also, there are indications of the existence of stock-scion interaction, especially in the micronutrient concentrations in the scion leaves. In peach, Brown and Cummins (1989) reported a small but significant effect of rootstock on foliar nutrient concentration. They also observed a narrow range in foliar N and P levels regardless of the rootstock. Creste and Lima (1995) also showed no rootstock effect on macronutrient concentration in *Citrus unshiu*. On the other hand, Jadczuk *et al.* (1995) reported that in non - bearing apple trees, rootstock was an important contributor to the tree nutrition. Cultivar differences in response to different rootstocks resulting in varying levels of nutrients was reported in peach by Knowles *et al.* (1984). In the present study also clonal response to rootstock effect varied for certain elements.

4.3.3 Biochemical studies

Biochemical analysis of different parameters in the budgrafted plants of five clones (18 months old) was carried out and the results are summarised in this section.

4.3.3.1 *Pigment composition*

Chlorophyll in the leaf samples was estimated from 18 months old budgrafted plants of five clones of *Hevea.* Chlorophyll a, b, total chlorophyll and a/b ratio are shown in Table 19. Significant differences were observed in the mean values of all these components in these five clones. Total chlorophyll, Chlorophyll a and b contents were the highest in RRII 105 and Gl1 followed by RRII 208. Clonewise analysis revealed that RRIM 600 and GT1 have significantly less chlorophyll a content ($p < 0.05$) than RRII 105, RRII 208 and Gl1. Chlorophyll b was also significantly less in RRIM 600 and GT1 than RRII 105 and Gl1 ($p < 0.05$). Total chlorophyll also showed the same trend as that of chlorophyll b except between RRIM 600 and RRII 208 which was also significantly different at 5% level. a/b ratio of the five clones showed that RRII 105 and Gl1 have significantly low values than GT1 and RRIM 600 ($p < 0.05$). Chlorophyll a was not showing much variation among the plants within a clone in all the five clones. Clones having the highest chlorophyll a content (RRII 105 and Gl1) showed the lowest CV (1.4 and 1.8%

respectively). But chlorophyll b was showing higher CV among the different components estimated. Same pattern of variations as that of chlorophyll a was reflected in total chlorophyll content in all the five clones studied. Significant clonal differences in the total chlorophyll content observed in the present study indicated that pigment composition is a clonal character.

Moreover, the clones RRII 105 and Gl1 showed same values of chlorophyll a, b, total chlorophyll and a/b ratios. It is worth mentioning that Gl1 is the mother parent of RRII 105 and the same pigment composition exhibited by these two clones indicated its genetic nature. In addition low CV (7.2 and 8.6%) of the total chlorophyll content among the plants of RRII 105 and Gl1 respectively indicated that stock has no profound influence on pigment composition in these two clones. Comparatively high variations in total chlorophyll content exhibited by the plants within a clone may be due to the high variations noticed in chlorophyll b content. Chlorophyll formation was dependent on various factors in the metabolic process (Kramer and Kozlowsky, 1979). The ratio of chlorophyll a to b also varies in the same plants due to changes in the environmental factors, seasons, etc. But the chlorophyll a/b ratio and other characteristics of the pigments were reported to be similar in coniferous and deciduous tree species and herbaceous species (Alberte *et al.*, 1976). In general, a higher proportion of chlorophyll a to b occurs in sun than in shade leaves.

Table 19. Chlorophyll composition (mg/cm^2) of leaves in 18 months old budgrafted plants of five clones of *Hevea*

Clones	Chlorophyll a		Chlorophyll b		Total chlorophyll		Chlorophyll a/b	
	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)
GT1	2.3	12.7	1.4	35.2	3.7	21.8	1.8	15.9
RRIM 600	2.4	7.9	1.4	14.8	3.8	10.5	1.7	6.7
RRII 105	2.8	1.8	2.2	14.7	5.0	7.2	1.3	12.4
RRII 208	2.7	6.3	1.9	27.1	4.6	14.8	1.4	21.0
Gl1	2.8	1.4	2.3	15.5	5.1	8.6	1.3	13.4

4.3.3.2 *Carbohydrates*

(i) Reducing sugar

Reducing sugar in the leaves showed appreciable variations in the five clones studied (Table 20). Gl1 showed the lowest value followed by RRII 105 and RRIM 600. The highest concentration of reducing sugar was observed in RRII 208 and it was significantly higher than RRII 105, Gl1 and RRIM 600 ($p < 0.05$). Gl1 had significantly low concentration of reducing sugar when compared with that of GT1. Considerable CV was observed among plants within clones and lowest CV was observed in the clone RRII 208.

(ii) Total sugar

Total soluble sugar concentration was not showing similar trend in these clones as reducing sugars (Table 20). Highest sugar concentration was observed in RRII 105 and is significantly different from the clone RRIM 600 only. All the other clones showed no significant differences. Considerable CV was also observed among plants within clones.

(iii) Phenols

Phenol concentrations in the leaves of five clones were shown in Table 20 and clones differed in their phenol concentrations. GT1 showed the highest

Table 20. Biochemical composition of leaf samples in 18 months old budgrafted plants of five clones of *Hevea*

Clones	Reducing sugar (mg/gm)		Total sugar (mg/gm)		Phenols (mg/gm)		Amino acids (mg/gm)	
	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)
GT1	122.1	24.9	339.5	18.3	71.9	35.0	24.9	13.1
RRIM 600	105.8	25.6	308.5	18.8	39.0	34.6	20.4	42.2
RRII 105	100.0	23.8	388.9	18.1	55.2	18.5	18.7	47.1
RRII 208	146.3	10.8	336.1	44.3	47.0	37.2	15.6	55.7
Gl1	80.3	27.7	335.6	30.2	47.0	16.5	10.8	23.1

concentration followed by RRII 105, RRII 208 and G11. Phenol concentration of GT1 was significantly higher than RRIM 600 and G11 and of RRII 105 was significantly higher than RRII 600 ($p < 0.05$). CV showed higher values among clones RRII 208, RRIM 600 and GT1. RRII 105 and G11 showed lower values when compared with other three clones.

(iv) Amino acids

Amino acids also showed considerable variations among clones and plants within clones as shown in Table 20. Amino acid concentration of GT1 was the highest among the five clones and lowest was observed in G11. G11 was significantly lower than that of GT1, RRII 105 and RRIM 600 ($p<0.05$). RRII 208 also was lower than GT1 ($p < 0.05$). High CV was observed in the amino acid concentration among the budded plants of different clones except GT1. Highest concentration of total sugar in the leaves of budgrafted plants of RRII 105 may be due to the greater growth vigour of this clone as expressed by its shoot biomass. Such a trend was exhibited by other clones also. Regression analysis of the data showed a positive relationship ($r=0.96$, $p < 0.001$) between shoot biomass and total sugar content of these five clones (Fig.39). Hence it is assumed that the variations observed in the total sugar content of the different clones may be due to the wide range of growth vigour existed in the plants. Similar observations were reported by Brown *et al.* (1985) in apple trees. Possible stock-scion interactions affecting carbohydrate

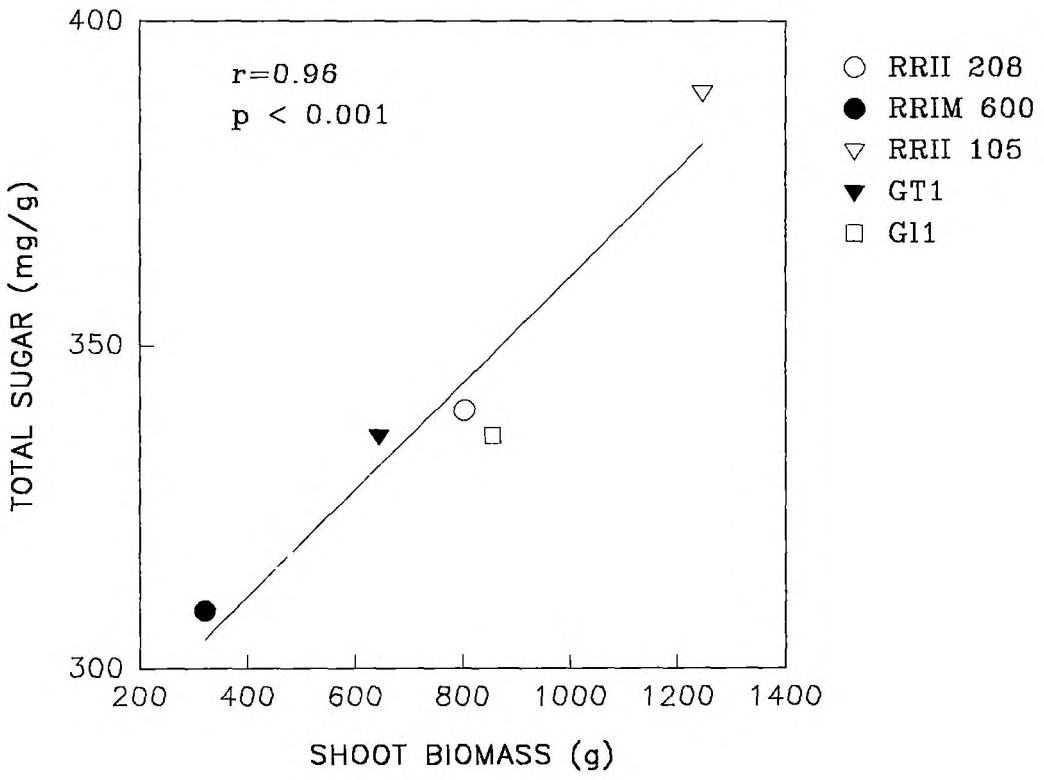


Fig.39: Realtionship between shoot biomass and total sugar content of leaves in budded plants of five clones of Hevea.

distribution in young apple trees was studied by them and showed that the effect of scion on the tree's dry weight and carbohydrate content were not changed by different rootstocks and vice versa.

Analysis of different biochemical factors of plant parts reflect the various metabolic processes. Changes in these parameters can be attributed to the differences in the metabolic activities of the plants which may be influenced by genetic / environmental factors. In the present study significant differences existed among the five clones with regard to all the biochemical parameters observed viz, reducing sugar, total sugar, phenol and amino acids. In spite of the homogeneity of the scion materials of each clone as mentioned earlier in this Chapter, considerable CV observed among plants of each clone in all these parameters indicated the stock influence or existence of stock-scion interaction.

4.3.3.3 *Isozyme studies*

Three enzyme systems viz, peroxidase, catalase and esterase were analysed in the budgrafted plants of clones RRII 105, GT1, RRII 208, RRIM 600 and G11 and are shown in the zymograms (Plates 5 to 10). In every gel, well number one represents the mother plant (budwood plant from which the buds were taken for budding) and well numbers two to seven represent the budded plants of a clone. All the five clones exhibited considerable variations among the plants in isozyme pattern of the three enzyme systems. Also, the isozyme expressions in the six plants of each clone showed variations from

the mother plant from which these plants were propagated.

Concentrations of the total protein in the leaf extracts of five clones are given in the following Table 21. Total protein concentration also showed appreciable variations among plants within clones.

Table 21. Total soluble proteins in the leaves of five clones of *Hevea brasiliensis*

Clones	Total soluble proteins (mg/g) Sample number						
	1	2	3	4	5	6	7
GT1	96.1	79.7	105.5	94.1	153.5	70.5	71.2
RRIM 600	138.5	67.2	122.3	71.3	183.3	126.5	75.9
RRII 105	101.6	112.8	82.6	116.7	93.3	174.7	104.5
RRII 208	100.6	106.0	76.8	87.7	129.0	75.6	93.4
Gl1	167.6	124.0	74.3	72.2	81.3	203.7	155.5

(i) Esterase

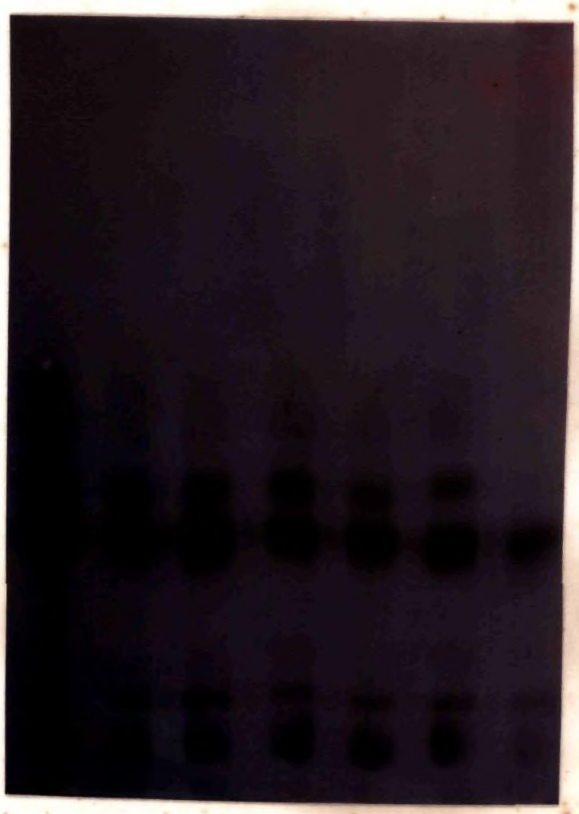
Esterase isozyme analysis in the five clones showed variations in the number of bands among clones and plants within clones (Plates 5 & 6). In the clone GT1, the number of bands varied from two to five in the budded

Plate 5. Esterase isozyme banding pattern of three clones of *Hevea brasiliensis*

(a) RRII 208 (b) RRIM 600 (c) G11

Plate 6. Esterase isozyme banding pattern of two clones of *Hevea brasiliensis*

(a) RRII 105 (b) GT1



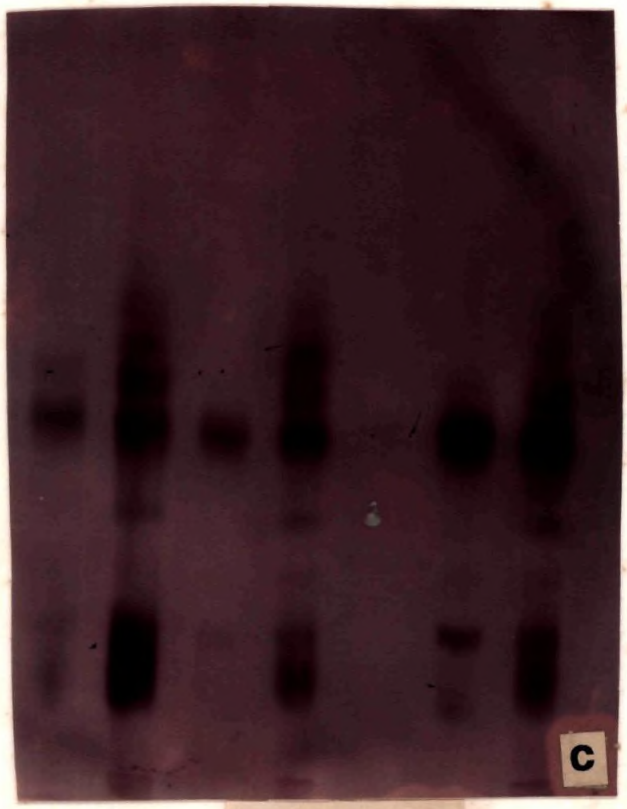


PLATE 5

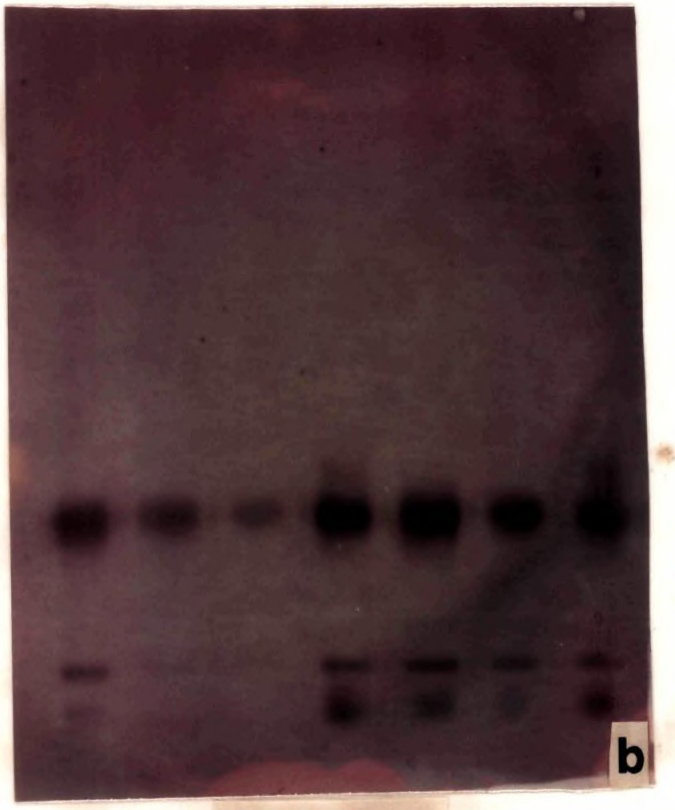


PLATE 6

plants while the mother plants showed four bands. Gl1 showed the highest number of bands among the five clones and within the six plants of the clone the number of bands was even reduced to one in sample number three. Maximum of five bands could be detected in the clone RRII 105. In this also the number of bands ranged from two to five as against five bands present in their mother plant. RRIM 600 exhibited high variations in the esterase isozyme expressions and the number of bands varied from two to eight. Four to seven bands were detected in the six plants of the clone RRII 208 while eight bands were present in their mother plant.

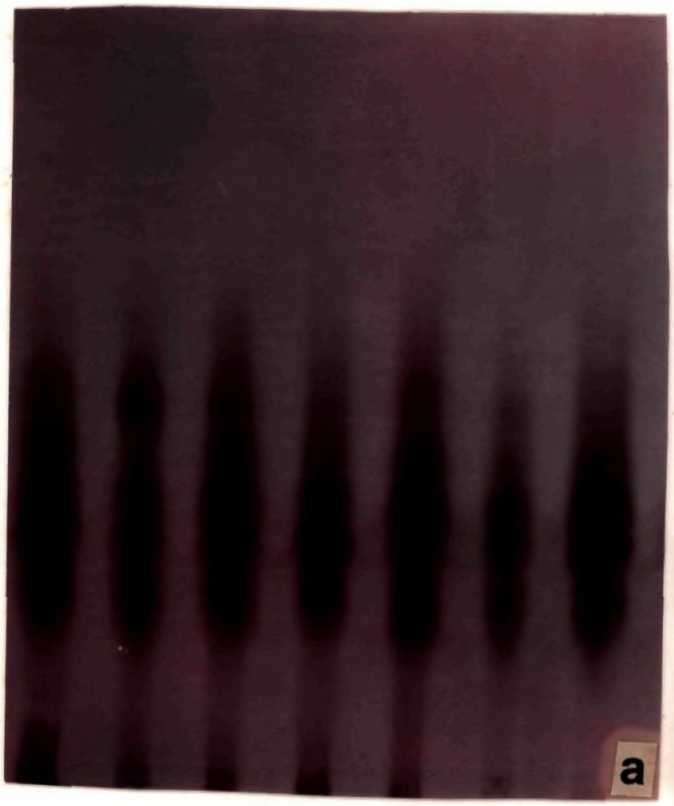
Analysis of esterase isozymes in the budgrafted plants of five clones of *Hevea* indicated high variations among plants and showed significant differences from their mother plant. Variations in the isozyme expressions were observed not only in the number of bands, but also in the intensity of bands. One or more specific bands were present in all the plants of each clone but showed varying intensities from very light to dark. These variations observed in this study indicate that isozyme polymorphism of esterase enzyme exists among the budgrafted plants of all the five clones studied.

ii) Peroxidase

Peroxidase enzyme showed remarkable variations in their isozyme expressions (Plates 7 & 8). The maximum number of bands varied from five in RRII 105 to eight in RRII 208. In GT1 the six budded plants expressed three ~~to~~

Plate 7. Peroxidase isozyme banding pattern of three clones of *Hevea brasiliensis*
(a) RRII 208 (b) RRIM 600 (c) G11

Plate 8. Peroxidase isozyme banding pattern of two clones of *Hevea brasiliensis*
(a) RRII 105 (b) GT1



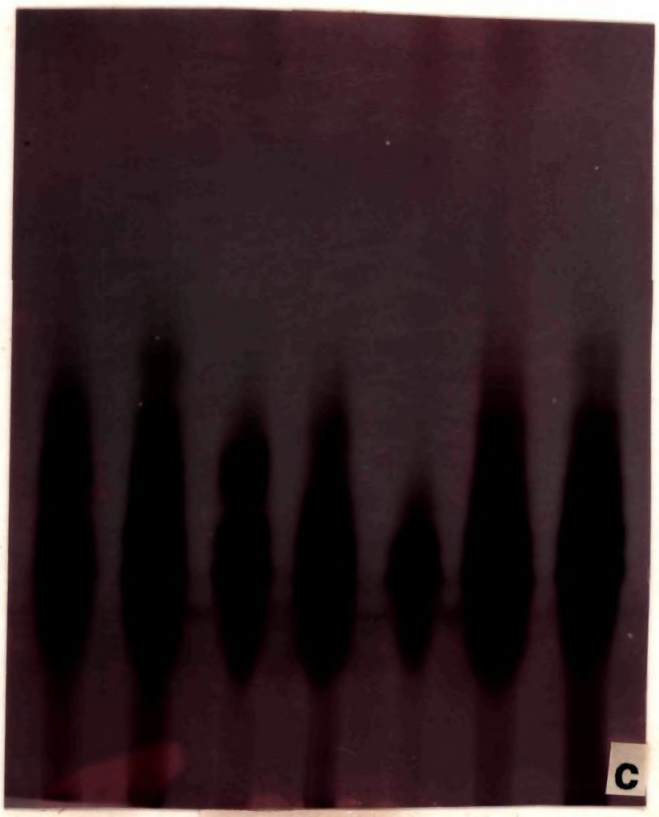
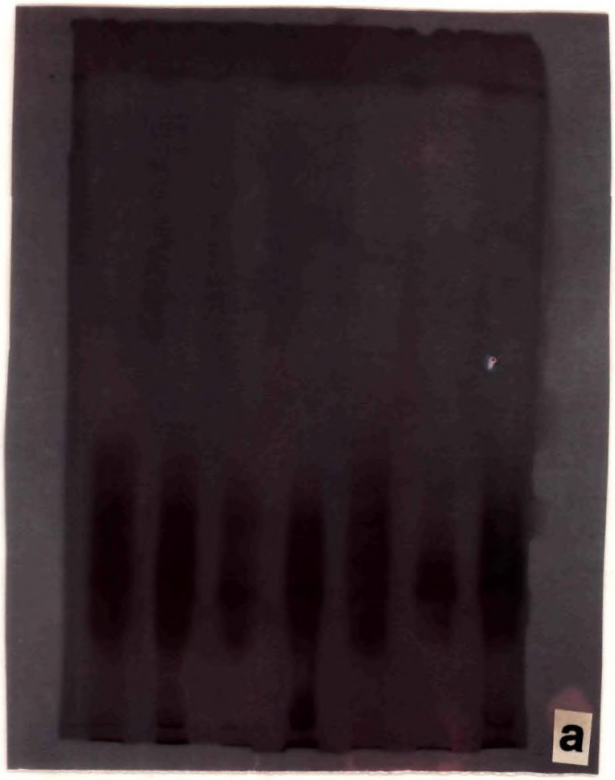


PLATE 7



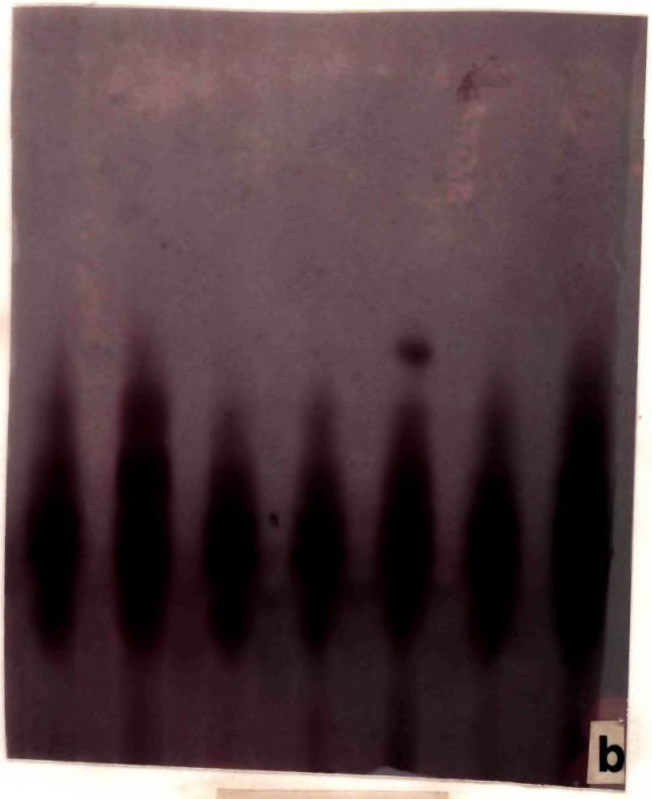


PLATE 8

to six bands and their mother plant showed six bands. An additional band could be detected in one of the budded plants (sample three) as shown in Plate 8. RRII 105 showed a range of two to five bands among the budded plants as against four bands expressed by their mother plant. RRIM 600 also showed high variations ranging from two to five bands in the budded plants while five bands were detected in its mother plant. Three to six bands could be observed in the budded plants of the clone Gl1 and their mother plant showed five bands. Highest number of bands was detected in RRII 208 and in this also the number varied from four to eight.

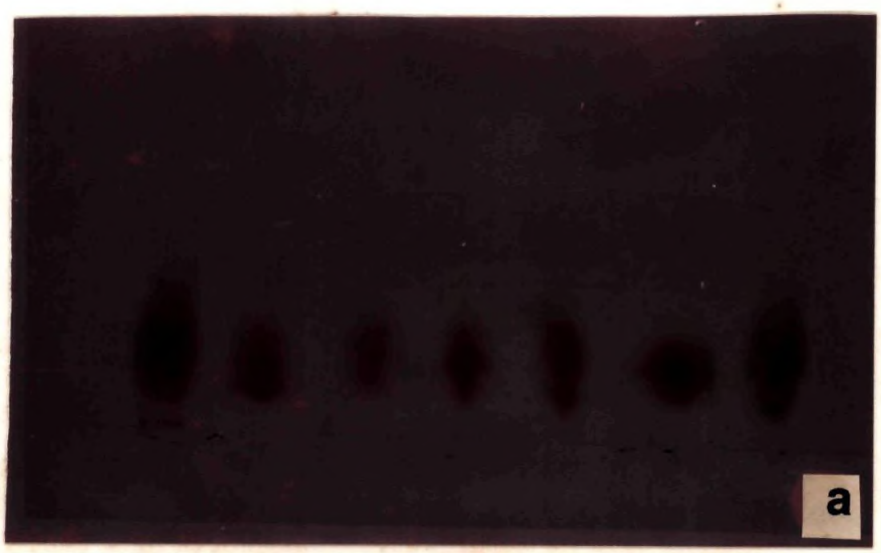
Isozyme expressions of peroxidase also showed high variations among clones and within clones in the number of bands as well as the intensity of bands. But one or more specific bands could be detected with varying intensities in all the plants of each clone indicating the relevance of peroxidase isozyme as a genetic marker.

(iii) Catalase

Among the three enzyme systems analysed catalase showed a limited number of isoforms and hence the variations in isozyme expressions are mainly reflected in the intensity of the bands (9&10). Maximum number of bands observed in the five clones ranged from three to four. In RRII 105 two to three bands were observed among plants while one to three bands could be detected in GT1. RRIM 600 and Gl1 showed two to four bands among the budded plants.

Plate 9. Catalase isozyme banding pattern of three clones of *Hevea brasiliensis*
(a) RRII 208 (b) RRIM 600 (c) G11

Plate 10. Catalase isozyme banding pattern of two clones of *Hevea brasiliensis*
(a) RRII 105 (b) GT1



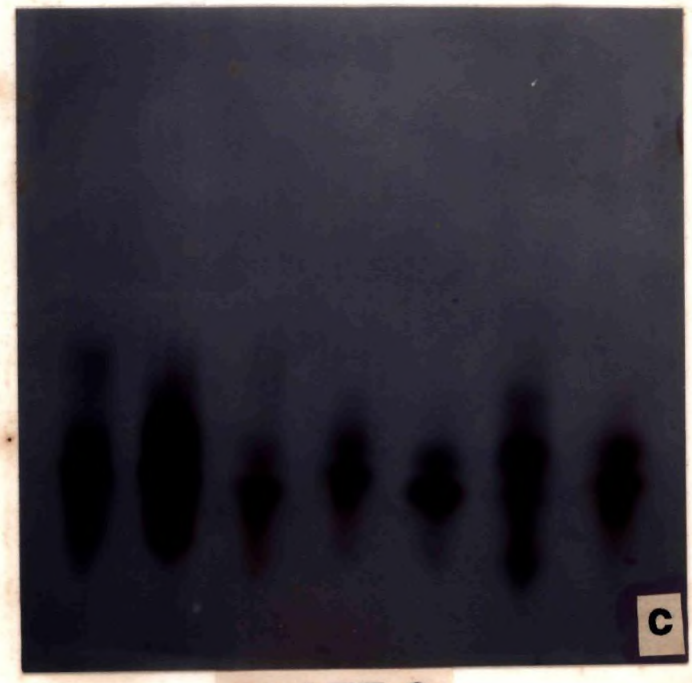


PLATE 9

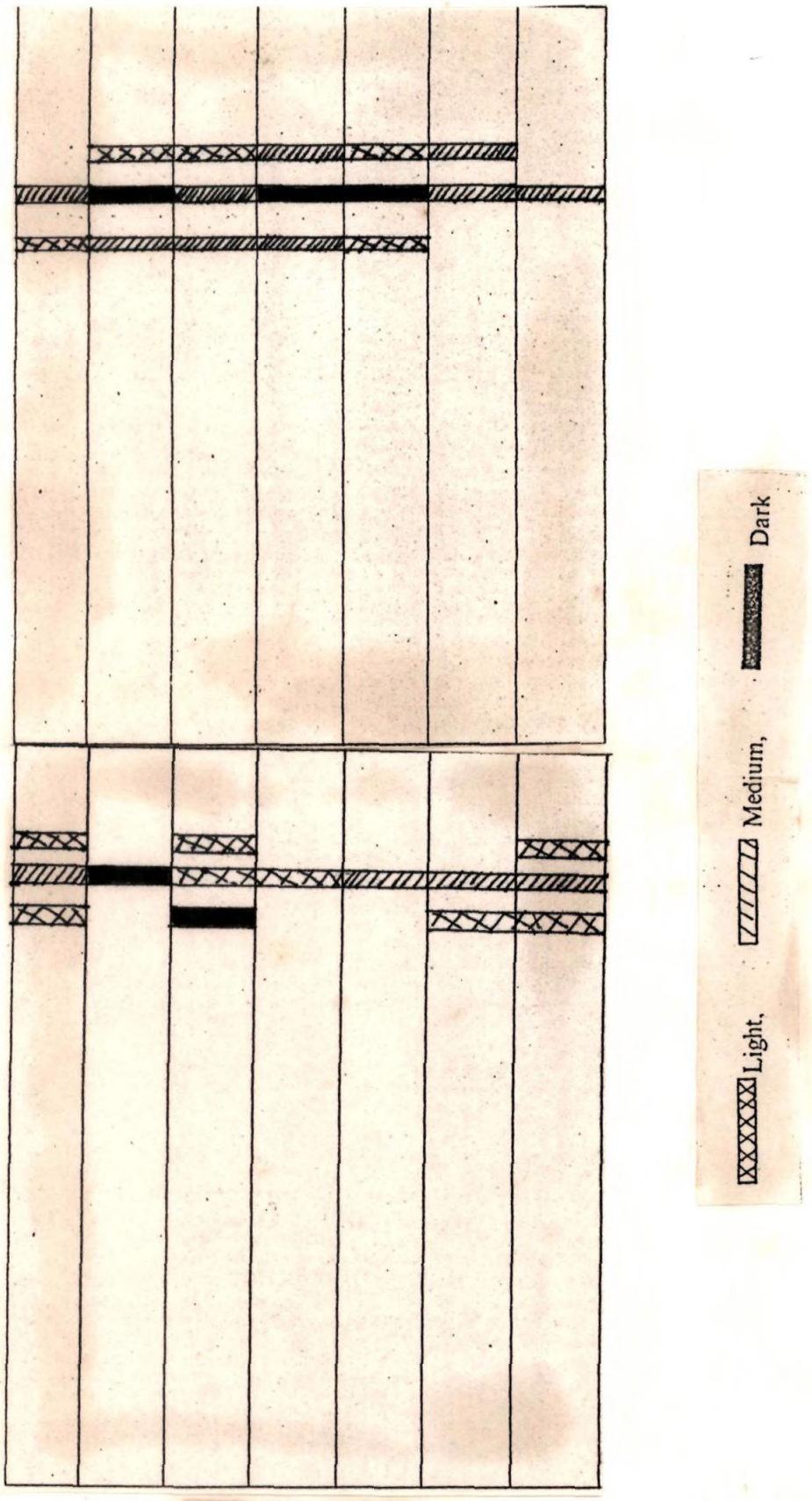


PLATE 10

RRII 208 exhibited less variations in the catalase isoforms and except one sample all the others including the mother plant showed only two bands. But the intensity of the bands showed remarkable variations. Here also specific bands could be detected in all the plants of each clone suggesting their use as reliable markers in clone identification.

All the three isozymes analysed in the present study revealed different banding patterns among clones and plants within clones with respect to number of bands and intensity of the bands. The set of bands observed in a gel after staining generally represent multiple forms of an enzyme having the same catalytic specificity. The variability in the intensity of bands reflects changes in specific activity or total amount of enzyme. Isoforms number of an enzyme reflects the number of subcellular compartments in which the same reaction is required (Gottlieb, 1982). Changes in the structural genes coding for polypeptides was suggested as the reason for the differences in the electrophoretic mobilities by Crawford (1981).

From the observations of the present study it can be concluded that there was a definite indication of genetic polymorphism in the scion which appears to be rootstock induced. Irrespective of the concentration of the total protein in the leaf extracts there were specific bands present or missing in one or more of the six budded plants propagated from a single mother plant. In other words, evenwhen the total protein content in the extract was less there were

additional bands appearing and *vice versa*. This suggests that low concentration of the protein was not responsible for the missing bands and rather specific bands were missing evenwhen the total protein concentration was high suggesting genetic polymorphism.

Isozyme technique is considered as a valuable tool for large scale use which can provide genetic fingerprints at enzyme level. Moreover, isozyme polymorphism is being used successfully to detect the genetic variability among cultivars (Cardy and Kannenberg, 1982; Chaparro *et al.*, 1987). Peroxidase zymograms were reported to be of value in distinguishing apple rootstocks (Weller and Costante, 1986). Other enzyme systems which exhibit considerable isozyme polymorphism were used by Saminy and Cummins (1992) for distinguishing apple rootstocks. Since rootstock influences the relative growth rate, ultimate size, anchorage, etc. to a greater extent, rootstock selection and planting homogeneous rootstocks having genetic uniformity is desirable. In this context they suggested isozyme analysis as an useful technique for ascertaining the genetic purity of the rootstocks. From studies of isozyme analysis in *Hevea,* Yeang *et al.* (1995) suggested that polyclonal seeds (cross pollinated) from the selected mother clone might be effective as rootstocks for certain parental combinations. Isozyme analysis was reported by them as the most reliable, simplest and cheapest *Hevea* clone identification method currently available. Estillai *et al.* (1990) suggested that isozyme markers associated with traits like rubber content, rubber quality and yield will facilitate Guayule

breeding. Qualitative zymogram studies of five enzymes in diploid, triploid and tetraploid clones of *Hevea* brasiliensis by Sreelatha *et al.* (1993) showed marked variations in banding patterns indicating the usefulness of isozymes for clone characterisation. Since isozyme polymorphism reflects the genetic variability, isozyme markers may be of immense value in demonstrating the degree of stock-scion interaction in *Hevea*.

Capscisin synthesis in *Capsicum annum* is a genetically controlled trait. Graft induced changes in capscisin content reported by Yagishita and Hirata (1987) and Yagishita *et al.* (1990) suggested the transmission of this characteristic from stock to scion and found that this transmitted character was stable. Isozyme analysis of Aspartate aminotransferase, leucine aminopeptidase, acid phosphatase, alkaline phosphatase and phosphogluco isomerase was reported in budded plants of *Hevea* clone RRII 105 by Krishnakumar *et al.* (1992). They also observed polymorphic isozyme expressions caused by stock-scion interaction.

4.4 CONCLUSIONS

1. Growth vigour, viz, height, stem diameter, total leaf area per plant, shoot biomass, etc. of the scion was found to be influenced by the rootstock.

2. Rootgrowth of the budded plants seemed to be influenced by the scion.

3. Stock-scion interaction exists in the root-shoot ratio of the budded plants.

4. Rootstock influences the CER of the scion. However, no significant relationship exists between stomatal conductance in plants before and after budding.

5. Mineral analysis of leaves before and after budding showed that stock has no profound influence on the mineral content of the scion except few cases. Fe content seemed to be influenced by stock-scion interaction especially in RRII 105.

6. Pigment composition and other biochemical parameters viz, reducing sugars, total sugars, phenols and amino acids of the scion indicated stock influence / stock-scion interaction.

7. Isozyme analysis of three enzymes, viz, esterase, peroxidase and catalase indicated genetic polymorphism in the scion which appears to be rootstock induced.

Chapter 5

COMPARATIVE STUDIES OF OWN ROOTED AND BUDDED PLANTS OF *HEVEA*

5.1 Introduction

The existence of stock-scion interaction in the budgrafted plants of *Hevea brasiliensis* and its impact on growth and productivity has been reviewed in the previous Chapters (Chapter 1 and Chapter 4).

Owing to the highly heterozygous nature of the rubber seeds, plants derived from them do not breed true to type. Hence it is not possible to obtain homogeneous seedling rootstocks for carrying out stock-scion interaction studies. Producing own-rooted plants of different clones of *Hevea* is ideal for such studies. Though it is possible to raise rooted plants of *Hevea* by stem cuttings it will be expensive as mentioned in Chapter 3. Air-layering can be successfully carried out using sphagnum moss for obtaining own-rooted plants as is evident from the results shown in Chapter 3. Studies on own-rooted plants

of different clones of *Hevea* may be useful in assessing the characters of that particular clone, since they are independent of any rootstock influence.

The ability of own-rooted plants of *Hevea* clones to absorb mineral nutrients compared with the same clones on seedling rootstocks is unknown. Cation exchange capacity of roots plays a vital role in the absorption of mineral nutrients (Crooke, 1964). Comparison of cation exchange capacity of own-rooted clonal plants, budded plants of the same clones and seedlings (before budding) on which these clones are budded will give a clear picture of the influence of stock or scion on these parameters. Similarly, foliar nutrient levels in these own-rooted and budgrafted plants reveal the role of stock/scion in the nutrient status of the budded plants.

The rooting pattern of the own-rooted plants needs specific evaluation since the performance of the vegetatively propagated plants depends on the desirable growth of the root system. No previous reports appear to be available either on the performance of own-rooted plants or their root system , raised through air-layering in *Hevea.* In view of this, comparative studies on the cation exchange capacity of own-rooted and budded plants and rooting pattern of these plants were carried out.

5.2 Materials and methods

The experiment on comparison of root CEC and foliar nutrient contents

of own-rooted and budded plants of *Hevea* comprised of three treatments, viz, seedlings (ungrafted), budded and own-rooted plants. Own-rooted plants of three clones viz, RRII 105, RRIM 600 and GT1, polyclonal seedlings and these seedlings budgrafted with the same three clones were used for the study. Upkeep and maintenance of these plants, manuring, etc., are as described in Chapter 2. The experiment was laid out in a completely randomised block design. All the observations were taken at the age of twelve months.

Cation exchange capacity (CEC) of lateral roots (dry roots) was measured in the above three types of plants as described in Chapter 2. N, P, K, Ca, Mg, Fe and Mn in the leaves of these own-rooted and budded plants were estimated as described in Chapter 2. The plants were uprooted and the rooting pattern was evaluated.

5.3 Results and discussion

5.3.1 Root system of one year old own-rooted plants

Observations on the root system of one year old own-rooted plants showed one or two strong roots with profuse lateral root growth. These main roots grew to a length of even more than 150 cm in some cases. In general, tap root was lacking in all these plants. But comparative evaluation of the root system of these plants and budded plants (Plate 11 a&b) indicated that the main roots of own-rooted plants grow much deep in the soil. The root system of rooted

stem cuttings of *Hevea* was evaluated earlier by Yoon and Leong (1975). Their studies on fourteen-month-old rooted cuttings showed that lateral roots were radiating in all directions or emerging from one side of the plants. It was also reported that treatment with basal ends of the cuttings inserted into six cm long polythene tubing before inserting into the sand bed for rooting only served to restrict the spread of the lateral roots initially. Root system of one year old own-rooted plants raised through air-layering in the present study seemed to be different from the root system reported in the stem cuttings. The roots lay interwoven with the sphagnum moss which are tied together inside the polythene film at the early stages probably directs growth of these roots downwards (Plate 11c). Another observation which can be considered as a promising characteristic of these plants is the profuse lateral root growth with fine rootlets. Studies by Soong (1976), on the morphology of *Hevea* root system at different ages of the tree conducted at Rubber Research Institute of Malaysia showed that rubber tree possesses an extensive rootsystem that can exploit large volumes of soil. It has a well developed tap root and lateral roots and also large numbers of fine rootlets. These fine rootlets often termed as feeder roots are the main absorbing zones of the rootsystem. Hence their development will influence the growth and nutrition of the rubber tree. The development and distribution of large number of such fine rootlets observed in the plants raised through air-layering may have beneficial effects on growth and nutrients absorption.

Plate 11 (a). Root system of one year old own-rooted plants of *Hevea* raised through air-layering

(b). Root system of one year old budgrafted plants of *Hevea*

(c). Rooted air-layer

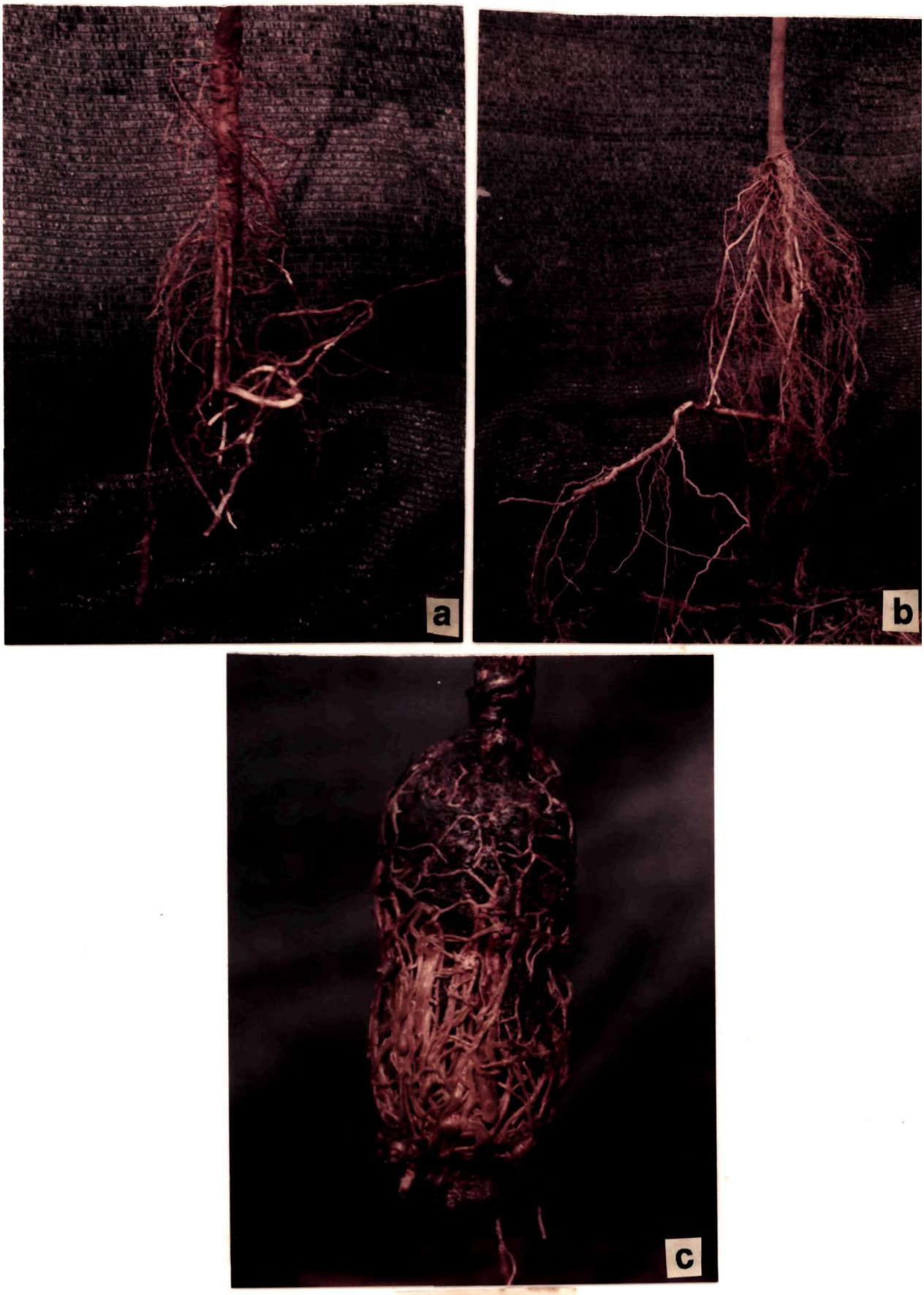


PLATE 11

5.3.2 Cation exchange capacity (CEC) of roots

Cation exchange capacity of lateral roots was compared in own-rooted plants of three clones of *Hevea*, viz., RRII 105, RRIM 600 and GT1, seedlings (before budding) and same seedlings budded with the above three clones (Table 22). Among the three clones studied, RRII 105 and GT1 showed higher CEC of roots in both own-rooted and budded plants than that of RRIM 600 (Table 22). There was no variation in CEC of roots between own-rooted and budded plants of clones RRII 105 and RRIM 600. However, the root CEC of the plants after budding with GT1 showed higher value than the same plants before budding and its own-rooted plants.

The results indicate that root cation exchange capacity may be a clonal character. Moreover, the comparative studies carried out with own-rooted, seedlings and budded plants clearly revealed that scion was controlling the cation exchange capacity of roots in all the three clones. RRIM 600 own-rooted plants showed lowest root CEC among the different treatments / clones observed. Further, budding the seedlings with RRIM 600 lowered the CEC of roots of the plants (Table 22). This observation further supported the influence of scion on root CEC of stock plants. Comparatively low root CEC exhibited by budded plants of RRIM 600 and influence of scion on root cation exchange capacity of stock plants were reported earlier by Sobhana *et al.* (1980).

Table 22. Root cation exchange capacity of own-rooted, seedlings (ungrafted) and budded plants of three clones of *Hevea.*

Root type	CEC (meq/100g dry root)		
	RRII 105	RRIM 600	GT1
Own-rooted	37.26 a	26.08 a	34.33 a
Seedlings (ungrafted)	32.18 b	34.75 b	31.44 b
Budded plants	39.27 a	27.47 a	38.76 c

Means followed by the same letter in columns are not statistically different at 5% level (Duncan's Multiple range test).

The histograms (Figs. 40&41) represent similarities in root CEC in the own-rooted and budded plants of the three clones and also the influence of scion on stock plants.

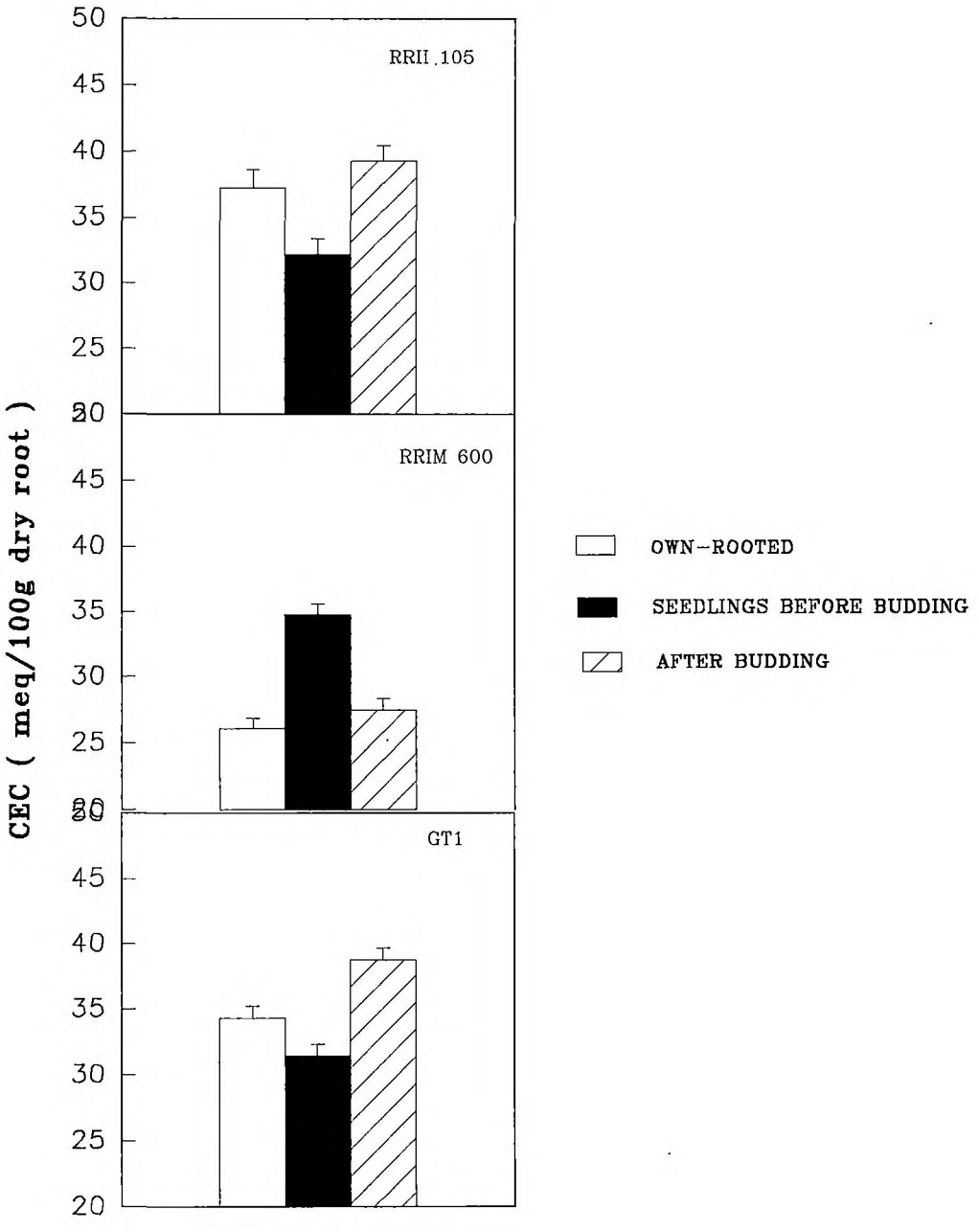


Fig.40: Root cation exchange capacity of own-rooted, plants before and after budding of three clones of Hevea.

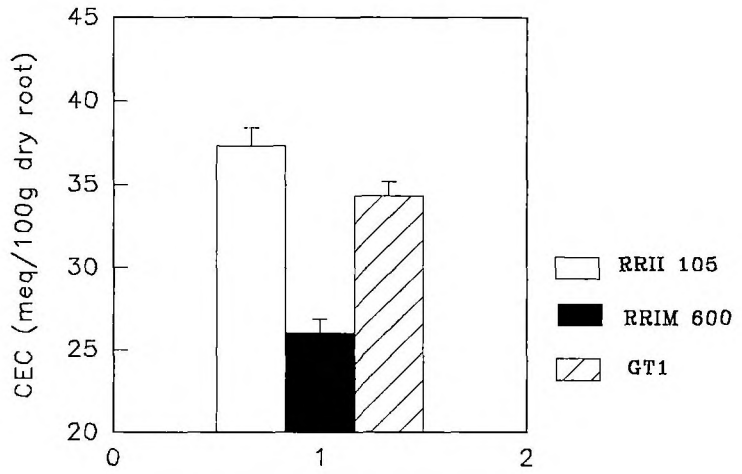


Fig.41a. Root cation exchange capacity of own-rooted plants of three clones of Hevea

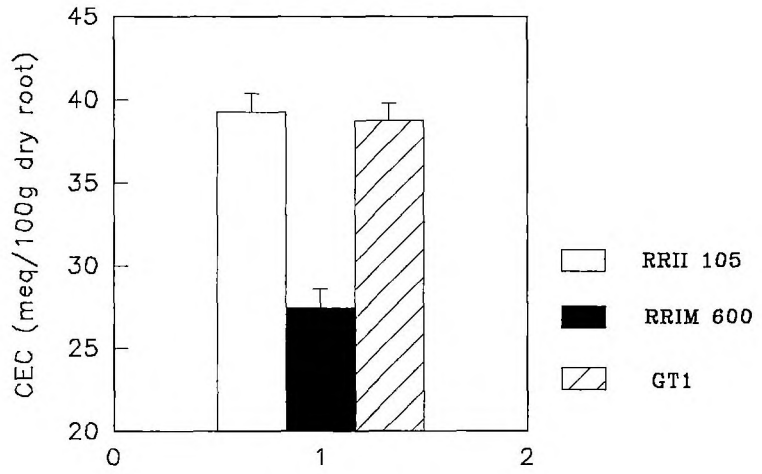


Fig.41b. Root cation exchange capacity of budded plants of three clones of Hevea

5.3.3 Mineral nutrition

(i) Nitrogen

When foliar N content of own-rooted and budded plants of three clones, viz., RRII 105, RRIM 600 and GT1 were compared budded plants have significantly high leaf N in all the clones (Table 23). Among the own-rooted plants foliar N was higher in GT1. However, there was no significant variation in foliar N among the budded plants of these clones.

Table 23. Comparison of foliar N, P and K contents in own-rooted and budded plants of three clones of *Hevea.*

Clone	Foliar elements (dry wt. basis)					
	N(%)		P(%)		K(%)	
	Own-rooted	Budded plants	Own-rooted	Budded plants	Own-rooted	Budded plants
RRII 105	2.20a	3.12a	0.17a	0.19a	0.86a	0.95a
RRIM 600	2.31a	3.16a	0.19a	0.19a	0.92a	1.01a
GT1	2.66b	3.32a	0.20a	0.20a	1.21b	0.92a

Means followed by same letter in columns are not statistically different at 5% level (Duncan's Multiple range test).

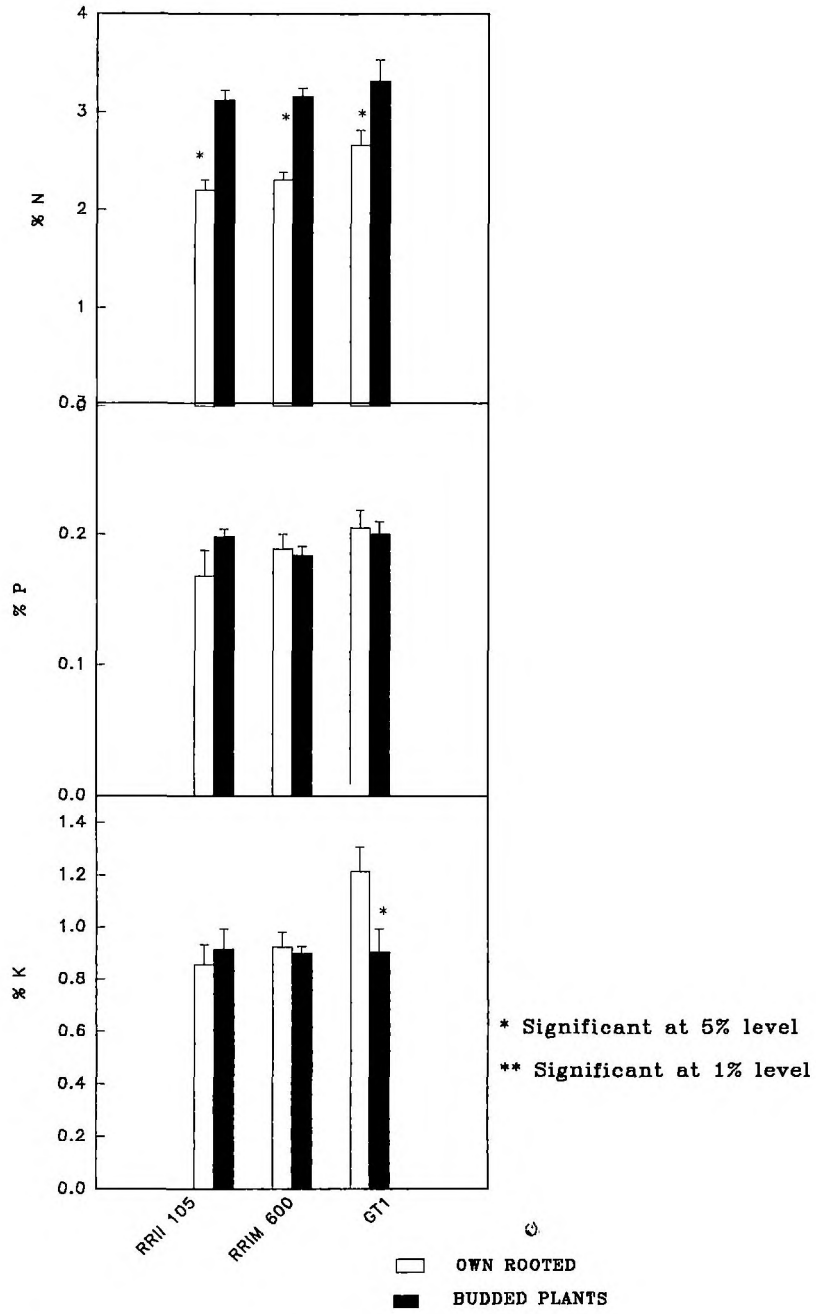


Fig.42:Comparison of foliar N,P and K in own-rooted and budded plants of three clones of Hevea.

(ii) Phosphorus

Leaf P concentration in both own-rooted and budded plants of the three clones did not show any variation in our present study. Between clones also, no variation was observed in foliar P content (Table 23).

(iii) Potassium

Own-rooted plants of GT1 had significantly high foliar K than RRII 105 and RRIM 600 (Table 23). But there was no clonal difference in foliar K contents in budded plants of these three clones. When own-rooted plants and budded plants of these clones were compared significant difference could be observed in leaf K content of GT1 only (Fig.42).

(iv) Calcium

Calcium levels in the leaves of own-rooted plants of GT1 was significantly higher than plants of the same clone budded on different rootstocks (Fig. 43). Though the difference was not statistically significant, the mean of foliar Ca levels were higher in own-rooted plants than the budded plants of the other two clones RRII 105 and RRIM 600 also (Table 24).

There was no significant variation in foliar Ca levels of own-rooted plants among these three clones. However, Ca level was significantly lower in budded plants of GT1 when compared with budded plants of

RRII 105 and RRIM 600.

(v) Magnesium

Foliar Mg content was significantly higher in own-rooted plants of all the three clones than the budded plants of the same clones on different rootstocks (Fig.43). However, there was no clonal variation in foliar Mg contents in both own-rooted as well as budded plants of RRII 105, RRIM 600 and GT1 (Table 24).

Table 24. Comparison of foliar Ca and Mg contents in own-rooted and budded plants of three clones of *Hevea.*

Clones	Foliar elements (dry wt. basis)			
	Ca(%)		Mg(%)	
	Own- rooted plants	Budded plants	Own-rooted plants	Budded plants
RRII 105	0.98 a	0.91a	0.27a	0.17a
RRIM 600	1.002a	0.76a	0.24a	0.17a
GT1	1.04 a	0.55b	0.28a	0.21a

Means followed by same letter in columns are not statistically different at 5% level (Duncan's Multiple range test).

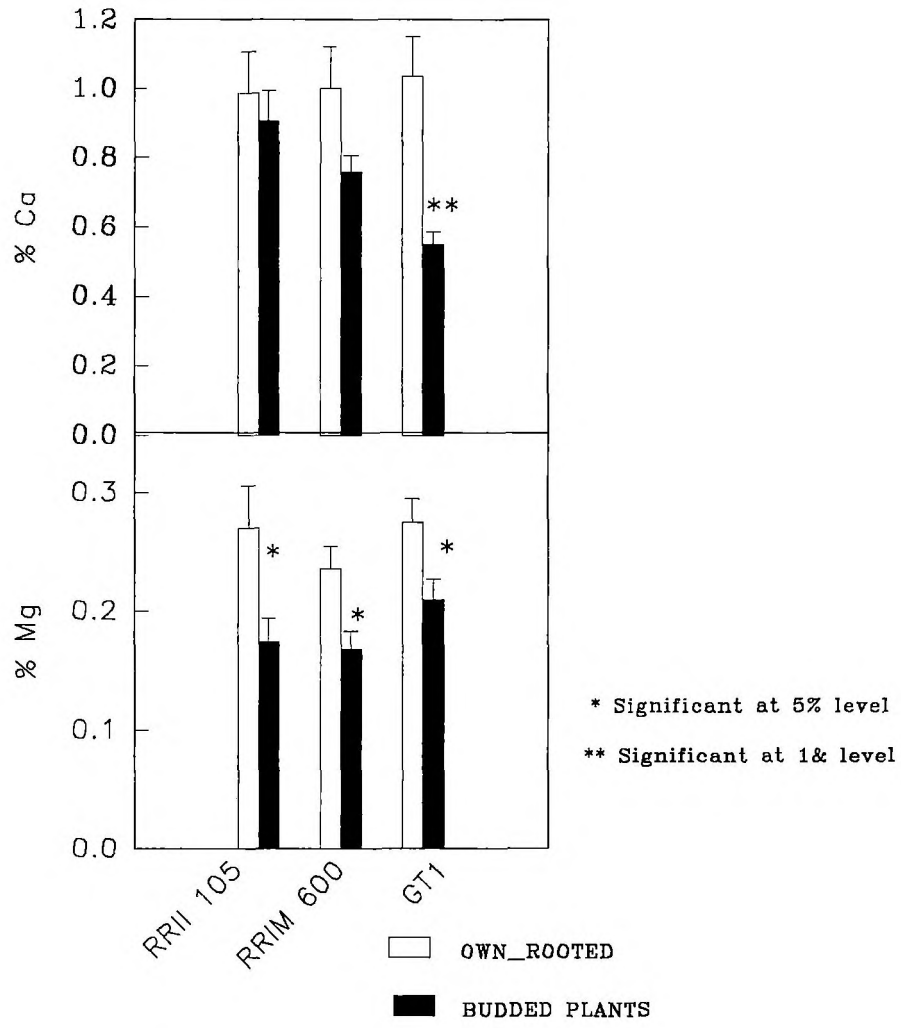


Fig.43:Comparison of foliar Ca and Mg in own-rooted and budded plants of three clones of Hevea.

(vi) Micronutrients

Analysis of foliar micronutrients, viz., Fe and Mn in own-rooted and budded plants of the clones RRII 105, GT1 and RRIM 600 revealed that significant variations existed in the foliar concentrations of these two elements. Of special interest was the significantly very high Iron content in the leaves of own-rooted plants of RRII 105 when compared with the budded plants of thesame clone (Table 25).

Table 25. Comparison of foliar Fe and Mn contents in own-rooted and budded plants of three clones of *Hevea*.

Clone	Foliar elements (dry wt. basis) Fe(ppm)		Mn(ppm)	
	Own-rooted plants	Budded plants	Own-rooted plants	Budded plants
RRII 105	1298.37a	657.13a	174.36a	104.90a
RRIM 600	584.60b	560.77a	160.80a	89.50a
GT1	483.98b	659.83a	202.98a	93.27a

Means followed by same letter in columns are not statistically different at 5% level (Duncan's Multiple range test).

Foliar Fe in own-rooted plants of GT1 was significantly lower than the budded plants of the same clone on different rootstocks (Fig.44). When

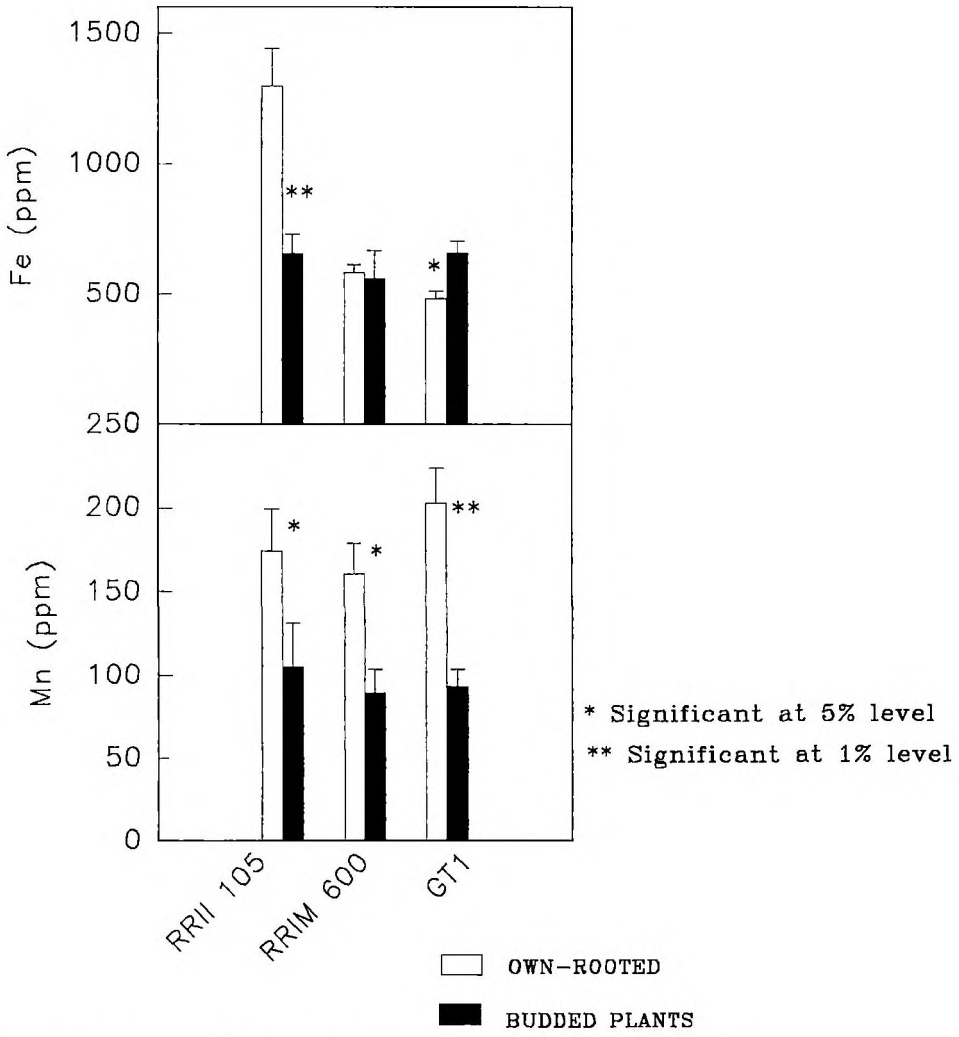


Fig.44: Comparison of foliar Fe and Mn in own-rooted and budded plants of three clones of Hevea

clonewise comparisons were made between the own-rooted and budded plants of the three clones significant variations existed in foliar Fe among the own-rooted plants of these clones, ie, RRII 105 having the highest Fe level and GT1 the lowest(Table 25). However, there was no clonal variation in foliar Fe in budded plants of these three clones.

When foliar Mn levels of own-rooted and budded plants of three clones were compared , the levels were higher in own-rooted plants in RRIM 600 and GT1. The mean level was also high in RRII 105 though not significant. But there was no clonal variation in foliar Mn levels in own-rooted as well as budded plants of these three clones.

In the present study, foliar N, P, K, Fe and Mn concentrations of budded plants of three clones were having fairly similar ranges, regardless of the rootstocks/scions. Rootstock effect on nutrient uptake of certain scions was reported in *Hevea* earlier by Teng and Pushparajah (1974). They also reported that certain rootstocks reflected poor uptake of some nutrient elements but not with other elements. A nutritional study on apple rootstocks showed that rootstock effects were observed for all leaf nutrient elements except Cu (Simmons, 1989). The higher leaf N and K content of own-rooted plants of GT1 observed in the present study showed the higher absorption ability of N and K of this clone. The levels of Ca and Mg also were high in own-rooted plants of GT1 than its budded plants. Higher levels of leaf Ca and Mg observed in

own-rooted plants of *Hevea* in the present study are in conformity with the earlier reports in peaches (Couvillon,1982). These differences in Ca and Mg are thought to be due to the presence of a graft union since leaf tissue from the scion grafted onto own-rooted cutting of the same clone had lower Ca levels than the leaf tissue of own-rooted plant of that clone (Couvillon,1982). Uptake and translocation of Ca may be influenced by factors affecting water movement in the plant (Knowels *et al.*, 1984). The principal pathway of Ca movement across the cortex is in the apoplasm (Haynes,1980). Any disturbance in the movement may restrict the entry of these ions from apoplasm into symplasm. Possibly, the graft combinations with GT1 may hinder Ca transport more than the other two clones resulting in significantly lower foliar Ca level in the budded plants of the same clone.

Cation exchange capacity of the root is thought to be a selective factor which allows the mineral composition of the plant to be controlled (Crooke and Knight, 1962). Some authors have directly related root CEC to the mineral content of the plant (Smith and Wallace, 1956a, b; and Frejat *et al.*, 1967). Despite the high root CEC in the budded plants of RRII 105 and GT1 foliar Ca and Mg concentrations in these clones were not showing higher values in our present study. The scion influence being similar to the root influence implies that the CEC of the conducting tissues regulates movement of mono and divalent cations to shoots (Wallace and Mueller, 1980).

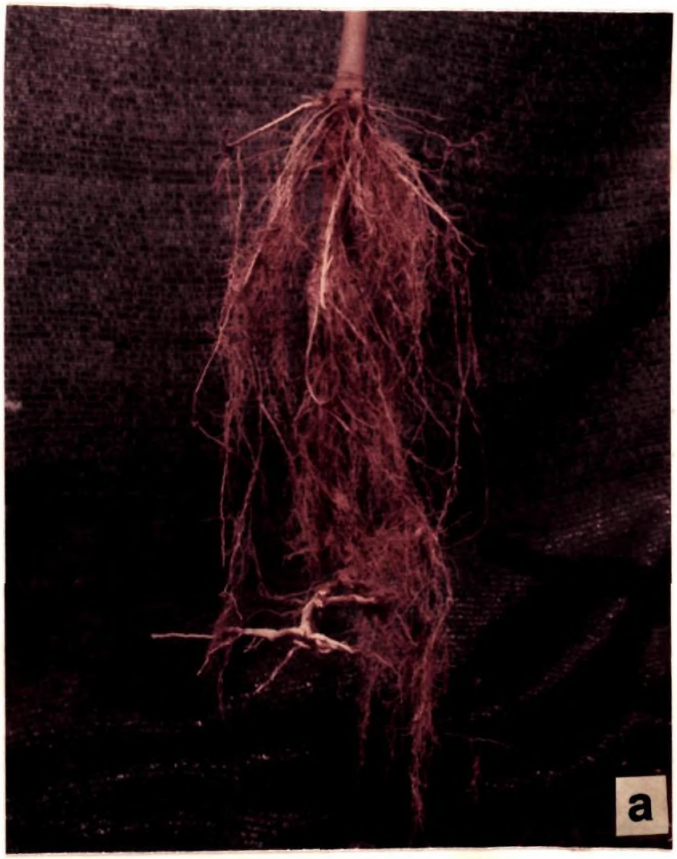
The mean level of leaf Ca in own-rooted plants of RRII 105 was the lowest among the own-rooted plants of the three clones in spite of the highest root CEC observed in the clone. Highest leaf Fe content, almost two times higher than the other two clones, in the own-rooted plants of RRII 105 may contribute to the lower leaf Ca level. Trace metals at high levels can inhibit Ca uptake by plants eventhough Ca often regulates their uptake and influence (Wallace *et al.,* 1971). Moreover, it was reported that when Fe concentration was greatest, Ca concentration was lowest and vice versa in oats cultivars and the differential absorption - transport of Ca by these cultivars may be associated with their ability to absorb - transport Fe (Brown,1979). Trivalent cations like Fe^{+3} and Al^{+3} held in the apoplasm of roots inhibit the uptake of divalent cations such as Ca^{+2} (Johnson and Jackson,1964; and Clarkson and Sanderson,1971). Eventhough, leaf Fe was significantly high in own-rooted plants of RRII 105 it was not significantly different from other clones in the budded plants. This indicated that though the absorption efficiency of Fe seems to be a clonal character it is not reflected in the budded plants since Fe level may be affected by stock-scion interaction.

5.4 Plants with double root system

An attempt was made to induce rooting in the scion portion of the budded plants in addition to the usual stock roots. This experiments was carried out with an assumption that these plants having the double root system may

Plate 12 (a). Budgrafted plant of *Hevea* with roots from both scion and Rootstock

(b). Enlarged view of the double root system



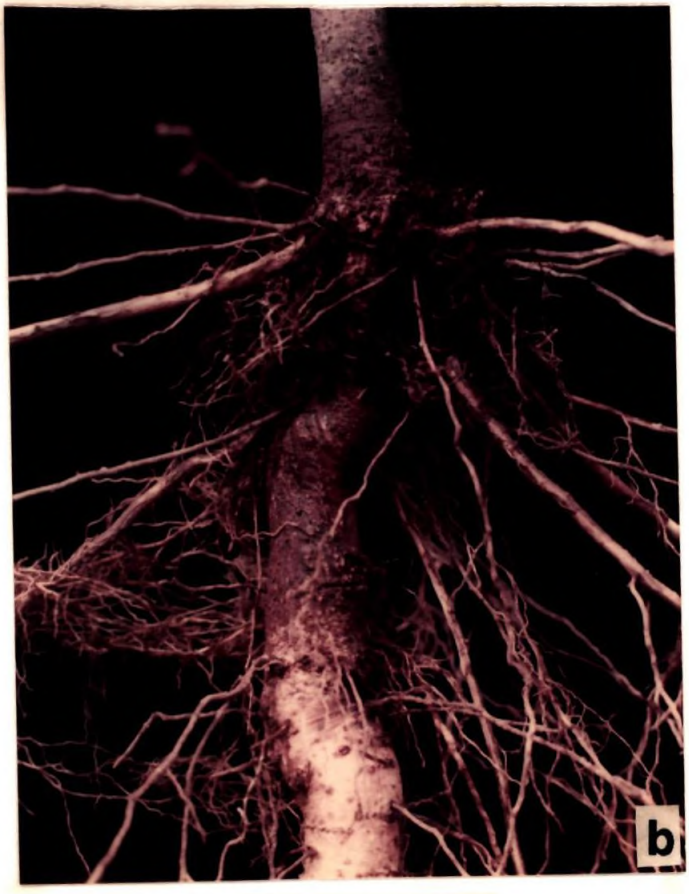


PLATE 12

dilute the influence of rootstock to some extent. The method of raising plants with double-root system is described in Chapter 2. A preliminary study was conducted in five clones viz, RRII 208, RRII 118, GT1, PCK 2 and PB 260. Both the rootsystems were developed in these clones and profuse lateral root growth was observed in the scion portion in addition to the usual tap root of the rootstocks (Plate 12 a&b).

5.5 CONCLUSIONS

1. Though tap roots are lacking in plants raised through air-layering, one or two strong roots with profuse lateral root growth were observed.

2. Cation exchange capacity of roots in the budded plants seemed to be determined by the scion.

3. Own-rooted and budded plants differ in the foliar nutrient concentration and rootstock has not much influence on the mineral nutrition of the budded plants except the existence of stock-scion interaction in few elements.

4. It is possible to raise plants having double-root system, ie. roots in the scion and stock, which will enable further detailed studies on stock-scion interaction.

SUMMARY

Hevea brasiliensis, the major source of natural rubber, is propagated commercially by budgrafting. This method of propagation results in considerable variability due to stock-scion interaction. Vegetative propagation by stem cuttings is not practiced commercially due to the lack of tap root system and difficulty in inducing rooting. One of the constraints for studying stock-scion interaction in *Hevea* is the lack of sufficient homogeneous clonal rootstocks. A good mist propagation system, which is very expensive, is essential for the production of rooted cuttings. In view of the above, experiments were carried out to study the (1) Physiology of rooting in *Hevea* (2) Stock-scion interaction in *Hevea* (3) Performance of own-rooted plants of *Hevea.*

Air-layering studies were carried out in three clones of *Hevea,* viz, RRII 105, RRIM 600 and GT1 to standardise the technique to obtain optimum rooting success. The results of the experiments showed that sphagnum

moss soaked in water was found to be the best rooting medium and root formation was observed in the air-layers after about 45 days of layering.

To find out the clonal response and age on rooting, air-layering studies were carried out in twelve clones of *Hevea* at three ages of growth. Variability of rooting in these clones ranged from 0-95%. Survival of the transplanted air-layers also showed considerable variability. Based on rooting success in one year old plants, six clones (above 75% rooting success) were grouped as easy-to-root, two as medium-to-root (45-75%) and four as hard-to-root (below 45%) types. It was observed that age of the plant has a definite effect on percent of rooting and survival of rooted layers. Generally first and second year old plants gave better rooting percent and survival. Percent of rooting and mean number of roots per layer exhibited a strong positive relationship. Likewise percent of rooting and survival of rooted layers of these clones at all the three ages indicated a strong positive relationship. Moreover, survival of rooted layers and mean number of roots per layer also showed positive relationship.

Biochemical characterisation of easy-to-root and difficult-to-root clones revealed marked differences in sugar and phenol content of bark. Percent of rooting showed positive relationship with sugar and phenol content.

Growth regulators viz, IBA, NAA and IAA (at four concentrations 100, 250, 500 and 1000 ppm) and Rootex (a commercial formulation of IBA) were

tried to find out the effect of these on rooting in air-layers of three clones of *Hevea.* There was an appreciable increase in the percent of rooting as well as mean number of roots per layer with the application of these growth regulators. The optimum concentration of these hormones was different in different clones. The highest concentration of 1000 ppm used in the present study was found to be inhibiting the percent rooting as well as mean number of roots per layer in all the clones studied.

Stock-scion interaction studies were carried out in eighteen months old budgrafted plants of five clones of *Hevea,* viz, RRII 105, RRII 208, RRIM 600, GT1 and Gl1. Growth characteristics viz, plant height, stem diameter, total number of leaves, leaf area, biomass, root:shoot ratio, etc., physiological aspects viz, CER, gs, transpiration rate, mineral nutrition, etc. and biochemical parameters viz, carbohydrates, amino acids, phenols, total chlorophyll, isozymes, proteins, etc. were studied.

Highly significant positive correlation was observed between height of the rootstocks (before budding) and height of the scion after budding. Similarly stem diameter of the rootstocks also showed positive correlation with stem diameter of the scion. Moreover, significant positive correlation existed between stem diameter of the rootstock at budgrafting and scion height at 18 months after budgrafting in the five clones. Likewise, height of the rootstocks at budgrafting also showed a significant positive correlation with the stem diameter of the scion

at 18 months after budgrafting. This further confirms that the vigor (both height and diameter) of the rootstock plants exhibit a strong positive influence on scion growth. Total leaf area of the plants before budding showed a strong positive relationship with that of the scion. The above ground biomass also showed similar relationship. Rootgrowth of the budgrafted plants of five clones at 18 months after budgrafting showed that scion controls the root growth of the budgrafted plants. Root : shoot ratio revealed that there was no significant differences among the five clones. Moreover, the root : shoot ratio is maintained in all the clones irrespective of the higher or lower root / shoot growth.

Carbon dioxide exchange rate (CER) of the rootstocks (before budding) and budgrafted plants after 18 months of budding showed a positive correlation indicating the influence of rootstock on CER of the scion. Clonal differences were also observed in the CER of leaves. Stomatal conductance (gs) did not vary much among the five groups of rootstock plants on which the five clones were budgrafted, but a higher CV was observed among the budgrafted plants of these clones. There was no significant relationship between gs of the rootstock plants before budding and budgrafted plants.,

Foliar N, P, K, Ca, Mg, Fe and Mn analysis of seedlings (rootstocks before budding) and 18 months old budgrafted plants of five clones revealed clonal differences for certain elements only. The degree of nutrient variability among the plants within a clone in different clones was different for various

elements. Significant differences observed among the clones in foliar N, P and K contents indicated that these nutrient contents are determined by the scion. No relationship observed in the foliar content of the above elements in the rootstock plants and scion indicated that rootstock had no influence on foliar N, P and K contents of the budgrafted plants. Foliar Ca and Mg content showed no significant differences among the different clones studied. A positive relationship was exhibited by the clone GT1 alone, between the foliar Mg content of rootstock and scion. But the stock influence on foliar Mg is not reflected in all the clones studied, indicating that clones may be specific towards the stock influence in different parameters.

Micronutrient analysis (Fe and Mn) of leaves of rootstock before budding and scion after 18 months of budgrafting indicated no significant relationship. But high CV in foliar Fe among the budgrafted plants of RRII 105 and an appreciable reduction in CV among the budgrafted plants than their corresponding stock plants in clones GT1 and RRIM 600 indicated the possibility of stock-scion interaction . The observation of positive relationship between foliar Mn of rootstocks (before budding) and scion after 18 months of budgrafting in the clone Gl1 alone indicated that there may be clonal differences in the response to stock influence.

Biochemical studies were carried out in 18 months old budgrafted plants of five clones. Pigment composition viz, chlorophyll a, chlorophyll b, total

chlorophyll, chlorophyll a/b ratio were studied and significant differences were observed in the mean values of all these components. Significant clonal differences in the total chlorophyll content observed in the present study indicated that pigment composition is a clonal character and rootstock has no profound influence on pigment composition of the scion.

Carbohydrates viz, reducing sugar contents in the budgrafted plants showed appreciable variations in the five clones studied. But total sugar content was not showing similar variations. Phenol and amino acid concentration also showed variations among clones and plants within clones. Wide variations exhibited in the concentration of reducing sugar among clones may be due to the differences in growth vigor of the plants. Considerable CV observed among plants within a clone in the biochemical parameters viz, reducing sugar, phenol and amino acids indicated stock influence or existence of stock-scion interaction.

Isozyme studies of three enzymes viz, peroxidase, catalase and esterase were carried out in budgrafted plants of five clones. All the three isozymes analysed in the present study revealed different banding patterns among clones and plants within clones with respect to number of bands and intensity of the bands. From the observations it can be concluded that there was a definite indication of genetic polymorphism in the scion which appears to be rootstock induced. Irrespective of the concentration of the total proteins in the

leaf extracts, there were specific bands present or missing in one or more of the six budded plants propagated from a single mother plant. In other words, evenwhen the total protein content in the extract was less there were additional bands appearing and *vice versa.* This suggests that low concentration of the protein was not responsible for missing bands and rather specific bands were missing evenwhen the total protein concentration was high suggesting genetic polymorphism.

Comparative studies of the performance of own-rooted plants raised by air-layering and budgrafted plants of three clones viz, RRII 105, RRIM 600 and GT1 were carried out. Studies on the root system of these two types of plants revealed that though tap root system is lacking in the own-rooted plants raised through air-layering, one or two strong roots with profuse lateral root growth were observed and these main roots grow deep in the soil.

Cation exchange capacity (CEC) of roots of seedlings (before budding), budgrafted plants and own-rooted plants of three clones were compared. The results indicated that root CEC may be a clonal character and scion was controlling the CEC of roots in all the three clones.

Foliar N, P, K, Ca, Mg, Fe and Mn of the above plants were also analysed and observed that budded plants of these three clones were having fairly similar ranges of these elements, regardless of the rootstocks/scion.

An attempt was made to induce rooting in the scion portion of the budded plants in addition to the usual stock roots. This experiment was carried out with an assumption that these plants having the double root system may dilute the influence of the rootstock to some extent. A preliminary experiment was conducted in five clones viz, RRII 208, RRII 118, GT1, PCK 2 and PB 260. Both the rootsystems were developed in these clones and profuse lateral root growth was observed in the scion portion in addition to the usual tap root of the rootstocks.

REFERENCES

Acharyya,N. and Dash,P.C. (1972). Effect of two plant growth substances on cashew air-layers. *Current Science,* **41**(4): 534-535.

Ahmed,H.S. and Al-Shurb,M.Y. (1984). Effect of rootstock on the leaf mineral content of citrus. *Scientia Horticulturae,* **23**: 163-168.

Alberte,R.S., Mc Clure.P.R. and Thornber,J.P. (1976). Photosynthesis in trees. Organisation of chlorophyll and photosynthetic unit size in isolated gymnosperm chloroplasts. *Plant Physiology,* **58**: 341-344.

Allurwar,M.W. and Parihar,S.K. (1992). Comparative study of root systems of common root-stocks of orange. *Journal of Soils and Crops,* **2**(1): 100-101.

Altman,A. and Wareing,P.F. (1975). The effect of IAA on sugar accumulation and basipetal transport of C^{14}-labelled assimilates in relation to root formation in *Phaseolus vulgaris* cuttings. *Physiologia Plantarum,* **33**: 32-38.

Alvino,A., Zerbi,G. and Turci,E. (1989). The effect of rootstocks and water table on the nutritional status of cv. Maycrest peach. *Advances in Horticultural Sciences,* **3**(2): 51-54.

Anand,V.K. and Heberlein,G.T. (1975). Seasonal changes in the effects of auxin on rooting in stem cuttings of *Ficus infectoria. Physiologia Plantarum,* **34**: 330-334.

Andrew,M.T. and Brent,T. (1980). Leaf isozyme as genetic markers in date palm. *American Journal of Botany,* **67**: 162-167.

Arulsekar,S. and Parfitt,D.E. (1986). Isozymes analysis procedures for stone fruits, almond, grape, walnut, pistachio and fig. *Hortscience,* **21**: 928-933.

Arulsekar,S., Parfitt,D.E. and McGrahanan,G.H. (1985). Isozyme gene markers in Juglans species: Inheritance ofGPI and AAT in *J.regia* and *J.hindsii*. *Journal of Heredity*, **76**: 103-106.

Arulsekar, S., McGrahanan, G.H. and Parfitt, D.E. (1986). Inheritance of phosphoglucomutase and esterase isozymes in Persian walnut (*Juglans regia* L.). *Journal of Heredity*, **77**(3): 220-221.

Asokan, M.P., Sobhana, P. and Sethuraj, M.R. (1988). Tissue culture propagation of rubber (*Hevea brasiliensis* (Willd. Ex Adr. De Juss.) Muell. Arg.) clone GT (Gondang Tapen) 1. *Indian Journal of Natural Rubber Research*, **1**(2): 10-12.

Audus, L.J. (1953). *Plant growth substances.* Interscience Publishers, Inc., New York.

Balakrishnamurthy, G. and Rao. V.N.M. (1988). Changes in phenols during rhizogenesis in rose (*Rosa bourboniana* Desp.). *Current Science*, **57**(17): 960-962.

Banerjee, D.P., Chatterjee B.K., Rao,D.P. and Dutta,D. (1982). Propagation of cashewnut by air-layering. *National seminar on plant propagation*, December 27-29, 1982, West Bengal, p.71.

Barden, J.A. and Ferree, D.C. (1979). Rootstock does not affect net photosynthesis, dark respiration, specific leaf weight and transpiration of apple leaves. *Journal of American Society for Horticultural Science*, **104**(5): 526-528.

Bargioni,G. and Baroni,G. (1995). Scion inclination in *Malus domestica* Borkh. and *Prunus* spp. Influences root growth and distribution. *HortScience*, **30**(3):517-520.

Barone,E., Marco,L.D., Marra,F.P. and Sidari,M. (1996). Isozymes and canonical discriminant analysis toidentify pistachio (*Pistacia vera* L.) germplasm. *Hortscience*, **31**(1): 134-138.

Bartolini,G. (1994). Interrelation of carbohydrates , rooting and survival of hardwood cuttings. *Advances in Horticultural Science*, **8**(3): 131-133.

Basu, R.N., Bose, T.K., Roy, B.N. and Mukhopadhyay, A. (1969). Auxin synergists in rooting of cuttings. *Physiologia Plantarum*, **22**: 649-652.

Baugher,T.A., Suman Singha., Leach,D.W. and Walter,S.P. (1994). Growth, productivity, spur quality, light transmission and net photosynthesis of 'Golden Delicious' apple trees on four rootstocks in three training systems. *Fruit Varieties Journal,* **48**(4): 251-255.

Beakane,A.B. and Rogers,W.S. (1956). The relative importance of stem and root in determining rootstock influence in apples. *Journal of Horticultural Science,* **31**: 99-110.

Bergamini,A. and Angelini,S. (1988). Analysis of the dimensions of apple leaves as a function of cultivar, rootstock and type of branch. *Societa Orticola Italiana,* (1988), 475-484.

Bergamini,A., Angelini,S. and Bigaran,F. (1986). Influence of four different rootstocks on the stomatal resistance and leaf water potential of Golden Delicious clone B. subjected to different irrigation regimes. *Societa Orticola Italiana,* (1988),533-544.

Bhandary,K.R. and Kololgi,S.B. (1960). Studies on seasonal effect of growth regulators in combination on rooting of air layers of Guava. L-49 Lalbagh. *Journal of Horticultural Science,* **5**(4): 12-18.

Bhatt,R.I. and Chundawat,B.S. (1982). Studies on effect of time of ringing and rooting media on rooting and survival of air-layers of guava (*Psidium guajava* L.). *National seminar on plant propagation,* December 27-29, 1982, West Bengal.

Bhatia,C.R., Buialti,M. and Smith,H.H. (1967). Electrophoretic variation in proteins and enzymes of the tumor forming *Nicotiana glauca x N.langsdorfii* and its parent species. *American Journal of Botany,* **54**:1237-1241.

Bhujbal, B.G. (1972). Effective concentration of IBA in the air-layering of guava. *Research Journal of Mahatma Phule Agricultural University,* **3**: 53-56.

Bian,C.T. (1976). Vegetative propagation of cashew (*Anacardium occidentale* L.). *Proceedings of National Plant Propagation Symposium,* Kuala Lumpur, Malaysia, pp. 99-105.

Bid,N.N. and Mukherjee,S.K. (1969). Varietal response to etiolation and growth regulator treatment in air-layering of Mango (*Mangifera indica L.). Indian Journal of Agricultural Sciences,* **39**: 1013-1019.

Bose,T.K., Mukhopadhyay,T.P. and Basu,T.K. (1982). A note on the effect of ascorbic acid and IBA on rooting of cuttings. *Indian Journal of Plant Physiology,* **25**: 310-312.

Bould, C. and Campbell, A.I. (1970). Virus, fertilizer and rootstock effects on the nutrition of young apple trees. *Journal of Horticultural Science,* **45**: 287-294.

Boyhan,G.E., Nirton,J.D. and Pitts,J.A. (1995). Establishment, growth, and foliar nutrient content of plum trees on various rootstocks. *HortScience,* **30**(2): 219-221.

Brancadoro,L., Valenti,L., Reina,A. and Scienza,A. (1994). Potassium content of grapewine during the vegetative period: the role of the r o o t s t o c k. *Journal of Plant Nutrition,* **17**(12): 2165-2175.

Bringhurst, R.S., Arulsekar,S., Hancock, Jr.,J.F. and Voth,V. (1981). Electrophoretic characterisation of strawberry cultivars. *Journal of American Society for Horticultural Science,* **106**: 684-687.

Brown, J.C. (1979). Role of Calcium in micronutrient stresses of plants. *Communications in Soil Science and Plant Analysis,* **10**(1&2): 459-472.

Brown,C.S., Young,E. and Pharr,D.M. (1985). Rootstock and scion effects on the seasonal distribution of dry weight and carbohydrates in young apple trees. *Journal of American Society for Horticultural Science,* **110**(5): 696-701.

Brown,C.S., Young,E. and Pharr,D.M. (1985). Rootstock and scion effects on carbon partitioning in apple leaves. *Journal of American Society for Horticultural Science,* **110**(5): 701-705.

Brown,S.K. and Cummins,J.N. (1989). Rootstock effect on foliar nutrient concentrations of "redhaven" peach trees. *Hortscience,***24**(5):769-771.

Brown,P.H., Zhang,Q.L. and Ferguson,L. (1994). Influence of rootstock on nutrient acquisition by pistachio. *Journal of Plant Nutrition,* **17**(7):1137-1148.

Bussi,C., Besset,J. and Duc,A. (1995). Rootstocks effects on growth and fructification of peach trees (*Prunus persica* (L) Batsch). *Fruits* (Paris), 50(2): 125-132.

Bussi,C., Huguet,J.G. and Besset,J. (1995). Rootstocks effects on growth and fruit yield of peach. *European Journal of Agronomy,* **4**(3): 387-393.

Buttery,B.R. (1961). Investigations into the relationship between stock and scion in budded trees of *Hevea brasiliensis. Journal of Rubber Research Institute of Malaysia,* **17**: 46-76.

Cardy,B.J. and Kannenberg. (1982). Allozymic variability among maize inbred lines and hybrids. Application for cultivar identification. *Crop Science,* **22**: 1016-1020.

Carron, M.P., Enjarlic, F., Lardent, L. and Derchamps, A. (1988). Rubber (*Hevea brasiliensis* Muell. Arg.). In: *Biotechnology in Agriculture and Forestry.* (ed). Bajaj, Y.P.S., Berlin, 222-245.

Carver,B.F., Burton,J.W. and Wilson,R.F. (1987). Graft-tranmissible influence on fatty acid composition of soybean seed. *Crop Science,* **27**(1): 53-56.

Castle, W.S. and Krezdorn, A.H. (1975). Effect of citrus rootstocks on root distribution and leaf mineral content of 'Orlando' tangelo trees. *Journal of American Society for Horticultural Science,* **100**(1): 1-4.

Castro,P.R.C. *et al.* (1990). Comparative growth analysis of *Hevea brasiliensis* Muell. Arg. scion and rootstock in the nursery. *Anais da Escola Superior de Agricultura Luiz de Queiroz,* **47**(1): 29-45.

Chandra, J.P. and Yadava, M.P.S. (1986). Clonal propagation of mysore gum (*Eucalyptushybrid). Indian Forester,* 783-791.

Chandrababu, R., Balasubramanyan,S. and Azhakiamanavalan,R.S. (1982). The influence of growth substances in successful air-layering of certain horticultural crops. *National seminar on plant propagation,* December 27-29, 1982, West Bengal. p. 68.

Channaveerappa, G.S. and Gowda, J.V.N. (1984). Studies on vegetative propagation of *Michelia champaka* L. by air-layering. *Progressive Horticulture,* **16**(1-2): 97-100.

Chaparro,J.X., Durham,R.E., Moore,G.A. and Sherman,W.B. (1987). Use of isozyme techniques to identify peach x 'non pariel' almond hybrids. *Hortscience,* **22**: 300-302.

Chaplin,M.H. and Westwood,M.N. (1980). Effects of *Pyrus* species and related genera rootstocks on mineral uptake in 'Barlet' Pear. *Journal of Plant Nutrition,* **2**(3): 335-346.

Chauhan,P.S. and Dua,I.S. (1982). *Improvement of Forest Biomass,* P.K. Khosla (Ed) Pragathi Press, Delhi: 187-191.

Chevallier,M.H. (1988). Genetic variability of *Hevea brasiliensis* germplasm using isozyme markers. *Journal of Natural Rubber Research,* **3**(1): 42-53.

Chhonkar,V.S. and Singh,R. (1967). Effect of plant regulators on air-layering in cashewnut (*Anacardium occidentale* L.) . *Indian Journal of Horticulture,* **24**: 26-29.

Cobianchi,D., Liverani,A, and Marangoni,B. (1988). Nutrient status of Flavortop and Redhaven peaches on various rootstocks in different agronomic situations. *Societa Orticola Italiana* (1988), 151-167.

Combe,J.CL. and Gener,P. (1977). Effect of stock family on the growth and production of grafted *Hevea. Journal of Rubber Research Institute of Sri Lanka,* **54**(1): 83-92.

Conkle,M.T., Hodgkiss,D.P., Nunnally.B.C. and Hunter,C.S. (1982). In: *Starch gel electrophoresis of conifer seeds: A laboratory mannual.* Pacific South West Foerest and Range Experiment Station, Berkley, California.

Copes, D.L. (1970). Initiation and development of graft incompatibility symptoms in Douglas-fir. *Silvae Genetics,* **19**: 77-82.

Copes, D.L. (1978). Isozyme activities differ in compatible and incompatible Douglas-fir graft unions. *Forest Science,* **24**: 297-303.

Costante, J.F., Lord, W.j., Howard,D. and Connington, L. (1983). Influence of planting depth on growth, root suckering and yield on interstem apple trees. *HortScience,* **18**(6): 913-915.

Couvillon,G.A. (1982). Leaf elemental content comparison of own-rooted peach cultivars to the same cultivars on several peach seedling rootstocks. *Journal of American Society for Horticultural Science,* **107**(4): 555-558.

Couvillon,G.A. (1985). Propagation and performance of inexpensive peach trees from cuttings for high density plantings. *Acta Horticuturae,* **173**:271-282.

Couvillon, G.A. (1988). Rooting response to diffrent treatments. *Acta Horticulturae,* **227**: 187-196.

Crawford,J.D. (1983). Phylogenetic and systematic inferences from electrophoretic studies. In: *Isozymes in Plant genetics and breeding.*Part A(Eds. S.D.Tyanskley and T.J.Orton), Elsevier, Amsterdam, 257-288.

Creste, J.E., Lima. and L.A.DE. (1995). Effect of rootstock and shoot type on foliar macronutrient contents in Satsuma tree (*Citrus unshiu,* Marc.). *Pesquisa Agropecuaria Brasileira,* **30**(1): 75-79.

Crooke,W.M. (1964). The measurement of the cation-exchange capacity of plant roots. *Plant and Soil,* **21**: 43-49.

Crooke, W.M. and Knight, A.H. (1962). An evaluation of the published data on the mineral composition of plants in the light of cation - exchange capacities of their roots. *Soil Science,* **93**: 365-373.

Crossa-Raynaud and Audergon. (1987). Apricot rootstocks. p.295. In: *Rootstocks for fruitcrops.* Roy C. Rom and Robert F. Carlson (eds.), A Wiley - Interscience Publication. New York.

Cummins, J.N. and Aldwinkle, H.S. (1983). Breeding apple rootstocks. p.294-394.In: J.Janick(ed).*Plant Breeding Reviews,* AVI,Westport, Conn.

Davies, F.T., Jr. (1984). Shoot RNA, cambial activity and IBA effectivity in seasonal rooting of juvenile and mature *Ficus pumila* cuttings. *Physiologia Plantarum,* **62**(4): 571-575.

Degani, C., El-Batsri, R. and Gazit, S. (1990). Enzyme polymorphism in mango. *Journal of American Society for Horticultural Science,* **115**:844-847.

Delas,J., Pouget,R. (1989). Rootstock / scion interactions and the mineral nutrition of the grape wine. *CTIFL-INRA* (1989),207-218.

Deloire,A. and Hebant,C. (1982). Peroxidase activity and lignification at the interface between stock and scion of compatible and incompatible grafts of *Capsicum* on *Lycopersicum. Annals of Botany,* **49**: 887-891.

Devlin and Witham (1986). Water loss: Transpiration. In: *Plant Physiology.* Delvin and Witham (eds.), CBS Publishers. Delhi.

Desai,J.B. and Patil,V.K. (1984). Studies on air-layering in jackfruit *(Artocarpus heterophyllus Lam.). Indian Journal of Forestry,* **7**: 177- 181.

Dhua,R.S. and Sen,S.K. (1984). Role of etiolation, auxinic and non-auxinic chemicals on root initiation of air-layers of jack fruit *(Artocarpus heterophyllus Lam.). Indian Journal of Horticulture,* **41**: 117-119.

Dijkman, M.J. (1951). *Hevea: Thirty Years of Research in the Far East.* University of Miami Press, Coral Gabler, Florida.

D'Khili,B., Michaux-Ferreire,N. and Grenan,S. (1995). Histochemical study on the incompatibility of micrografting and green grafting of grapewines. *Vitis,* **34**(3): 135-140.

Dutta,P. and Mitra,S.K. (1991). Seasonal variation in rooting of air-layers of guava.*Science and Culture,* **57**: 209-210.

Dutta, B.N., Sen,S. and Chatterjee, B.K. (1982). Effect of growth regulators and phenolic compounds on air-layering of water-apple (*Syzygium javanica* L. cv. Baruipur). *National seminar on plant propagation,*December 27-29, 1982, West Bengal.

El-Shazly,S.M., Alcaraz,C.F. and Carpena.O. (1992). Effects of some citrus rootstocks on leaf mineral contents of young and adult lemon trees. In *Proceedings of the International Society of Citriculture: V o l u m e 1.Taxonomy, breeding and varieties, rootstocks and propagation; Plant physiology and ecology. 7th International Citrus Congress, Acireale, Italy,1992.*

Ellstrand,N.C. and Lee,J.M. (1987). Cultivar identification of cherimoya (*Annona cherimola* Mill.) using isozyme markers. *Scientia Horticulturae,* **32**:25-31.

Estilai,A., Hashemi,A. and Waines,J.G. (1990). Isozyme markers for cultivar identification in guayule. *Hortscience,* **25**:346-348.

Facteau,T.J., Chesnut,N.E. and Rowe,K.E. (1996). Tree, fruit size and yield of 'Bing' sweet cherry as influenced by rootstock, replant area and training system. *Scientia Horticulturae,* **67**:13-26.

Fallahi,E.,Westwood,M.N., Chaplin,M.H. and Richardson,D.G. (1984). Influence of apple rootstocks and K and N fertilizers on leaf mineral composition and yield in a high density orchard. *Journal of Plant Nutrition,* **7**(8): 1161-1177.

Fernandez,R.T., Perry,R.L. and Ferree,D.C. (1995). Root distribution patterns of nine apple rootstocks in two contrasting soil types. *Journal of American Society for Horticultural Science,* **120**(1): 6-13.

Ferree,D.C. and Carlson,R.F. (1987). Apple rootstocks. In: *Rootstocks for fruit crops.* p.107. Roy C. Rom and Robert F. Carlson (eds.). A Wiley-Interscience Publication. John Wiley and Sons, New York.

Ferree,D.C., Schmid,J.C. and Morrison,C.A. (1992). An evaluation over 16 years of Delicians strains and other cultivars on several rootstocks and hardy interstems. *Fruit Varieties Journal,* **36**:37-45.

Gan,Y.Y., Zaini,S. and Idris,A. (1981). Genetic variation in the grafted vegetatively propagated mango(*Mangifera indica). Pertainids,* **4**:53-62.

Gaudillere,J.P., Moing,A. and Carbonne,F. (1989). Study comparing the photosynthesis of two plum (*Prunus domestica* L.) varieties grafted on four rootstocks. *CTIFL-INRA* (1989),185-193.

George,P.J., Panikkar,A.O.N. and Joseph,M.G. (1980). Rubber yielding plants other than *Hevea brasiliensis.* In: *Handbook of Natural Rubber Production in India,* (Ed.P.N.Radhakrishna Pillai), Rubber Research Institute of India, Kottayam.

George,A.P. and Nilssen,R.J. (1987). Propagation of *Annona* species: A review. *Scientia Horticuturae,* **33**:75-85.

Ghosh,S.H. and Ghosh,N.H. (1989). Effect of stock plant vigour on the growth of scion in *Hevea brasiliensis. Phytobreedon,* **5**(1): 46-48.

Gliemeroth,K. (1962). A physiological method of determining the interrelations between rootstock and scion of grafted fruit trees. *Tagungsber. dtsch. Akad. Landwwiss. Berlin,* **35**: 29-34.

Goncalves, P.DE S., Martins, A.L.M., Gorgulho, E.P., Bortoletto, N. and Bermond, G. (1994). Influence of six rootstocks on growth of six scion clones of rubber: a preliminary report. *Pesquista Agropecuaria Brasileira,* **29**(4): 553-560.

Goncalves,P.DE S., Martins,A.L.M., Bortoletto,N., Ortolani,A.A., and Bermond,G. (1994). Vigour evaluation of six different populations of *Hevea* rootstocks. *Pesquista Agropecuaria Brasileira,* **29**(4): 543-552.

Gottlieb,L. (1982). Conservation and duplication of isozymes in plants. *Science,* **216**: 373-380.

Gowda,V.N. (1983). Nutrient status of Coorg mandarin (*Citrus reticulata* L.) and Kinnow mandarin (a citrus hybrid) as influenced by different rootstocks. *Thesis Abstracts. Haryana Agricultural University,* **9** (4): 346.

Guilbault,G.G. (1976). *Handbook of enzymatic methods of analysis.* Marcel Dekker (Ed.) Inc. New York. pp.147.

Gupta, B.B. , Kumar, A. and Negi, D.S. (1989). Rooting response of branch cuttings of *Melia azerarach* L. *Indian Journal of Forestry,* **12**(3): 210-214.

Gupta, P.K., Nadgir,A.L., Mascarenhas, A.F. and Jaganathan,V. (1980). Tissue culture of forest trees: Clonal multiplication of *Tectona grandis* L. (Teak) by tissue culture. *Plant Science Letters,* **17**: 259-268.

Gurumurti, K., Gupta, B.B. and Kumar, A. (1984). Hormonal regulation of root formation. In: *Hormonal regulation of plant growth and development* (Ed. S.S. Purohit) Agrobotanical Publishers (India), 387-400.

Gurumurthi,K. and Stanley Jagadees,S. (1992). Micropropagation of *Azadirachta indica* A. Juss. from buds of coppice shoots. *Regional workshop on vegetative propagation biotechnologies for improvement.* Nataraj Publishers. 137-140.

Hambrick, C.E.III, Davies Jr. F.T. and Pemberton, H.B. (1991). Seasonal changes in carbohydrate/nitrogen levela during field rooting of *Rosa multiflora* 'Brooks 56' hardwood cuttings. *Scientia Horticulturae,* **46**: 137-146.

Haridas, P., Nair, U.C.K. and Lai, R.D. (1992). Commercial potential of nursery grafting for higher yield and improved quality of tea. *Journal of Plantation Crops,* **20**(Suppliment): 175-178.

Hartmann, H. and Kester, D.E. (1976). *Plant propagation: Principles and practices.* Prentice-Hall International Inc., New Jersey.

Hartney,V.J. (1980). Vegetative propagation of Eucalyptus. *Auastralian Forest Research,* **10**: 191-211.

Heller,A., Borochov,A. and Halevy,A.H. (1994). Factors affecting rooting of *Coleonema aspalathoides. Scientia Horticulturae,* **58**: 335-341.

Herrin.B.B. and Carter,J. (1995). The response of stem girdling and covering material on air-layering propagation of rubber plants. *HortScience,* **30**(4): 914.

Hicks,P.H., Sleper,D.A., Randall,D.D. and Crane,C.F. (1982). Peroxidase isozyme differences in tall fescue cultivars and allopolyploid accessions. *Euphytica,***31**:175-181.

Higgs,K.H. and Jones,H.G. (1990). Response of apple rootstocks to irrigation in south - east England. *Journal of Horticultural Science,* **65**: 129-141.

Higgs,K.H. and Jones,H.G. (1991). Water relations and cropping of apple cultivars on a dwarfing rootstock in response to imposed drought. *Journal of Horticultural Science,* **66**(3): 367-379.

Hirst,P.M. and Ferree,D.C. (1995). Effect of rootstock and cultivar on the growth and precocity of young apple trees. *Fruit Varieties Journal,* **49**(1): 34-41.

Hirst,P.M. and Ferree,D.C. (1995). Rootstock effects on the flowering of 'Delicious' apple. II. Nutrient effects with specific reference to phosphorus. *Journal of the American Society for Horticultural Science,* **120**(6): 1018-1024.

Hoang, N.V. (1985). Early selection in three-part-tree combination. *Proceedings of the International Conference,* 1985, Kuala Lumpur, pp. 175-186.

Holevas,C.D., Stylianides,D.C. and Michaelides,Z. (1985). Nutrient element variability in the leaves of almond trees in relation to variety rootstock and the vegetative part of the tree. *Options Me'diterraneennes,* **1**:111-120.

Howard, B.H. (1986). Factors affecting the rooting response of fruit tree cuttings to IBA treatment. *Acta Horticulturae,* **179**(2): 829-840.

Howell,G.S. (1987). Vitis rootstocks. In: *Rootstocks for fruit crops.* Roy C. Rom and Robert F. Carlson (eds.). A Wiley-Interscience Publication, John Wiley and Sons, New York.

Jadczuk,E., Kobylin'ska,J. and Zelazo,N. (1995). Effect of rootstock upon leaf mineral composition of non-bearing 'Jonagold' apple trees. *Acta Horticulturae,* **383**: 345-352.

Jaafar,H. and Pakianathan,S.W. (1979). Stimulation of lateral root production and budbreak with growth regulators in *Hevea* budded stumps. *Journal of Rubber Research Institute of Malaysia,* **27**(3): 143-154.

Jambhale,N.D. and Patil,S.C. (1996). Micropropagation of elite Eucalyptus types through shoot tip culture. *Indian Forester,* **122**(1): 61-64.

Jayasekera,N.E.M. and Senanayake,Y.D.A. (1971). A study of growth parameters in a population of nursery rootstock seedlings of *Hevea brasiliensis* Muell. Arg. C V Tjir 1. *Quarterly Journal of Rubber Research Institute of Ceylon,* **48**: 66-81.

Jose,P.A., Thomas,J. and Krishnan,P.N. (1995). Vegetative propagation of *Ocheinauclea missionis* (Wall . Ex G. Don) Ridsid. A rare and threatened tree species of western ghats. *Indian Forester,* **121**(12): 1159-1164.

Juntilla,O. (1988). Effect of rootstock on phtoperiodic control of elongation growth in grafted ecotypes of salix. *Physiologia Plantarum,* **74**: 39-44.

Kaimakan,I.V. (1977). The effect of rootstock on changes in pear leaf ultrastructure. *Referativavi Zhurnal 12,55.854.*

Kaplankiran,M., Ozsan,M. and Tuzcu,O. (1985). Effect of the relationship between the rootstock and the scion on the content of carbohydrates in various rootstocks. *Doga Bilim Dergisi,D,(Tarim ve Ormancilik),* **9**(3): 261-268. *(Horticultural abstracts,* 1986, 2843.).

Karthikakuttyamma,M. (1976). *Plant and soil analysis. A Laboratory Mannual.* Rubber Research Institute of India, Kottayam.

Kato,M. and Tokumaru,S. (1979). An electrophoretic study of esterase and peroxidase isozymes in *Brassica raphanus. Euphytica,* **28**: 339-349.

Katzfuss,M. (1962). The mineral content of the bark of several apple and peach rootstocks in relation to their growth effect on scion varieties. *Tagungsber. dtsch. Akad. Landw. wiss. Berlin.* 35: 35-39.

Kempanna,C., Lingaraj, D.S. and Chandrasekharaiah, S.R. (1961). Propagation of *Gliricidia maculata,* H.B. and K by air-layering with the aid of growth regulators. *Science and Culture,* **27**: 85-86.

Kephart,S.R. (1990). Starch gel electrophoresis of plant isozymes: A comparative analysis of techniques. *American Journal of Botany,* **77**(5): 693-712.

Khosla,P.K., Chauhan,P.S. and Rajinder Sood. (1979). Air-layering studies in some forest trees. *Indian Journal of Forestry,* 2: 161-164.

Knowels,J.W.,Dozier,W.A.,Evans,C.E. Carlton,C.C. and McGuire,J.M. (1984). Peach rootstock influence on foliar and dormant stem nutrient content. *Journal of American Society for Horticultural Science,* **109**(3): 440-444.

Koike,H. and Tsukahara,K. (1993). Studies on root system and growth of 'Fuji' apple trees on dwarfing interstocks and rootstocks. *Journal of the Japanese Society for Horticultural Science,* **62**(1): 49-54.

Kojima,K., Takahara,T., Ogata,T. and Muramatsu,N. (1995). Relationship between growth characteristics and endogenous ABA, IAA and GA_3 levels in citrus rootstocks. *Journal of the Japanese Society for Horticultural Science,* **63**(4): 753-760.

Korovin,V.A (1971). The relationship between the leaf chlorophyll content in apple and scion-rootstock compatibility. *Selskokhozyaistvennaya Biologiya,* **6**(4): 610-611.

Kramer,P.J. and Kozlowski,T.T. (1979). Internal factors affecting growth. In: *Physiology of woody plants.* (P.J.Kramer and T.T.Kozlowski eds.) pp.546-627. Academic Press, Inc. New York.

Krishnakumar,R., Asokan,M.P. and Sethuraj,M.R. (1992). Polymorphic isozyme expression caused by stock-scion interaction in *Hevea brasiliensis* clone RRII 105. *Indian Journal of Natural Rubber Research,* **5**(1&2):161-171.

Kruczynska,D., Olszewski,T., Czynczyk,A. and Staszak,A. (1990). Preliminary results of rootstock and interstem combination effect on growth, yield and leaf mineral content of two apple cvs. 'Delicates' and 'Empire'. *Acta Horticulturae,* **274**: 257-266.

Kul'tabaev,E.T. (1985). Sugar dynamics in leaves of different rootstocks under different growing conditions. *Referativnyi Zhurnal,***55** (*Rastenievodstvo*), 11.55.597.

Kumar, P. (1989). Vegetative propagation in palas (*Butea monosperma*) through air-layering. *Indian Journal of Forestry,* **12**(3): 188-190.

Kumar, N.V. and Chauhan, K.S. (1972). Role of juvenility and auxins on the rooting of cuttings of guava (*Psidium guava* L.). *Proceedings of the 3rd International Symposium on Tropical and Subtropical Horticulture,* **2**: 7-14.

Kumar, D.P., Vijayakumar, N. and Sulladamath, U.V. (1985). Studies on nutrient status of parent plants in relation to rooting behaviour of *Ficus elastica* cultivars. *South Indian Horticulture,* **33**(4): 283-285.

Kunwar, R. and Singh, R. (1983). The influence of different rootstocks on the mineral composition of srinagar mandarin (*Citrus reticulata* Blanco) leaves. *Journal of Plant Nutrition,* **6**: 405-412.

Kurian,R.M., Reddy,V.V.P., and Reddy,Y.T.N. (1996). Growth, yield, fruit quality and leaf nutrient status of thirteen year old 'Alphonsa' mango trees on eight rootstocks. *Journal of Horticultural Science,* **71**(2); 181-186.

Kurien,S., Gosami,A.M. and Deb,D.L. (1994). Scionic influence on root activity in citrus using a radiotracer technique. *Fruits (Paris),* **49**(4): 261-267.

Lam. L.V. and Hai,T.T. (1993). Crown budding ; prospects and early results in Vietnam. *Indian Journal of Natural Rubber Research,* **6**(1-2): 92-96.

Larsen,F.E., Higgins,S.S. and Robert,F.J. (1987). Scion / interstock / rootstock effect on sweet cherry yield, tree size and yield efficiency. *Scientia Horticulturae,* **33**: 237-247.

Lasminingsih,M. (1989). Band pattern of protein of some rubber clones. *Bullettin Perkebunan Rakyat,* **5**(1): 34-38.

Lassner,M.W. and Orton,T.J. (1983). Detection of somatic variation. In: *Isozymes in Plant Genetics and Breeding.* Part A.(Eds. S.D.Tanksley and T.J.Orton).Elsevier Science Publishers, B.V., Amsterdam, pp.209-218.

Lauri,D.A. and Joseph,W.S. (1990). Inheritance studies and clonal fingerprinting with isozymes in sugarbeet. *Crop Science,* **30**: 1064-1072.

Layne, R.E.C., Jackson, H.O. and Stroud, F.D. (1977). Influence of peach seedling rootstocks on defoliation and cold hardiness of peach cultivars. *Journal of American Society for Horticultural Science,* **102**(1): 89-92.

Lebrun, P. andChevallier, M. H. (1988). Starch and polyacrylamide gel electrophoresis of *Hevea brasiliensis: A laboratory manual.* IRCA-CIRAD Publishers, Montpellier, France, pp.1-44.

Lehman,J.L., Young,E. and Unrath,C.R. (1990). Growth dyanamics of young apple trees as influenced by scion and rootstock vigour. *Journal of Horticultural Science,* **65**: 123-127.

Leong,S.K., Ooi,C.B. and Yoon,P.K. (1976). Further development in the production of cuttings and clonal rootstocks in *Hevea. Proceedings of National Plant Propagation Symposium,* Kuala Lumpur, 1976,154.

Leong,W. and Yoon,P.K. (1978). Effect of interstock on growth of *Hevea. Journal of Rubber Research Institute of Malaysia,* **26**(3), 99-104.

Leong,S.K. and Yoon,P.K. (1979). Effects of bud types on early scion growth of *Hevea. Journal of Rubber Research Institute of Malaysia,* **27**(1):1-7.

Leong,S.K. and Yoon,P.K. (1984). The role of cuttings and clonal rootstocks in *Hevea* cultivation. *Proceedings of the International Rubber Conference,* 1984, Colombo, Sri Lanka,71-85.

Linehan,D.J. (1984). Micronutrient cation sorption by roots and uptake by plants. *Journal of Experimental Botany,* **35** :1571-1574.

Lingaraj, D.S. (1960). Propagation of *Althea rosea,* Benth. and Hook.(Holly Hock) by air-layering with the aid of growth regulators. *Current Science,* **29**: 488.

LLoyd,J., Kriedemann,P.E. and Aspinall,D. (1990). Contrasts between *Citrus sp.* in response to salinisation : an analysis of photosynthesis and water relations for different rootstock-scion combinations. *Physiologia Plantarum,* **78**(2): 236-246.

Lockard, R.G. and Schneider, G.W. (1981). Stock-scion relationships and the dwarfing mechanism in apple. *Horticultural Reviews,* **3**: 315-375.

Lord,W.J., Green,D.W., Damon(Jr),R.A. and Baker,J.H. (1985). Effect of stem piece and rootstock combinations on growth, leaf mineral concentrations, yield and fruit quality of 'Empire' apple trees. *Journal of the American Society for Horticultural Science,* **110**:422-425.

Loreti, F., Massai. R. and Morini, S. (1993). Relationship between effects of rootstocks and planting system on nectarines. *Acta Horticulturae,* **349**: 155-158.

Lowry, O.M., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin ciocalteu reagent. *Journal of Biological Chemistry,* **93**: 265.

Madhusudanan, K.N. (1987). Amino acid changes during rooting of pine apple propagule. *Indian Journal of Plant Physiology,* **30**(2): 220-222.

Marattukalam, J.G. and Saraswathiamma, C.K. (1992). Propagation and planting. In: *Natural Rubber: Biology, Cultivation and Technology.* M.R.Sethuraj and N.M.Mathew (eds.). Elsevier Science Publishers, Amsterdam, The Netherlands, 1997.

Markert, C. (1975). Biology of isozymes. In: *Isozymes:* C.Markert(ed). Academic Press, New York,1-9.

Markert, C. (1977). Isozymes: The development of a concept. *Current topics in Biological and Medical Research,* **1**: 1-17.

Markert, C. and Moller, F. (1959). Multiple forms of enzymes: tissue, ontogenetic and tissue specific patterns. *roceedings of National Academy,* U.S.A.45:753-763.

Masa, A. (1989). Biochemical affinity between the scion cultivar Albarino (*Vitis vinifera* L.) and different rootstocks. *Connaissance de la Vigne et du Vin,* **23**(4): 207-214. *(Horticultural Abstracts,* 1990,8004.).

Mc Cready, R.M., Guggolz, J., Silviera,V. and Owens, H.S. (1950). Determination of starch and amylase in vegetables: Application to peas. *Analytical Chemistry,* **22**:1156-1158.

Medrado, M.J.S., Appezzato-da-gloria, B. and Costa, J.D.(1995). Anatomical changes in rubber tree cuttings *(Hevea brasiliensis* clone RRIM 600) in response to different rooting techniques. *Scientia Agricola,* **52**(1): 89-95.

Menedez, R.A., Larsen, F.E. and Frittsr, JR. (1986). Protein and isozyme electrophoresis and isoelectric focusing for the characterisation of apple clones. *Scientia Horticulturae,* **29**(3): 211-220.

Minocha, J.L., Kumar, R.R. and Mehta, A. (1991). Morphological, cytological and peroxidase isozyme studies in diploids , hybrids and amphidiploids of *Vigna radiata and V.munga. Indian Journal of Genetics,* **51**: 429-437.

Misra, R.S. and Agrawal, A.K. (1975). Root induction in air-layers of Kaghzi kalan with special reference to plant growth regulators. *Progressive Horticulture,* **1**(3): 81-86.

Misra, K.K. and Jaiswal, H.R. (1993). Propagation of *Anthocephalus chinensis* (Lam) R. Rich. Ex. Walp. by air-layering with the aid of indole butyric acid. *Indian Forester,* **119**(7): 587-589.

Moore, S. and Stein, W.H. (1948). Photometric method for use in the chromatography of amino acids. *Journal of Biological Chemistry,* **153**: 176 375. 367 – 388

Morinaga, K. and Ikeda, F. (1990). The effects of several rootstocks on photosynthesis, distribution of photosynthetic product and growth of young satsuma mandarin trees. *Journal of the Japanese Society for Horticultural Science,* **59**(1): 29-34.

Mowrey, B.D. and Werner, D.J. (1990). Developmental specific isozyme expression in peach. *HortScience*, **25**: 219-222.

Mukherjee, S.K. and Chaterjee, B.K. (1978). Effect of etiolation and growth regulators on air-layering of Jackfruit (*Artocarpus heterophyllus* Lam.). *Indian Journal of Horticulture,* **35**(1): 1-4.

Mukherjee, S.K., Rao, D.P., Chakladar, B.P. and Chatterjee, B.K. (1986). Effect of growth regulators , invigoration and etiolation on rooting of air-layers of bael (*Aegle marmelos* Corre.). *Indian Journal of Horticulture,* **43**: 9-12.

Nagpal, R. and Puri, S. (1986). Effects of auxins on air-layers of some agro-forestry species. *Indian Journal of Forestry,* **9**: 232-236.

Nanda, K.K., Purohit, A.N., Adarsh Bala and Anand, V.K.(1968a). Seasonal rooting response of stem cuttings of some forest tree species to auxins. *Indian Forester,* **94**: 154-162.

Nanda, K.K. and Anand,V.K. (1970). Seasonal changes in auxin effects on rooting of stem cuttings of *Populus nigra* and its relationship with mobilization of starch. *Physiologia Plantarum,* **23**: 99-107.

Nanda, K.K., Anand, V.K. and Parshotaam Kumar (1970). Some investigations of auxin effects on rooting of stem cuttings of forest plants. *Indian Forester,* **96**: 171-187.

Nanda, K.K. and Kochar, V.K. (1984). *Vegetative propagation of plants.* Kalyani Publishers, New Delhi.

Natali, S., Xiloyannis, C. and Babieri, A. (1985). Water consumption of peach trees grafted on four different rootstocks. *Acta Horticulturae,* **173**: 355-362.

Nelson, N. (1944). A photometric adaption of the somogyi method for the determination of glucose. *Journal of Biological Chemistry,* **153**: 375.

Ng, A.P., Ho, C.Y., Sultan, M.O., Ooi, C.B., Lew, H.L. and Yoon, P.K. (1981). Influence of six rootstocks on growth and yield of six scion classes of *Hevea brasiliensis. Proceedings of RRIM Planter's Conference,*1981, Kualalumpur, Malaysia, pp.134-151.

Nielson, G. (1985). The use of isozymes as probes to identify and label plant varieties and cultivars. In: *Isozymes. Current topics in Biological and Medical Research* (eds.J.Ratazzi., G.Scandalios and G.S.Whitt). **12**:1-32.

Nunez-Elisea, R., Caldeira, M.L., Ferreira, W. and Daven-Port, T.L. (1992). Adventitious rooting of 'Tomy Atkins' mango air layers induced with naphthalene acetic acid. *HortScience,* **27**(8): 926.

Nyirenda, H.E. and Karyanga, C.W. (1984). Effect of rootstock on components of yield in tea (*Cammelia sinensis* (L) o.kuntze). 2. Stem circumference and number of branches. *Journal of Horticultural Science,* **59**(4): 589-594.

Oberly, G,H. and Poling, B. (1978). Effect of rootstocks on apple leaf mineral element composition. *Compact Fruit Tree,* **11**: 22-25.

Ogata, T., Kataoka, I., Sugiura, A. and Tomana, T. (1989). Effect of several rootstocks on mineral nutrient absorption of peach, nectarine and plum. *Journal of Japanese Society for Horticultural Science,* **57**(4): 608-614.

Okie, W.R. (1987). Plum rootstocks. In: *Rootstocks for fruit crops.* Roy C.Rom and Robert F.Carlson (eds.). A Wiley Interscience Publication, John Wiley and Sons, New York. p.321.

Om, H. and Pathak, R.K. (1983). Influence of stock and scion on macro-nutrient contents of apple leaves. *Indian Journal of Plant Physiology*, **16**(4): 337-343.

Omarov, M.D. and Erokhina, A.I. (1989). The distribution of the persimmon root system in the soil in relation to rootstocks. *Subtropicheskie Kul'tury*, **3**: 78-82. (*Horticultural Abstracts*, 1990, 2948).

Othman, H., Leong, S.K. and Samsuddin, Z. (1988). Root-shoot balance of *Hevea* planting materials. *In: Plant roots and their environment. Proceedings of an ISSR Symposium, 21-26. August 1988, Uppsala, Sweden.*

Ozerol, N.H. and Titus, J.S. (1965). The determination of total chlorophyll in methanol extracts. *Transactions of the Illinois State Academy of Science*, **58**: 39.

Pakianathan, S.W., Wong, T.K. and Hafsah Jaafar. (1979). Use of indole butyric acid on budded stumps to aid earlier root initiation and growth. *Proceedings of RRIM Planter's Conference*, Kuala Lumpur, 275-302.

Pal, R.S. and Srivastava, R.P. (1982). Possibilities of propagating citrus cultivars and rootstocks by mound layering/stooling. *National seminar on plant propagation*, December 27-29, 1982, West Bengal, p.72.

Pal, M., Mishra, M., Bakshi, M. and Bhandari, H.C.C. (1996). Cheap substitutes of auxin for the clonal propagation of *Casuarina equisettifolia*. *Indian Forester*, **122**(11): 999-1003.

Palanisamy, K.and Kumar, P. (1997). Seasonal variation on adventitious rooting in branch cuttings of Pongamia pinnata pierre. *Indian Forester*, **123**(3): 236-239.

Panahi, B., Sheibani, A. and Peat, W.E. (1996). The effect of combinations of three scions and three rootstocks on yield and quality of Iranian Pistachio nuts. *HortScience*, **31**(4): 634. (Abstract).

Paranjothi, K. and Gandhimathi, H. (1975 a). Tissue and organ culture of *Hevea*. *Proceedings of International Rubber Conference*, **2**: 59-84.

Parfitt, D.E. and Arulsekar, S. (1985). Identification of plum x peach hybrids by isoenzyme analysis. *HortScience*, **20**: 246-248.

Parker, J. (1949). Effects of variations in the root-leaf ratio on transpiration rate. *Plant Physiology,* **24**: 739.

Patel, N.B. and Pasaliya, Y.M. (1995). Effect of plant growth regulators and advanced ringing on rooting in air-layering of guava (*Psidium guajava* L.) cv. Lucknow-49. *Recent Horticulture,* **2**(2): 33-36.

Pathak, S., Saroj, P.L., Prasad, J. and Pathak, R.K. (1991). Studies on rooting potentiality of custard apple (*Annona squamosa* L.) through stool layering. *Narendra Deva Journal of Agricultural Science,* **6**(1): 85-87.

Patil, S.B. and Chakrawar, V.R. (1979). Vegetative propagation of seedless lemon by air-layering. *Punjab Horticultural Journal,* **14**: 119-124.

Piccotino, D., Massai, R., Baroni, G. and Bovo, M. (1992). Root system conformation and growth of kiwifruit as affected by propagation technique. *Acta Horticulturae,* **297**: 391-399.

Pilone, N. (1992). Rootstock effects on citrons under different conditions. *Informatore Agrario,* **48**(18): 127-131.

Piper, C.S. (1950). *Soil and plant analysis.* University of Adelaide, Adelaide

Poessel, J.L. (1989). Selection for graft compatibility in apricot trees; biochemical characterisation of compatible and incompatible cutivars. *Erwerbsobstbau,* **31**(2): 35-40.

Polhamus, L.G. (1962). *Rubber: Botany, production and utilization.* Interscience Publishers, Inc. New York.

Poling, E.B. and Oberly, G.H. (1979). Effect of rootstock on mineral composition of apple leaves. *Journal of American Society for Horticultural Science,* **104**: 799-801.

Prasad, A. and Singh, R.D. (1972). *Punjab Horticultural Journal,* **22**: 64-71. (Cited from *"Propagation of Tropical and Subtropical Horticultural Crops"*. T.K.Bose, S.K.Mitra and M.K.Sadhu (eds.). p.257, Naya Prakash, Calcutta.

Protopapadakis, E.E. (1988). Effect of rootstocks on isoenzymic composition of citrus. In *Sixth International Citrus Congress, Middle-East, Tel Aviv, Israel, 1988, Volume I. Rehovot,Israel; Balaban Publishers* (1988), 609-614.

Puri, S. and Nagpal, R. (1988). Effect of auxins on air-layers of some agroforestry species. *Indian Forestry,* **11**(1): 28-32.

Rajagopal, R. (1993). *Physiomorphological studies of Ficus elastica Roxb. and Datura innoxia Mill. in relation to Vitamin B and C application.* Ph.D Thesis, Aligarh Muslim University, Aligarh, India.

Ram, S. and Sirohi, S.C (1982). Effect of growth regulators on air-layering in mango. *National seminar on plant propagation,* December 27-29, 1982, West Bengal. p.72.

Rao, M.B.N., Satyanarayana, G., Rameswar, A., Shivraj, A. and Padmanabham, V. (1988). Biochemical basis for root-regeneration in ringed shoot cuttings of cashew (*Anacardium occidentale* L.). Plants of different ages- cofactor activity and total phenol content. *Journal of Plantation Crops,* **17**: 65-68.

Rao, M.B.N., Satyanarayana, G., Shivraj, A., Gnanakumari, N. and Padmanabham, V. (1988). Interaction of souce plant age and shoot ringing on rooting of cashew (*Anacardium occidentale* L.) cuttings. *Journal of Horticultural Science,* **63**(3): 517-519.

Ranaware, V.S., Nawale, R.N., Khandekar, R.G. and Magdum, M.B. (1995). Effect of season on air-layering of cinnamon (*Cinnamomum zeylanicum* Blume). *Indian Cocoa, Arecanut and Spices Journal,* **19**(3): 81- 84.

Ranney, T.G. and Whitman,E.P.II. (1995). Growth and survival of 'Whitespire' Japanese Birch grafted on rootstocks of five species of birch. *Hortscience,* **30**(3): 521-522.

Rao, M.B.N. and Satyanarayana, G. (1989). Biochemical basis for root-regeneration in ringed shoot cuttings of cashew (*Anacardium occidentale* L.). Plants of different ages- Auxin activity and carbohydrate contents. *Journal of Plantation Crops,* **17**(2): 127-130.

Reddy, Y.T.N., Kohli, R.R. Singh, G. and Bhargava, B.S. (1989). Effect of rootstocks on growth, yield and leaf nutrient composition of mango (*Mangifera indica* L.). *Fruits (Paris,* **44**(7-8): 409-413.

Rio, C.D., Rallo, L. and Caballero, J.M. (1991). Effects of carbohydrate content on the seasonal rooting of vegetative and reproductive cuttings of olive. *Journal of Horticultural Science,* **66**(3): 301-309.

Roberts, A.N. and Blaney, W.E. (1967). Qualitative, quantitative and positional aspects of interstock influence on growth and flowering of apple. *Proceedings of American Society for Horticultural Science*, **91**: 39-50.

Rom, C.R. (1987). In: *Rootstocks for Fruit Crops*. Eds. Rom,C.R. and Carlson, R.F. John Wiley & Sons, Inc.

Roux, Y,Le. and Pages, L. (1994). Development and polymorphism of the root systems of young rubber . *Canadian Journal of Botany,* **72**(7): 924-932.

Roux, Y,Le. (1996). Root system archetecure of *Hevea brasiliensis:* comparative study of seedling and microcutting. *CIRAD*(CP-RU-IA 31.14).

Rubber Research Institute of Malaya (1959b). Propagation and planting technique- Vegetative propagation of *Hevea brasiliensis. Annual Report Rubber Research Institute of Malaysia.* pp. 41-43.

Rubber Research Institute of Malaya (1962). Growth of various planting materials. *Annual Report Rubber Research Institute of Malaysia,* pp.58.

Rubber Research Institute of Malaya (1982). Stock-scion relationships. *Annual Report RRIM.* pp.38.

Rubber Statistics. (1996). Published by Rubber Board, Kottayam.

Sabyasachi Rath and Mishra S.P. (1996). Effect of growth regulator on varying age of shoots on success of marcotage in jackfruit. *Orissa Journal of Horticulture,* **24**(1/2): 79-82.

Sadhu, M.K., Naskar, B.B. and Basu, R.N. (1972). Auxin synergists in the rooting of air-layers of tropical fruit trees. *Indian Agriculture.* **16**:251-257.

Sagy, G.A. and Omokhafe, K.G. (1996). Evaluation of rootstock and scion compatibility in *Hevea brasiliensis* (3). Paper presented at the *IRRDB Seminar* 5-8 November 1996, Sri Lanka, p.13.

Salesses, G. and Alkai, N. (1985). Simply inherited grafting incompatibility in peach. *Acta Horticulturae,* **173**: 57-62.

Samarappuli, L., Yogaratnam, N., Karunadasa, P. and Mitrasana, U. (1996). Root development in *Hevea brasiliensis* in relation to management practices. *Journal of Rubber Research Institute of Sri Lanka,* **77**: 93-111.

Saminy, C. and Cummins, J.N. (1992). Distinguishing apple rootstocks by isozyme banding patterns. *Hortscience,* **27**(7): 829-831.

Santamour, F.S.JR. (1988). Cambial peroxidase enzymes related to graft incompatibility in red oak. *Journal of Environmental Horticulture,* **6**(3): 87-93.

Saraswathi Amma, C.K., Marattukalam, J.G., Thomas, V. and George, M.J. (1991). Comparative studies on the rootsystem of stem cutting and budgraft of rubber at 15 years of growth. *Rubber Board Bulletin,* **26**(4): 25-27.

Saric, M.R., Zorzic, M. and Buric. D. (1977). The influence of rootstock and scion on ion uptake and distribution. *Vitio,* **16**(3): 174-183.

Satyanarayana, N., Cox, S. and Sharma, V.S. (1992). Field performance of grafts made on fresh tea clonal cuttings. *Journal of Plantation Crops,* **20** (Supplement): 151-156.

Scandalios, J.G. (1964). Tissue specific variation in maize. *Journal of Heredity.,* **55**: 281-285.

Scandalios, J.G. (1968). Genetic control of multiple molecular forms of catalase in maize. In: *Annals of the New York Academy of Sciences.*

Schmid, P.P.S. and Feucht, W. (1985). Carbohydrates in the phloem of *Prunus avium / Prunus cerasus* graftings and of homospecific controls. *Angewandle Botanik,* **60**(3/4): 201-208. (*Horticultural Abstracts,* 1987,7541.).

Schmid, P.P.S. and Feucht, W. (1985). Proteins, enzymes and polyphenols in plants of *Prunus avium / Prunus cerasus* graft combinations showing incompatibility symptoms. *Gartenbauwissenschaft,* **50**(3): 104-110.

Schneider, G.W., Chaplin, C.E. and Martin, D.C. (1978). Effects of apple rootstocks, tree spacing and cultivar on fruit and tree size , yield and foliar mineral composition. *Journal of the American Society for Horticultural Science,* **103**(2): 230-232.

Scott, T.A. and Melvin, E.H. (1953). Determination of Dextran with Anthrone. *Analytical Chemistry,* **25**(11): 1656-1661.

Senanayake, Y.D.A. (1975). Yield variability in clonal rubber (*Hevea brasiliensis* Muell. Arg). *Journal of Plantation Crops,* **3**(2): 73-76.

Seneviratne, A,N. and Samarakoon, S.M.A. (1995). The role of snag on the growth of scion in budgrafted plants of *Hevea* with special reference to young buddings. *Journal of Rubber Research Institute of Sri Lanka,* **76**: 21-35.

Seneviratne, P., Nugawela, A., Samarakoon, S.M.A. and Ramavikrama, D. (1996). The growth of stock plants and its effect on the scion growth of young buddings of *Hevea brasiliensis* (Muell Arg). *Journal of Plantation Crops,* 24(2): 119-125.

Sestak, Z., Catsky, J. and Jarvis, P.G. (1971). *Plant photosynthetic production: Manual of methods.* Dr.W.Junk, The Hangue, Netherlands.

Sharfuddin, A.F.M. and Husain, A. (1973). Effect of growth regulators, rooting media and wrapping materials on the success of air-layering in litchi. *Bangladesh Horticulture,* **1**(2): 45-51.

Sharma, D.D. and Chauhan, J.S. (1991). Effect of different rootstocks and training systems on the mineral composition of 'Delicious' apple leaves. *Journal of Horticultural Science,* **66**(6): 703-707.

Sharma, S.K. and Singh, R. (1989). Photosynthetic characteristics and productivity in citrus. II. Effect of rootstocks. *Indian Journal of Horticulture,* **46**(4): 422-425.

Shishido, Y., Zhang, X. and Kumakura, H. (1995). Effects of rootstock varieties, leaves and grafting conditions on scion growth in eggplant. *Journal of the Japanese Society for Horticultural Science,* **64**(3): 581-588.

Shklarman, Y., Safran, H. and Sagee, O. (1992). Microscopic study of graft union characteristics and water translocation in young citrus buddings. In: *Proceedings of International Society of Citriculture: Volume 1. Taxonomy, breeding and varieties, rootstocks and propagation , plant physiology and ecology: 7th International Citrus Congress, Acireale, Italy,* 8-13, 1992.

Simmons, R.K. (1989). Growth characteristics of apple dwarfing rootstocks as related to lenticels, roots and the vegetative tissues within the union of stock and scion. *Acta Horticulturae,* **243**: 87-88.

Singh, A. (1980). Stock-scion relationships and incompatibility. In: *Fruit Physiology and production* .pp.153-159. Kalyani Publishers, New Delhi.

Singh, S.P. (1985). Study of carbohydrate and nitrogen fractions in semihardwood cuttings of *Pyrostegia venusta* at root emergence. *Haryana Journal of Horticultural Sciences,* **14**(3/4): 199-203.

Sinha, M.M., Awasthi, D.N., Tripathi, S.P. and Mistra, R.S. (1986). Vegetative propagation of apple cutivars on their own roots: Effect of IBA concentrations on the air-layering of three commercial cultivars: biochemical studies. *Indian Journal of Horticulture,* **43**: 94-97.

Sinha, R.R., Sobhana, P. and Sethuraj, M.R. (1985). Axillary buds of some high yielding clones of *Hevea* in culture. *First IRRDB Hevea Tissue culture workshop,* Kuala Lumpur, 1985, 16.

Sita, G.L. and Vaidyanathan, C.S. (1979). Rapid multiplication of Eucalyptus by multiple shoot production. *Current Science*, **48**: 350-352.

Sivanadyan, K., Haridas, G. and Pushparajah, E. (1975). Reduced immaturity period of *Hevea brasiliensis. Proceedings of International Rubber Conference,* Kuala Lumpur.1975.

Skene, K.G.M. (1975). Cytokinin production by roots as a factor in the control of plant growth. In: *'The development and Function of roots'.* (J.G.Torrey and D.T.Clarkson, eds.), pp. 365-396. Academic Press, New York.

Smith, J.S.C. and Smith, O.S. (1992). Fingerprinting crop varieties. *Advances in Agronomy,* **47**: 85-140.

Smith, D.R. and Thorpe, T. (1975). Root initiation in cuttings of *Pinus radiata* seedlings. II. Growth regulator interactions. *Journal of Experimental Botany,* **26**: 193-202.

Sobhana, P., Nair, N.U., George, M.J. and Sethuraj, M.R. (1980). Effect of scion on root growth, cation exchange capacity of roots and mineral uptake in *Hevea brasiliensis. International Rubber Conference India,* 1980, Kottayam, India.

Sobhana, P., Sinha, R.R. and Sethuraj, M.R. (1986). Micropropagation of *Hevea brasiliensis*- Retrospect and prospect. VI. *International Congress of Plant Tissue and Cell Culture,* Minnesota, 1986, 145.

Sobhana, P., Rajagopal, R., Sethuraj, M.R. and Vijayakumar, K.R. (1995). A note on vegetative propagation of *Hevea brasiliensis* by air layering. *Indian Journal of Natural Rubber Research,* **8**(1): 70-72.

Soong, N.K. (1976). Feeder root development of *Hevea brasiliensis* in relation to clones and environment. *Journal of Rubber Research Institute of Malaysia,* **24**(5): 283-298.

Sreelatha, S., Saraswathy Amma, C.K., Vijayakumar, K.R., Thomas, M., Nair, N.U., Simon, S.P. and Sethuraj, M.R. (1993). Isozyme studies on different cytotypes of *Hevea brasiliensis. Indian Journal of Natural Rubber Research,* **6**(1&2): 24-27.

Stolz, L.P. (1968). Factors influencing root initiation in an easy- and a difficult-to root chryasanthemum. *Proceedings of American Society for Horticultural Science,* **92**: 622-626.

Sujatha, V.S., Srivastava, K.N. and Mori, T.A. (1991). Isozyme variation in Muskmelon *(Cucumis melo* L.). *Indian Journal of Genetics,***51**: 438-440.

Sundaram, K.S. and Rangasamy, P. (1994). Studies on rooting of cuttings of Timla fig tree. *South Indian Horticulture,* **42**(6): 374-375.

Suryanarayana, V. and Rao, K.V. (1982). Effect of growth regulators on air-layering of fig and pomegranate. *National seminar on plant propagation,* December 27-29, 1982, West bengal, p.70.

Suryanarayana, V. and Rao, K.V. (1982). Effect of growth regulators on air-layering of mango, lemon and ber. *National seminar on plant propagation,* December 27-29, 1982, West Bengal, p. 69.

Suzuki, T. and Kohno, K. (1983). Changes in nitrogen levels and free aminoacids in rooting cuttings of mulberry (*Morus alba). Physiologia Plantarum,* **59**: 455-460.

Swain, T. and Hillis, W.E. (1959). The phenolic constituents of *Prunus domestica.* The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture,* 10: 63-68.

Sylversten, J.P. and Graham, J.H. (1985). Hydraulic conductivity of roots, mineral nutrition and leaf gas exchange of citrus rootstocks. *Journal of the American Society of Horticultural Science,***110**: 865-869.

Szali, J. (1978). Effect of rootstock and integrated variety on catalase activity in Jonathan leaves. *Acta Agronomica Academiae Scientiarum Hungaricae,* **26**(3/4): 274-278.

Tahrouch-Skouri, S., Andary, C. and Macheix, J.J. (1993). Grafting induces a modification of phenolic metabolism in *Jasminum grandiflorum. Academie des Sciences, Series 3, Sciences de la Vie,* **316**(3): 293-297.

Tayde, G.S. (1985). Studies on root system of different citrus rootstocks as influenced by kinnow mandarin scion in nursery . *PKV Research Journal,* **9**(2): 74-76.

Templeton, J.K. (1960). Some aspects of the growth of *Hevea* buddings. *Proceedings of Natural Rubber Research Conference.,* 297-311.

Teng, T.K. and Pushparajah, E. (1974). The influence of clone, rootstock and crown budding on the mineral nutrition of *Hevea. IRRDB Symposium,* 1974, Cochin, pp.33/1-33/15.

Tewari, A. and Dhar, U. (1997). Studies on the vegetative propagation of the Indian butter tree (*Aisandra butyracea* (Roxb.) Bachni). *Journal of Horticultural Science,* **72**(1): 11-17.

Thaler, P. and Pages, L. (1996). Periodicity in the development of the root system of young rubber tree (*Hevea brasiliensis* Muell. Arg.) : Relationship with shoot development. *Plant, Cell and Environment,* **19**(1): 56-64.

Tinley, G.H. (1960). Vegetative propagation of clones of *Hevea brasiliensis* by cuttings. *Proceedings of Natural Rubber Research Conference,* 1960, Kuala Lumpur, Malaysia, pp.409-418.

Tiong, G.L. (1989). Some preliminary results of a study on culling of rootstock to improve growth and yield of grafted rubber. *The Planter,* **65**: 547-553.

Titova, N.V. and Shishkaner, G.V. (1976). Photosynthesis and pigment content in apple trees on different rootstocks.

Torres, A.M., Diedenhofen, V., Bergh, B.O.and Knight, R.J. (1978). Enzyme polymorphism as genetic markers in the avocado. *American Journal of Botany,* **65**:134-139.

Torres, A.M. and Tisserat, B. (1980). Leaf isozymes as genetic markers in date palms. *American Journal of Botany,* **67**:162-167.

Tukey, R.B., Langston, R. and Cline, R.A. (1962). Influence of rootstock, bodystock and Interstock on the nutrient content of apple foliage. *Proceedings of American Society for Horticultural Science,* **80**: 73-78.

Twyford, C.T., Viana, A.M., James, A.C. and Mantel, S.H. (1990). Characterisation of Diascoria food yams using isoelectric focusing of peroxidase and acid phosphatase isozymes. *Tropical Agriculture,* **67**: 337-341.

Ugolik, M. and Kantorowicz-Bak, M. (1993). Effect of rootstock on growth, yield and mineral element content in apple leaves. *Prace z Zakresu Nauk Rolniezych,* **75**: 161-169.

Uniyal, R.C., Prasad, P. and Nautiyal, A.R. (1993). Vegetative propagation in *Dalbergia sericea:* influence of growth hormones on rooting behaviour of stem cuttings. *Journal of Tropical Forest Science,* **6**(1): 21-25.

Val, J., Moreno, M.A., Tabuenca, M.C. and Monge, E. (1989). Photosynthetic pigments in young non-bearing pear and quince trees. *Acta Horticulturae,* **256**: 119-125.

Vallejos, C.E. (1983). Enzyme staining . In : *Isozymes in Plant Genetics and breeding,* Part A. (Eds. S.D. Tanskley and T.J. Orton). Elsevier, Amsterdam, pp.469-516.

Van Geyt, J.P.C.F. and Smed, E. (1984). Polymorphism of some marker enzymes of sugarbeet (*Beta vulgaris* L.) investigated by polyacrylamide gel electrophoresis and starch gel electrophoresis. *Zeitschrift fuer Pflazenzuchtug,* **92**: 295-308.

Vasconcellos, L.A.B.C. and Castle, W.S. (1994). Trunk xylem anatomy of mature healthy and blighted grapefruit trees on several rootstocks. *Journal of American Society for Horticultural Science,* **119**(2): 185-194.

Veeraraghavathatham, D., Rao, V.N.M. and Shanmugavelu, K.G. (1985). A physiological analysis of shy rooting behaviour of *Jasminum auriculatum* Vahl. cv. Parimullai stem cuttings. *South Indian Horticulture,* **33**(3): 177-181.

Vose, P.B. (1984). Effects of genetic factors on nutritional requirements of plants. In: *Crop Breeding a Complimentary Basis.* (Eds. P.B.Vose and S.G.Blixt), Pargamon Press, Oxford, pp67-114.

Vyvyan, M.C. (1930). The effect of scion on root. III. Comparison of stem and root worked trees. *Journal of Pomology and Horticultural Science,* **8**: 259-282.

Wallace, A. and Collaborators. (1971). In *Regulation of Micronutrient status of plants by chelating agents and other factors.* A.Wallace, Ed. and Pub., Los Angeles, Calif.pp.69-81.

Wallace, A. and Mueller, R.T. (1980). Calcium uptake and distribution in plants. *Journal of Plant Nutrition,* **2**(1&2): 247-256.

Walt, M. Van Der and Davie, S.J. (1995). Physiological changes associated with dwarfing rootstocks. *Inligtingsbulletin- Instituut vir Tropiese en Subtropiese Gewasse,* **276**: 10-16.

Webster, C.C. and Paardecooper, E.C. (1989). The botany of the rubber tree. In: *Rubber* : C.C.Webster and W.J.Baulkwill (eds.). Longman Scientific and Technical, England.

Weeden, N.F. and Lamb, R.C. (1985). Identification of apple cultivars by isozyme phenotypes. *Journal of American Society for Horticultural Science,* **110**: 509-515.

Weller, D.L. and Costante, F.J. (1986). Peroxidase zymograms of 16 apple rootstocks. *Canadian Journal of Plant Science,* **66**: 647-652.

Wendel, J.F. and Parks, C.R. (1983). Cultivar identification in *Camelia japonica* L. using allozyme polymorphism. *Journal of American Society for Horticultural Science,* **108**:290-295.

Went, F.W. (1938). Specific factors other than auxin affecting growth and root formation. *Plant Physiology,* **13**: 35-80.

Woodbridge, C.G. (1973). Effects of rootstocks and interstocks on nutrient levels in 'Barlette' pear leaves, on tree growth, and on fruit. *Journal of American Society for Horticultural Science,* **98**: 200-202.

Wutscher, H.K. and Hill, L.L. (1995). Performance of Hambin orange on 16 rootstocks in east-central Florida. *HortScience,* **30**(1): 41-43.

Wycherly, P.R. (1968). Breeding of *Hevea. Planter's Bulletin,* **99**: 159

Yadava, U.L. and Doud, S.L. (1978). Effect of rootstock on the bark thickness of peach scions. *HortScience,* **13**(5): 538-539.

Yagishita, N. and Hirata,Y. (1987). Graft-induced changes in fruit shape in *Capsicum annuum* L.: 1. Genetic analysis by cross. *Euphytica,* **36**: 809-814.

Yagishita, N., Hirata, Y., Mizukami, H., Ohashi, H. and Okochi, K. (1986). Characterisation of graft-induced changes of capsicin content in *Capsicum annuum* L. *Euphytica*, **34**: 297-301.

Yagishita, N., Hirata., Mizukami, H., Ohashi, H. and Yamashita, K. (1990). Genetic nature of low capsicin content in the variant strains induced by grafting in *Capsicum annuum*.L. *Euphytica*, **46**: 249-252.

Yeang, H.Y., Wickneswari, R., Sunderasan, E., Leong, S.K., Asri,H.A., Napi, D.M., Zamri, A.S.M. and Ghani, M.N.A. (1995). Isozymes in *Hevea* crop improvement. *IRRDB Symposium on physiological and molecular aspects of the breeding of Hevea brasiliensis.* 1995, England.

Yeet, Y.H., Gandhimathi, H. and Paranjothi, K. (1977). Protein and enzyme variation in some *Hevea* cultivars. *Journal of Rubber Research Institute of Malaysia.*, **25**(1): 9-18.

Yoon, P.K. and Leong, S.K. (1975). Induction of pseudo-tap roots of cutting and production of clonal rootstocks in *Hevea*. *Proceedings of International Rubber Conference,* 1975, Kuala Lumpur, Malaysia,pp.85-108.

Yoon, P.K. (1972). Technique of crown budding. *Rubber Research Institute of Malaya,* p.28.

Yoon, P.K. and Ooi, C.B. (1976). Deep planting of propagated materials of *Hevea*- It's effects and potentials. *Proceedings of National Plant Propagation Symposium,* 1976,pp.273-302.

Young, E. and Houser, J. (1980). Influence of Siberian C Rootstock on peach bloom delay, water potential and pollen meiosis. *Journal of American Society for Horticultural Science,* **105**(2): 242-245.

Ystaas, J., Hovland, O. and Kvale, A. (1995). Productivity and fruit quality of Aroma' apples as affected by tree density in single row, double row and triple row planting systems in a northern climate. *Soil and Plant Science, Acta Agriculturae Scandinavica, Section B,* **45**(2): 132-141.

Zimmerman, R.H. and Steffens, G.L. (1989). Management of self rooted tissue-cultured apple trees: 1. Orchard establishment and early growth. *Acta Horticuturae,* **239**: *117-120.*

Zimmerman, R.H. and Miller, S.S. (1991). Orchard growth and fruiting of micropropagated apple trees. *Journal of American Society for Horticultural Science,* **116**: 780-785.

Zimmerman, R.H. and Steffens, G.L. (1996). Long-term evaluation of micropropagated apple trees: vegetative growth, cropping and photosynthesis. *Scientia Horticulturae,* **66**: 69-76.