GENETIC STUDIES ON YIELD AND CERTAIN YIELD COMPONENTS IN THE PARA RUBBER TREE [*HEVEA BRASILIENSIS* (Willd. ex Adr. de Juss.) Muell. Arg.]

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भारतीय रबड़ गवेषण संस्थान
रबड़ बोर्ड, वाणिज्य एवं उद्योग मन्त्रालय, भारत सरकार
The Rubber Research Institute of India
Rubber Board, Ministry of Commerce and Industry, Govt. of India

CERTIFICATE

This is to certify that the thesis entitled **Genetic studies on yield and certain yield components in the para rubber tree [*Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg.]** is an authentic record of original research work carried out by **Mr. Thomas Sebastian** at the Rubber Research Institute of India, Kottayam, under my supervision and guidance during the period June 1998 to November 2003, in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** in the Faculty of Science, Mahatma Gandhi University. The work presented in this thesis has not been submitted for the award of any other degree or diploma earlier. It is also certified that Mr. Thomas Sebastian has fulfilled the necessary requirements for submission of thesis and has passed the qualifying examination.

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DECLERATION

I hereby declare that this thesis entitled **Genetic studies on yield and certain yield components in the para rubber tree [*Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg.]** has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles for recognition.

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Thomas Sebastian

Dedicated to my parents and brothers

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ABBREVIATIONS

AC	-	Ash Content
AFLP	-	Amplified Fragment Length Polymorphism
AE	-	Acetone Extract
ASTM	-	American Society for Testing and Materials
CD	-	Critical Difference
cm	-	Centimeter
CTAB	-	Hexadecyl Triethyl Ammonium Bromide
CV	-	Coefficient of Variation
dATP	-	Deoxy Adenosine Triphosphate
dCTP	-	Deoxy Cytosine Triphosphate
dGTP	-	Deoxy Guanosine Triphosphate
dTTP	-	Deoxy Thymidine Triphosphate
DRC	-	Dry Rubber Content
DRY	-	Dry Rubber Yield
EDTA	-	Ethylene Diamine Tetra Acetic acid
GA	-	Genetic Advance
GC	-	Gel Content
GCV	-	Genotypic Coefficient of Variation
GI	-	Girth Increment on tapping
$g\ t^{-1}\ t^{-1}$	-	Gram per tree per tap
$g\ plant^{-1}\ 10t^{-1}$	-	Gram per plant per ten tappings
h	-	Hour
H^2	-	Heritability (broad sense)
ha	-	Hectare
IFR	-	Initial Flow Rate
IS	-	Indian Standards
KRS	-	Kohong Rubber Station
LTP	-	Length of Tapping Panel
LVR	-	Latex Vessel Rows
LVR-RB	-	Latex Vessel Rows in Renewed Bark
LVR-VB	-	Latex Vessel Rows in Virgin Bark
LY	-	Latex Yield

m	-	meter
M	-	Molar
ml t^{-1} t^{-1}	-	Millilitre per tree per tap
ml min^{-5} cm^{-1}	-	Millilitre per five minutes per centimeter of tapping cut
mm	-	millimeter
mM	-	Millimolar
MV	-	Mooney Viscosity
N_2	-	Nitrogen Content
NR	-	Natural Rubber
NRS	-	Non Rubber Substances
NS	-	Non Significant
%	-	Per cent
P_0	-	Initial Wallace Plasticity
PB	-	Prang Besar
PCR	-	Polymerase Chain Reaction
PCV	-	Phenotypic Coefficient of Variation
PI	-	Plugging Index
PRI	-	Plasticity Retention Index
PVPP	-	Polyvenil Polypyrolidone
RAPD	-	Random Amplified Polymorphic DNA
RFLP	-	Restriction Fragment Length Polymorphism
RBT	-	Renewed Bark Thickness
rpm	-	Rotation Per Minute
RRIC	-	Rubber Research Institute of Srilanka
RRII	-	Rubber Research Institute of India
RRIM	-	Rubber Research Institute of Malaysia
SDS	-	Sodium Dodecyl Sulphate
t	-	tones
2, 4, 5 - T	-	2, 4, 5 - trichlorophenoxyacetic acid
TSC	-	Total Solid Content
TE buffer	-	Tris - EDTA buffer
UPGMA	-	Unweighed Pair - Group Method using Arithmatic Average
UV	-	Ultra Violet
V	-	Volt
VBT	-	Virgin Bark Thickness

Chapter 1

INTRODUCTION

INTRODUCTION

The para rubber tree, *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. is a perennial tree belonging to the family, Euphorbiaceae and is the major source of natural rubber. Natural rubber is having varied industrial, technological and domestic uses. The unique and versatile properties of this material have made it highly indispensable for the modern life. Among the tree crops, no other plant species has influenced human life as much as natural rubber and the rubber plantation industry has now almost revolutionized the industrial world. Today, natural rubber cultivation is a good preposition of ecologically sustainable, socially acceptable and economically viable agriculature. Apart from latex, the rubber plantation is also being valued for its timber, which reduces the pressure on natural forests for timber and wood (Jacob, 2002).

Natural rubber has been found in the latex of over 2000 species of plants belonging to 311 genera of 79 families. The minor sources of natural rubber are *Manihot glaziovii* (Euphorbiaceae), *Ficus elastica* (Moraceae), *Parthenium argentatum* and *Taraxacum koksaghyz* (Compositae). In addition, many other species like *Castilla elastica* (Moraceae), *Cryptostegia grandiflora* (Asclepiadaceae) and *Funtumia elastica* (Apocynaceae) have been experimented as possible minor sources of natural rubber (Wycherley, 1992). The genus *Hevea* comprises ten species *viz., Hevea benthamiana, H. brasiliensis, H. camargoana, H. camporum, H. guinensis, H. microphylla, H. nitida, H. pauciflora, H. rigidifolia, and H. spruceana* (Schultes, 1970, 1977, 1987; Wycherley, 1992). Of the above ten species, only *H. brasiliensis*

produces 99 per cent of the world's natural rubber (Saraswathyamma, 2002). The quality and quantity of natural rubber produced by *H. brasiliensis* is superior to those of all other species.

H. brasiliensis is a native to the tropical rain forest of Central and South America rica and is one of the recently domesticated crop species in the world. *Hevea* is introduced to South East Asia in 1876 by Sir Henry Wickham and has been commercially cultivated in India since 1902 (Nair *et al.,* 1976). The original genetic material of *Hevea* is referred as 'Wickham gene pool'. Rubber is propagated by generative and vegetative means. Generative method is through seeds and vegetative method is through budgrafting.

The economic life span of the tree is very long with a gestation period of six to seven years (Plate 1). The rubber tree is sturdy, quick growing and tall (Plate 2). A warm humid equable climate (21° C to 35° C) and a fairly distributed annual rainfall of not less than 200 cm are necessary for the optimum growth. The tree grows successfully under slight varying conditions also. The tree is now grown in tropical regions of Asia, Africa, and America. Rubber tree has a well-developed taproot and laterals. The bark on tapping yields latex (Plate 3). Latex present in latex vessel rows in the bark of the tree trunk is exploited commercially for the extraction of latex (Plate 4 a and b). The cambium in between wood and bark is responsible for the increase in girth of the tree including bark renewal. The leaves are trifoliate with long stalks. Normal annual leaf fall known as "wintering" occurs in the case of mature trees during the period December to February in South India. Refoliation and flowering follow wintering. Some trees may occasionally show off-season flowering during September - October. The rubber tree is monoecious. Both male and female flowers are seen in the same inflorescence. Male flowers are much more numerous than female flowers which are bigger and found terminating the main branches of the

panicle. Pollination is by insects. Only a small proportion of the female flower set fruits and good number of flowers are shed during tender stage (Saraswathyamma, 1990). The fruits mature in about five to six months after pollination. They are three seeded and burst when mature, scattering the seeds 15 - 18 meters. The seeds weigh four to six grams. They possess a hard brown coat having characteristic mottling. Seeds of seedling trees and different clones vary in size, shape, weight and seed coat markings. The seeds belonging to a clone have characteristic size, shape and seed coat mottlings. It has been reported that seed coat can be utilized for the identification of clones (Polhamus, 1962; Saraswathyamma *et al.*, 1981; Mercykutty *et al.*, 2002 and Sebastian *et al.*, 2002).

Rubber plantation industry in India has registered commendable growth in production and productivity. India has attained the first position in terms of productivity with 1576 kilograms per hectare per year (Desalphine, 2002). The country holds third position in terms of production (631, 400 t) covering an area of 566, 558 ha and fourth in consumption of natural rubber (Krishnakumar, 2003). This achievement is mainly due to the development and proper utilisation of genetically improved planting materials. The development and release of RRII 105, the outstanding high yielder has contributed substantially for this progress. The potential and realised yield of some other RRII clones, also showed that a few of them perform extremely well in certain areas (Mathew, 2002).

Genetic improvement programme in tree crops, especially *Hevea* is laborious and a minimum period of 20 - 25 years is required for the evaluation and release of a clone. Earlier selections were made among the trees obtained from seeds that produced only 200 - 300 kilogram per hectare per year (kg $ha^{-1}yr^{-1}$). Later, new clones were developed adopting various genetic improvement programmes. These selections boosted annual production

to 2000 kg $ha^{-1}yr^{-1}$. Now there are clones having a production potential of around 4000 kg $ha^{-1}yr^{-1}$ (Saraswathyamma, 2002). Today India accounts for nine per cent of the world production of NR (Rubber Board, 2003).

There are several problems that hamper breeding and quick release of cultivars for large scale planting. They include seasonal nature of flowering and low fruit set, long breeding and selection cycle, lack of fully reliable early selection methods, etc. In India, flowering is restricted to a short period of two to three months; however, all the clones do not flower simultaneously (George *et al.*, 1967). This non-synchronization of flowering in some of the parent clones selected, limits the possibility of attempting all possible cross combinations.

During the past, the main objective in breeding was to improve productivity. Subsequently, improvement of secondary characters became one of the objectives in *Hevea* breeding programme. Yield in *Hevea* is determined by the volume of latex and the percentage of rubber it contains. The rubber yield in *H. brasiliensis* is a complex multifactorial trait and is a manifestation of various morphological, anatomical, physiological and biochemical characters of the tree (Pollinere, 1966). The choice of a suitable breeding method for improvement of yield and its components depends on genetic variability, association between characters, heritability and the value of expected genetic advance under selection.

Attempts have been made to study the relationship between yield and yield attributing factors and several yield components have been identified (Swaminathan, 1977; Markose, 1984; Simmonds, 1989; Saraswathyamma, 1990; Premakumari, 1992; Mydin, 1992; Licy, 1997). However, a dearth of knowledge still exists on the nature and extend of genetic and environmental control of these traits. Moreover, indepth information on the performance of many of the exotic clones in local agroclimatic region of India is scanty. Hence the

present investigation was taken up including a set of 13 *Hevea* clones (12 introduced clones along with RRII 105) with the following objectives:

- To evaluate the performance of a set of 12 exotic clones in the local agroclimatic condition.
- To examine the genetic variability for yield and yield components among these clones along with indigenous clone, RRII 105.
- To assess the relationship between yield and factors contributing to yield.
- To select genetically divergent genotypes for the utilization of them in the hybridization programme in future.
- To identify prepotent clones based on performance of seedling progenies for their utilization in polyclonal seed gardens.
- To study latex and rubber properties of the above 13 clones.
- To identify clones having high yield and desirable secondary attributes in comparison with outstanding clone RRII 105.

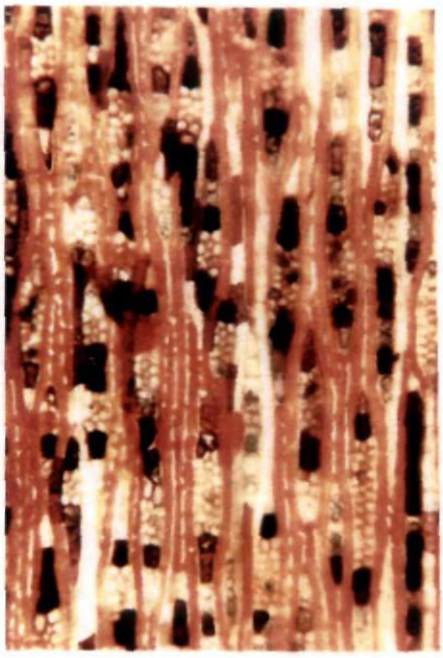
Selections based on the information obtained from genetic studies on yield and yield components can provide better candidates in the crop improvement programme in this species.

Plate 1. An immature *Hevea* plantation with cover crop

Plate 2. A view of the mature *Hevea* plantation

Plate 3. A mature tree under tapping

(a)



(b)

Plate 4. (a) Radial longitudinal section of *Hevea* bark showing the latex vessel rows

(b) Transverse longitudinal section of *Hevea* bark showing interconnections of latex vessels

Chapter 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1. Genetic base of *Hevea*

The genetic base of *Hevea* is narrow (Wycherley, 1968; Schultes, 1977; Allen, 1984). From this little genetic foundation spectacular yield improvement of about ten times have been achieved (Varghese, 1992). However, reports of Tan, (1987); Ong and Tan, (1987); Simmonds, (1989) elucidate that the genetic advance gained in the early breeding phases seems to have slowed down in the more recent phases of breeding. There are a number of factors that narrow down the genetic variation, *viz.*, (1) wider adaptation of clonal propagation by budding (2) directional selection for yield (3) and cyclical assortative breeding pattern. Such unidirectional selection for yield over the years ignoring the genetic variability with regard to secondary characters (Wycherley, 1969) has reduced the genetic variability in the population. In generation wise assortative mating best genotype in one generation is used as the parents for next and so on (Simmonds 1986a, 1989). So the parentage of the best clones can be traced back to a limited number of parent genotypes (Tan, 1987 and Varghese, 1992). However, efforts are in progress to broaden the genetic base of *Hevea* by the introduction of wild germplasm from the centre of origin and introduction of exotic clones. Moreover, the highly hetrozygous nature of the clone can also be exploited for enhancing the existing genetic variability.

Hybridization programme between clones in India started in 1954 with the available clones introduced earlier into the country (Nair and Panikkar, 1966). So far

around 5700 hybrid seedlings have been produced and about 1500 clones were developed (Varghese *et al.*, 1990) of which RRII 100 series (Nair and Panikkar, 1966; Nair and George, 1969; George *et al.*, 1980 and Nazeer *et al.*, 1986), 200 series (Saraswathyamma *et al.*, 1980) and 300 series (Premakumari *et al.*, 1984) are of commercial importance. Among the initial selections designated as RRII 100 series of clones (Nair and George, 1969) RRII 105 is the most outstanding clone. Hybridisation and ortet selection are the two conventional breeding methods practiced in *Hevea* that contributed to substantial increase in productivity. The identification of variability and successful implementation of bud grafting are the two developments that successfully led to the synthesis of early primary clones through ortet selection or mother tree selection.

2.2. Major yield components

2.2.1. Growth

Growth is one of the most important characteristics of a clone, next to yield. During the first few years, growth is mainly in length, and a rapid increase in girth becomes noticeable after the trees are a few years old. Different clones have different characteristic growth patterns (Polhamus, 1962). Girth measurements are considered an important criterion of tappability, since vigorous clones enable early opening of the trees for tapping (Ostendorf, 1932; Vollema and Dijkman, 1939; Dijkman, 1951; Peries, 1970; Webster and Paardekooper, 1989; Wright, 1998). Annual growth pattern of rubber trees during the immature and mature phases have been discussed in detail (Templeton, 1968, 1969; Pillay, 1980; Webster and Paardekooper, 1989; Sethuraj and Mathew, 1992; George and Jacob, 2000) and the relationship between girth increment rate and rubber yield in different clones have been well established. (Nga and Subramanian, 1974; Ong, 1981; Chandrasekar, 1994 and Tuy, 1997). In general, a growth retardation is observed during tapping, which also shows wide clonal variation.

Vollema (1941) reported that tapping does retard the growth of the *Hevea*. However, Schweizer (1941) indicated that rubber production in connection with growth increases. Annual girth increment showed difference among clones and high yielding clones generally showed low girth increment on tapping (Markose, 1984). However, Licy (1997) reported no such trend in a set of clones.

Analysis of monthly growth pattern and its duration in 13 *Hevea* clones from a traditional rubber growing tract in India, indicated that the peak growth period is about 2 months (Jul - Aug) and the active growth period is about 6 months (May - Oct.) (Chandrasekar *et al.*, 2002). Clonal differences in growth could be mainly attributed to genotypic differences. However growth is also determined by other factors like soil, climate, planting density, cultural operation etc. A few reports are also available on the monthly growth pattern of *Hevea* trees exposed to long drought and high summer temperature in subhumid tropies (Chandrasekar *et al.*, 1996; Chandrashekar *et al.*, 1998). The results showed that by analysing the growth of rubber, potentially drought tolerant clones can be identified.

2.2.3. Physiological components of yield

Harvesting of *Hevea* is carried out by the controlled wounding of the bark of the tree trunk. This process is known as tapping. The standard girth generally accepted for commencement of tapping is 50 cm at a height of 125 cm from bud union for all the bud-grafted materials.

Sethuraj (1968, 1981, 1977) identified initial flow rate, plugging index, dry rubber content and length of the tapping cut as major physiological yield components in *Hevea*, and established the relationship between yield with its major yield components. The

influence of initial flow rate on yield has been established (Sethuraj *et al.*, 1974; Yeang and Paranjothy, 1982). Sethuraj *et al.*, (1974), Sethuraj (1981) found initial flow rate to be a clonal character. Relationship between seasonal variation in initial flow rate and variation in yield in different clones of *Hevea* has been reported (Saraswathyamma and Sethuraj, 1975).

Plugging index is an index that measures the extent of time during which latex flows out or it indicates the intensity of flow restriction mechanism operating in the latex vessel after tapping (Milford *et al.*, 1969). It is also a major component of yield in *Hevea.* Clonal variation in plugging index have been established by Milford *et al.*, (1969); Paardekooper and Somosorn (1969); Sethuraj *et al.*, (1974); Saraswathyamma and Sethuraj, (1975). Yield has been found to be positively correlated to the initial flow rate (Paardekooper and Somosorn, 1969) and negatively correlated to plugging index (Milford *et al.*, 1969).

The dry rubber content (DRC) of natural rubber latex, as obtained from the tree, varies from 30 to 40% by weight. Clonal characteristics, age of tree, length of tapping cut, frequency of tapping, stimulant application, time of tapping, environmental conditions etc. are some of the factors that affected DRC of latex (Kang and Hasim, 1982). Seasonal variation in yield is a result of volume of latex and not that of rubber content. Paardekooper and Sookmook, (1969) have established a negative correlation between yield and rubber content, this indicate that high yielding character of a clone is a result of the low plugging and that because of the higher extraction of latex the rubber content can be maintained only at a lower level under a given regenerative capacity (Sethuraj, 1992). The dry rubber content (DRC) of latex varies in different clonal lattices (Ng *et al.*, 1979). It is reported that the rubber content of latex tends to become higher during the lowest yield periods like summer. Generally DRC is highest at the time of first opening and gradually reaches a stable condition.

Wittshire (1934) reviewed the earlier work on DRC variations. Schweizer (1936) noted a decrease in DRC during wintering which rapidly regained normal condition. Rebaillier (1972) also reported seasonal changes in DRC.

The length of tapping cut is another major component that control yield. The length of the tapping cut is determined by the girth of the tree for a given system of tapping (Sethuraj, 1992). pH of latex is considered to be an important factor regulating the metabolic activity of the laticiferous system and highly significant positive correlations have been obtained between pH and latex production under certain conditions (Brozozowska - Hanower *et al.*, 1979; Cretin *et al.*, 1980; Eschback *et al.*, 1984).

The factors that influence annual rubber yield from a unit area is determined by the average yield $tree^{-1}$ tap^{-1}, the number of trees and number of tapping per year (Sethuraj, 1992). In India, the period of peak yield is from September to January (Sethuraj, 1992). Seasonal variations in yield is being mediated through variation in both initial flow rate and plugging index (Milford *et al.*, 1969; Paardekooper and Somosorn, 1969; Saraswathyamma and Sethuraj, 1975 and Sethuraj, 1977). It was clearly demonstrated that the effect of drought and high temperature is mediated mainly through changes in plugging index than through changes in initial flow rate (Ninane, 1970).

The lowest value for rubber content is during monsoon season and highest values during dry seasons (drought season), which are the highest and lowest yielding periods respectively (Sethuraj, 1992). Negative relations between yield recorded over a period of one year and rubber content has been established (Heuser and Holder, 1931; Wittshire, 1934; Brozozowska - Hanower *et al.*, 1979).

It is evident that the annual yield of a clone or clones depends upon the average values of initial flow rate, plugging index and dry rubber content through different seasons of the year. The environmental factors leading to lower flow rate and higher plugging will reduce yield. The variation in rubber content is related to its interaction with plugging and the volume of the latex lost. When yield is reduced due to high plugging rubber content will increases (Sethuraj, 1992). The latex yield is dependent on the rate and duration of latex flow (Markose, 1984).

2.2.4. Anatomical components of yield

The major type of laticifers exploited commercially for its latex is secondary laticifers distributed in the bark of tree trunk. Bryce and Campbell (1917) have briefly illustrated the structure of *Hevea* bark. The outermost protective layers of tissues are called cork cells. Interior to the cork cells, there are two more distinguishable zones, an inner soft zone and an outer hard zone. Sclerified stone cells are present in the outer zone, which makes this region so harder. The latex vessels in *Hevea* appear as concentric rings, alternating with layers of phloem cells. The differentiation of laticifers from the cambial cells is a rhythmic process and a ring of laticifers is produced each time (Premakumari *et al.*, 1992). Latex vessels are developed by the activity of vascular cambium. Rao (1975), Premakumari *et al.*, (1981) reported that the rate of cambial activity shows seasonal variations. Thomas *et al.*, (2002) reported climatic variations have significant influence on cambial rhythm.

The number of latex vessel rings is a clonal character (Sanderson and Sutcliffe, 1921; Vischer, 1921, 1922 and Bobilioff, 1923) and the frequency of laticiter differentiation is genetically controlled (Premakumari *et al.*, 1992). Depending upon the clone, age of the tree, growth rate, seasonal factors etc. the number of latex vessels varies. Age of the clone is one of

the major factors that determine the frequency of distribution of latex vessel rows in virgin bark. Gomez (1982) reported that in the trees below five years, majority of the laticifer rings were concentrated in the first 4 - 5 mm, and only 40% being in the second. Between five and ten years, laticifer rings were concentrated near the cambium and it would be almost zero near the eight millimeter and by about 25 years about 75% of the latex vessel rings were oriented at the inner most five millimeter of the bark. Bark is regenerated due to the continued activity of vascular cambium. During this process new phloem tissues are produced and normal process of laticifer differentiation continues. The protective tissue lost by tapping is replaced by the formation and activity of a new phellogen below the cut surface (Bobilioff, 1923; Panikkar, 1974).

Various workers have already established highly significant correlations between yield and structural features. Bobilioff (1920), La Rue (1921) and Taylor (1926) reported significant correlations between yield and number of latex vessel rows in seedling progenies. Elaborate studies and yield component analysis proved that the number of latex vessel rows is the major single factor related to yield. When this character was related with girth and plugging index, that accounted for 75 per cent of the yield variations in young plants. A significant correlation between number of latex vessel rows and initial flow rate could be identified (Sethuraj *et al.*, 1974b). Laticifer area and orientation of latex vessel rows are reported to be the factors that influence yield of *Hevea* clones (Premakumari *et al.*, (1988).

2.3. Genetic studies on yield and major yield components

2.3.1. Variability, heritability and genetic advance

The success of any breeding programme usually depends upon the quantum of genetic variability present in the materials. The knowledge of genetic variability, heritability

and genetic advance in *Hevea* is very essential for a breeder to choose desirable parents and to decide the correct breeding methodology for crop improvement. Genetic studies in *H. brasiliensis* are time consuming, mainly due to the perennial habit of the planting materials. Simmonds (1969) reported that most of the differences between family yields could be accounted for by additive gene effect. Analysis of variance were carried out on yield and girth of seedling progenies of earlier hand pollination (Gilbert *et al.*, 1973; Nga and Subramanian, 1974; Tan *et al.*, 1975; Tan, 1975; Alika, 1980 and Liang *et al.*, 1980). In analysis of variance, magnitudes of sum of squares of relevant terms as well as variance components are used to quantify sources of variation.

The economically important characters like yield and components of yield are not determined by a single gene but it is polygenically controlled. To ascertain the influence of genes and various nongenic factors, detailed biometrical study is essential. However this is relatively limited. The knowledge of the phenotypic variance of a trait and its separation into genetic and environmental components is useful for helping breeders to design an effective selection method. Genotypic coefficient of variation indicates the relative magnitude of genetic diversity present in the material and helps to compare the genetic variability present for different characters. Mydin (1992) reported high genetic variability for volume of latex under stress, plugging index under stress, annual mean dry rubber yield, and dry rubber yield during stress and peak periods was indicated by high estimates of genotypic coefficient of variation. Markose (1984) reported high genotypic coefficient of variation for dry rubber yield, latex volume, bark thickness and latex vessel rows, however, DRC showed low GCV. Licy *et al.*, (1992) indicated high GCV for total volume of latex and lowest for bark thickness, among a set of 23 hybrid clones. Licy (1997) reported that the phenotypic

coefficient of variation was higher than genotypic coefficient of variation for all the characters studied. However it was closer for most of the characters suggesting less environmental influence. Moderate to high GCV is observed for the annual mean dry rubber yield, summer yield, peak yield, volume of latex, girth increment rate, number of latex vessel rows in virgin, and renewed bark, rate of latex flow and plugging index.

Heritability, is useful for comparing and improving the efficiency of selection methods. It is mathematically defined as the ratio between the additive variance (σ^2A) and the phenotypic variance (σP^2). Varying levels of heritability for yield and major yield components has already been reported (Liang *et al.*, 1980; Alika, 1982; Markose, 1984; Mydin, 1992 and Licy, 1997). It estimates the degree of resemblance between offsprings and parents. Heritability decreases with the increase in environmental component of variance for the character under selection (Varghese, 1992). The major functions of heritability estimates are to provide information on transmission of character from the parent to the progeny. Such estimates facilitate evaluation of hereditary and environmental effects in phenotypic variation and thus aid in selection. Heritability estimate can be used to predict genetic advance under selection so that breeder can anticipate improvement from different types and intensities of selection. Information in advanced generations on estimates of heritability and genetic advance on rubber yield and its components in *Hevea* is very limited (Simmonds, 1986a). Genetic advance under selection can be estimated from the given heritability value (Alika, 1982; Simmonds, 1989). Tan and Subramanian (1976) established additive inheritance for several seedling characters in the nursery. Gilbert *et al.*, (1973) and Nga and Subramanian (1974) noted that yield and girth variation can be largely accounted for by additive genetic variation.

Markose (1984) reported that broad sense heritability was high for dry rubber yield, latex volume, bark thickness and number of latex vessel rows. Mydin (1992) reported that additive gene effects offering scope for improvement through selection was indicated for dry rubber yield, latex flow rate and volume of latex girth increment rate, annual plugging index, plugging index under stress by the moderate to high heritability estimates along with high genetic advance for these traits. Non-additive gene action was indicated by the high heritability and low genetic advance for dry rubber content during the three periods, girth and bark thickness. Licy *et al.,* (1992), reported that nature and magnitude of genetic variability, heritability and heterosis were assessed in 23 F1 hybrid clones, derived from the cross between RRII 105 and RRIC 100 of *Hevea brasiliensis* at premature phase. Mean annual yield exhibited a high heritability with high genetic advance. Licy (1997) reported high heritability coupled with high genetic advance observed for some of the economic traits like dry rubber yield, volume of latex, rate of flow.

2.3.2. Genetic divergence

Multivariate analysis by means of Mahalanobis D^2 statistic has been recognized as a powerful tool in the hands of breeders to quantify the degree of divergence between genotypes, biological population at genotypic level.

Based on D^2 analysis for yield and various yield components a set of mature Wickham clones were grouped in to eight (Markose, 1984; Mydin *et al.,* 1992) and nine (Abraham *et al.,* 1997) genetically divergent clusters. Considerable genetic diversity was revealed by the wide range of D^2 values and intra and inter cluster distances. The forty clones were grouped into genetically divergent clusters irrespective of their country of

origin indicating the absence of any relationship between geographic diversity and genetic divergence (Markose, 1984; Mydin *et al.*, 1992).

2.3.3. Association of characters

The component traits are not independent in their action but are interlinked and in this complex genetic system, selection practiced for an individual trait might subsequently bring about a simultaneous change in the other. Thus an understanding of the association among component trait is essential to bring a rational improvement. Breeders regularly have to improve two or more traits simultaneously, such as high yield with desirable secondary attributes. Such traits often show correlated response.

A knowledge of the association of quantitative traits, especially of yield and its attributes will be of immense practical value in crop breeding programme. Selection pressure can be profitably exerted on any of these easily discernible characters having close association with yield (Kamalam *et al.*, 1978). Correlation studies between various yield components at nursery stage have established the influence of vigour, bark thickness and number of latex vessel rows on the yield of *Hevea* clones (Ho *et al.*, 1973; Narayanan and Ho, 1973).

Grantham (1925) and Heusser and Holder (1931) found a negative correlation between yield and dry rubber content. Lee and Tan (1979) found a close association between daily latex volume and yield of rubber and suggested that latex volume was a dominant factor determining yield. Correlations between yield and morphological characteristics of the planting materials have been attempted by various workers (Whitby, 1919; Sanderson and Sutcliffe, 1929; Dijkman and Ostendorf, 1929; Gilbert *et al.*, 1973; Narayanan *et al.*, 1973; Lee and Tan, 1979; Liang *et al.*, 1980; Liu, 1980; Filho *et al.*, 1982; Hamazah and Gomez, 1982 and Pavia *et al.*, 1982). Licy (1997) reported that genotypic correlation in general was higher than

phenotypic correlations in most of the cases, among the 18 characters studied, most of the correlations were found to be in the positive direction. At both phenotypic and genotypic level, summer yield, peak yield, volume of latex, number of latex vessel rows in both virgin and renewed bark and virgin bark thickness exhibited high positive association with annual mean dry rubber yield. The above characters in turn showed high positive association among themselves too. This suggests the scope for simultaneous improvement of these traits by selection, which in turn will improve yield as well. A positive correlation between initial flow rate of latex and number of latex vessel rows in a population of cross-pollinated families was estimated (Sethuraj *et al.*, 1984).

From the correlation studies it has been reported that yield and girth are related to each other and in general positively correlated (Narayanan and Ho, 1973; Liu, 1980). Significant positive correlations of dry rubber yield with volume of latex, latex vessel rows and virgin bark thickness have been reported (Wycherley, 1969; Narayanan *et al.*, 1974 and Markose, 1984). A negative correlation of girth increment with rubber yield has been observed (Narayanan *et al.*, 1973) for trees under tapping where the plant assimilates are partitioned in favour of latex formation rather than growth, particularly in case of clone having high yield potential.

Markose (1984) and Mydin (1992) reported that yield was positively and significantly correlated with latex volume, bark thickness, and number of latex vessel rows. The correlations of bark thickness and latex vessel rows with yield were found mediated through volume of latex at both genotypic and phenotypic levels, annual mean dry rubber yield showed moderate to high positive correlation with dry rubber yield during the stress and peak periods, volume of latex, dry rubber content and latex flow rate during the various

seasons, girth, girth increment rate, length of tapping panel and bark thickness and negative correlations with yield depression under stress and plugging index during three periods.

2.4. Latex and rubber properties

The chemical composition of freshly tapped *Hevea* latex is complex compared to synthetic lattices. This is because *Hevea* latex is a cytoplasm. In addition to the rubber hydrocarbon *Hevea* latex contains a large number of non-rubber constitutes in small quantities. Many of these are dissolved in the aqueous serum of the latex, others are adsorbed at the surface of the rubber particles and some are the non-rubber particles suspended in the latex. A typical composition of fresh latex is as follows.

Total Solid Content	-	41.5%
Dry Rubber Content	-	36%
Protein	-	1.4%
Neutral lipids	-	1.0%
Phospholipids	-	0.6%
Ash	-	0.5%
Inositol and Carbohydrates	-	1.6%
Other Nitrogen compounds	-	0.3%
Water	-	58.5%

There are 3 major particular components suspended in an ambient serum. They are the rubber particles, lutoids and the Frey-Wyssling particles. Rubber particles constitute 25 - 45% of the volume of the latex in fresh latex. The rubber particles in fresh latex are protected by a complex film containing proteins and lipids. The rubber, contained in the particles, is non water - soluble and occurs as molecular aggregates.

Natural rubber latex is a milky liquid that consists of extremely small particles of rubber suspended or dispersed in an aqueous medium. It is obtained from a great variety of plants, but *Hevea brasilensis* is the only commercial source of latex. The chemical structure of natural rubber is cis-1, 4-polyisoprene, it is a high molecular weight polymer. In addition to the pure rubber hydrocarbon, natural rubber contains various other substances like proteins, fats and fatty acids carbohydrates, mineral matter etc. Allen and Bloomfield (1963) reported the hydrocarbon content is about 94%. The presence of non-rubber substances, though they are in small concentrations, is reported to influence the chemical and physical properties of the hydrocarbon polymer. The properties of natural rubber depend upon the state of cross-linking. It has reported that the cis content of the polymer in NR is to be almost 100 per cent. However Tanaka, (1985) reported the presence of about three trans unit per chain.

The ultimate objective of *Hevea* breeding is to improve the yield potential and economically important secondary characters. But selections of genotypes having latex with good technological properties are also important in present scenario. Some technological characters of rubber such as it's viscosity or plasticity retention index (PRI) could be a selection target if improvement were deemed economically (Demange *et al.*, 2001). At present the selection is based mainly on biological characteristics such as yield of latex, girth and resistance to disease and wind damage. However a high yielding clone with vigorous growth and resistance to maladies need not always produce latex and rubber of desirable properties. A study of the properties of the clonal rubbers is of importance because proper understanding of these characters will enable plant breeders to consider related properties

in their choice of clones for planting. More emphasis is now being placed on the properties of the latex and rubber obtained from individual clones. Fuller (1988) reported that a major source of variability within and between natural rubber grades is probably the difference in the property of the latex derived from different clones. Several properties of clonal lattices have been studied and documented (Subramanian, 1975). The physical properties of clonal rubber have been less examined. Different clones have different characteristics and give rubber with different properties. The colour and composition of the latex and the plasticity of the rubber tend to be uniform within a clone and different for different clones (Martin, 1961). Seasonal factors and soil characteristics could also affect both the quantity and composition of the latex (Ebi and Kolawole, 1992). Reports on, systematic study of lattices and rubber properties of different clones are scanty.

2.5. Molecular markers

Molecular markers have a great potential for plant breeding as it promises to expedite the time taken to produce crop varieties with desirable characters. The efficiency of conventional plant breeding is greatly improved, as the selection is based not directly on the trait but on molecular markers linked to that trait. In *Hevea*, studies on the application of molecular markers have been initiated and a few reports on the potential use of this powerful technique are available. Molecular markers like isozyme have been utilized for various purposes in plant breeding.

Isozymes are multiple forms of an enzyme that differed by minor variations in amino acid composition reaction, but exhibit different physical or kinetic properties. Bergman (1987); Cousineau and Donnelly (1992); Granger *et al.,* (1992) reported that isozymes can provide useful information at the genomic level and are widely used to identify and discriminate

cultivars in many agricultural and horticultural crop species. Sreelatha *et al.*, (1993) utilized isozyme polymorphism for the identification of different cytotypes of *Hevea brasiliensis*.

The characterization and identification can be carried out on the basis of phenotypic differences of electrophoretic banding patterns. The advantage of isozymes is that these patterns can usually be interpreted in terms of loci and alleles. The phenotypic approach for characterizing and comparing populations has been criticized because it does not provide genome information (Crawford, 1983; Simpson and Withers, 1986). Therefore, isozymes are ideal genetic markers when estimating genetic variability and plant population. The advantages of isozymes over other biochemical markers are (a) allelic expression is generally co-dominant, free of epistatic interaction and usually unchanged by environmental effects (b) alleles of different loci are generally distinguishable (c) enzymatic systems to be studied are usually chosen for technical reasons independent of their level of genetic variability, as a result of this they can represent a random sample of the genome (d) allelic differences are always detected as mobility difference independent of the functioning and level of variability of each enzyme system.

Genetic diversity among wild and cultivated populations of *Hevea* was assessed using isozymes (Yeang *et al.*, 1998). Isozymes can be utilized for the identification of cultivars, (Yeet *et al.*, 1977), provide information on the organisation of gene pool in a large number of species (Gottileb, 1981). Isozyme markers are utilized in *Hevea* breeding programmes such as the assessment of genetic diversity and relatedness (Chevallier *et al.*, 1985; Chevallier, 1988; Besse *et al.*, 1994) estimation of outcrossing rates (Sunderasan *et al.*, 1994) and clone identification (Leconte *et al.*, 1994).

The potential use of isozymes in tree breeding programme (Adams, 1983) has not been exploited. This technique is used for the conformity of the planting materials in the bud wood gardens (Yeang, 1988; Leconate *et al.*, 1994). Isozymes offer most reliable single gene markers and they are often codominant in inheritance (Arulsekar and Parfitt, 1986).

Tanksley and Orton (1983), Nielsen (1984), Simpson and Withers (1986), Kahler and Price (1987), Chapman (1989), Hamrick and Godt (1990) etc. reviewed plant isozymes dealing with crop population, genetic variability and characterization. In addition to the characterization and identification of cultivars varieties and natural population, isozyme electrophoresis is used for (a) varietal uniformity assessment (Arus, 1983) (b) phylogenetic studies (Crawford, 1983; Simpson and Withers, 1986) (c) estimation of mating system and selection parameters (Tanksley and Orton, 1983; Brown *et al.*, 1990) (d) evaluation of seasonal variation (Evans and Sharp, 1986) and (e) genetic linkage mapping (Tanksley, 1983; Allard, 1990 and 1999).

Though Isozymes are the powerful tool for the characterization and estimation of genetic divergence, they have certain limitations in plant breeding. The two major drawbacks in using isozymes and protein as markers are (1) not all the genetic changes occurring at the DNA level are detected at the protein level (2) only one set of structural genes of organisation are represented in these proteins and this set may not be representative of the whole genome. Another limiting characteristic of isozyme systems in population studies is the relative low number, which can be observed by gel electrophorsis. Of about 3000 enzymes known in plants, only about 60 have been analyzed for isozyme polymorphism (Vellejos, 1983) listed 57 different isozyme system. Isozymes are also influenced by environment and development.

DNA markers are considered to be superior to examine the genetic relationship between clones / cultivars because of the availability of a larger number of potential polymorphic sequences. These markers do not depend on environment and development. These include restriction fragment length polymorphism (RFLP), simple sequence repeats (SSR) and random amplified polymorphic DNA (RAPD). These technique differ in their principles and generate varying amounts of information (Das *et al.*, 1999). Detection of DNA polymorphism by PCR based RAPD gained more importance due to its simplicity, efficiency, relatively easy to perform and non-requirement of prior DNA sequence information (Venkatachalam *et al.*, 2002). Recently the applicability of RAPD markers for genetic analysis in *Hevea* was evaluated in a set of 24 clones from the breeding pool of RRII (Varghese *et al.*, 1997). Among different clones selected RRIC 100 displayed the highest mean genetic distance. Use of this clone as a parent in hybridization programmes has resulted in highly heterotic hybrids (Licy *et al.*, 1996).

Application of molecular tools in rubber tree improvement is lagging behind because of limited knowledge of the genome. The genetics of the rubber tree has been poorly investigated (Lespinasse *et al.*, 2000). In *Hevea* the long juvenile period would make RAPD markers an extremely useful tool for identification of cultivars during propagation and planting (Varghese *et al.*, 1997).

A variety of molecular techniques have been used to study the extent of the genetic variation between different wild and cultivated *Hevea* clones. Among the different techniques, isozymes and RFLP were used for the assessment of genetic variability between wild and cultivated population (Chevellier, 1988 and Besse *et al.*, 1994) and RFLP was used to estimate phylogenetic relationship from mitchondrial DNA (Luo *et al.*, 1995) and to assess the genetic variability from ribosomal DNA (Besse *et al.*, 1993). Genes for powdery mildew

resistance was identified by the RAPD analysis (Shoucai *et al.*, 1994). Varghese *et al.*, (1997) also reported that DNA polymorphism could be detected within 24 *Hevea* clones. Recently, Lespinesse *et al.*, (2000) established the first genetic map of *Hevea brasiliensis* using RFLP, ALFP, microsatillite & isozyme markers. The results of DNA polymorphism in 37 cultivated *Hevea* clones using RAPD analysis involving 80 random oligonucleotide primers are reported by Venkatachalam *et al.*, (2002). The observed polymorphism may be useful for developing molecular markers helpful for screening various traits in *Hevea* improvement programmes. Even though morphological traits are also commonly used to determine genetic relationships, they do not provide good estimates of genetic distance because they are influenced by environment and are not variable enough to adequately characterize genetic differences among elite genotypes.

2.6. Progeny analysis

The propagation of *Hevea brasiliensis* is carried out using seeds or vegetative parts. Earlier the propagation of the crop was through seeds only. However, vegetative propagation using buds became common in later years (Marattukalam *et al.*, 2000). At present seeds are used mainly for producing rootstocks. Special types of seeds known as polyclonal seeds are used directly for propagation. Polyclonal seeds are hybrid seeds that are produced in polyclonal seed gardens. Here several clones are planted intermixed to maximize optimum cross-pollination. The clones planted in these gardens should possess desirable characters like high yield, disease resistance, vigour, ability to produce good seedling families and profuse production of seeds. The number of clones in the seed gardens usually varies from three to seven (Simmonds, 1986b). To maximize cross - pollination, special designs are adopted while planting (Marattukalam *et al.*, 2000). Superior clones are used as

parents in open - pollinating seed gardens in order to produce superior seedling progeny. The technique remains in use today. Saraswathyamma (1990) reported the evaluation of seedling progenies of male sterile clones in *Hevea* based on juvenile yield and secondary attributes.

Polyclonal seedlings resultant of cross pollination express heterosis or hybrid vigour. Polycross or synthetic seedling populations of polyclonal seed gardens of good clones have been successfully used as planting materials. Allogamy coupled with seed propagation increases variation through genetic recombination in seedling population. The seedling population have special agricultural merits in maintaining the genetic variability and adaptability of the population (Mydin, 1990; Varghese, 1992). Seedlings, though not comparable with high yielding clones in production potential, have a special agricultural merit for raising superior polycross progeny from special polyclonal seed gardens. The evaluation of such polycross population can be considered as selective breeding. Such 'multi parent' first generation synthetic varieties have been economically successful for many decades, predominantly due to additive genetic control of vigour and yield as well as high general combining ability (Simmonds, 1986b; Tan, 1987).

The genetic superiority of selected mother trees can be identified through progeny testing. The estimation of genetic superiority of mother parents through seedling progeny analysis is also referred as prepotency testing. Allard (1960) reported that prepotency is the capacity of a parent to impress characteristics on its offsprings so that they resemble that parent and each other more closely than usual where gene combination tend to cohere but do not recombine resulting in some sort of functional homozygosity (Harland, 1957). In cashew the study was carried out to estimate large number of cashew mother trees in

relation to the characters of their seedling progenies to formulate an efficient method of evaluation of mother trees for large scale production of quality seeds (George *et al.*, 1984). However in coconut palm, a study was undertaken to formulate an efficient method for evaluation of genetic superiority of mother plants through early seedling progeny analysis (Shylaraj and Gopakumar, 1987). Mydin (1990) reported that the prepotent ability of a clone to produce high quality seedlings could be determined by systematic and planned experiments like seedling progeny analysis. Mydin *et al.*, (1996) indicated that high mean performance of the progeny of a clone coupled with high proportion of superior seedlings within the progeny is indicative of the ability of the parent to transmit superior traits to its progenies. Based on the high performance index and high recovery of superior seedlings of certain clones were identified as likely prepotents. Mydin *et al.*, (2002) reported prepotent clones for use as seed garden components, based on half-sib progeny analysis of eleven clones recommended for planting in India. The two year old progenies showed significant variation for test tap yield, girth and bark thickness. Superior progenies were identified by performance index based on juvenile traits, the recovery of superior seedlings within each progeny was worked out. Out of the eleven clones evaluated five clones were identified as likely prepotent with high performance index and high recovery of superior and elite seedlings in their progeny.

Studies have indicated the scope for identification of likely prepotents on the basis of a performance index, computed for the seedling progenies of clones (Mydin *et al.*, 1990). Nine clones identified as likely prepotents on the basis of seedling progeny analysis at the age of two years (Mydin, 1992). Markose (1984) reported that no significant difference was noted in early growth behavior of clonal seedling progenies raised from open pollinated

seeds at 10 months growth in nursery. It appears that the expression of clonal characters in seedlings needs further growth in field.

2.7. Recent trends in *Hevea* breeding

In India, more than eighty per cent of the rubber plantation is being planted with a single clone i.e., RRII 105, developed by the Rubber Research Institute of India. The major reason for this is due to its yield potential. This clone is also occupying more than ninety percent of the new planting area. This practice has led to a situation of monoclonal planting for the last two decades. If this trend continues it can lead to serious consequences like disease epidemics common to such monoculture plantations. So as a precaution, Rubber Board recommended multiclonal planting to prevent epidemics or other damage vulnerable to such monoclonal cultivation. Liyanage *et al.,* (1989) reported that severe incidence of *Corynespora* leaf disease affected RRIC 103 in Sri Lanka leading to its withdrawal from the planting recommendations. RRII 105 has been reported to be infected by this disease in Karnataka region of India (Jacob, 1997). Even though, there is no alarming situation at present, there is a need for planning alternate measures to prevent possible danger (Saraswathyamma *et al.,* 2000). In order to enrich the genepool for utilization in hybridization programmes, RRII had introduced *Hevea* clones from other rubber growing countries. Saraswathyamma *et al.,* (1992 and 2000) reported that a total of 127 clones have been introduced in to India from other countries. India has obtained clones developed in China, Indonesia, Ivory Coast, Liberia, Malaysia, South America, Sri Lanka and Thailand. Clones developed in India were supplied to China, Ivory Coast, Malaysia, Sri Lanka and Thailand (Saraswathyamma and Maratattukalam, 1996; Saraswathyamma *et al.,* 2000). The recent approach in *Hevea* breeding is to develop not only a high yielding clone but clones having

other desirable secondary attributes along with yield. RRIM 928, RRIM 929, RRIM 931, RRIM 2014 etc. are some of the latex timber clones developed by Rubber Research Institute of Malaysia. In India RRII 5 and RRII 203 are noted for their average yield and good quantity of timber (Saraswathyamma *et al.*, 2000). As per the latest information the present clones are classified in various categories based on the performance (IRRDB, 2001). In Malaysia PB 280, PB 260 and PB 217 are included in category I, PB 280 considered as latex-timber clone. PB 255 in category II whereas, PB 310, PB 311, PB 312 and PB 314 in category III. In Vietnam PB 255 as class I clones, however in Indonesia PB 217 and PB 260 as latex clones in class I category. The yield performance of the clone PB 255 and PB 260 are high in Thailand as these clones were recommened in class I clone and PB 235 as class II clone. In Sri Lanka PB 217 is classified as group I and PB 235 and PB 260 in class II, the clone PB 255 is in third group. In Combodia, PB 235 was included in class I and PB 217 and PB 310 in class II clones, however PB 255 and PB 260 are class III category. The yield performance of PB 217 in Cote d' Ivorie was high hence it included under class I, PB 235 and PB 260 in class II whereas, PB 255 in class III category.

Improving the quality of latex is also important as natural rubber forms the row material for various industrial and technological purposes. Hence the study related to quality improvement of natural rubber of *Hevea* clones is also worthwhile. Synthesis of clones having combined effect of high production potential with excellent latex and rubber properties should prove to be rewarding. Molecular markers are importance in perennial crops like *Hevea* where the conventional genetic analysis is difficult due to long breeding and selection cycle and difficulties in raising next generation. Isozymes and DNA based RAPD markers are effective tools for analyzing the genetic relationship between clones.

The application of RAPD markers for genetic analysis was evaluated in a set of clones from the breeding pool of RRII. Among different clones, one which recorded the highest genetic distance, are utilized as parent in hybridization programme that resulted in highly heterotic hybrids. Characterization of latex and rubber properties is another trust area, which should be considered in breeding programme to improve the quality of raw rubber that extracted from the clones of *Hevea*. Different clones have different characteristics and give rubber with different properties.

Chapter 3

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1. Location of the study

The study was conducted at the Rubber Research Institute of India, (9° 32' N 76° 36'E) Kottayam, Kerala. It is a tropical low elevation region, the monthly mean temperature varies from 26 - 28°C. During the peak latex production period of November, daily temperature varies from 22°C to 31°C.

3.2. Experimental Materials

The study was conducted on 13 clones of *Hevea brasiliensis*. Details of the clones studied are given in Table 1. Out of the thirteen clones included in the study, three clones *viz.*, KRS 25, KRS 128 and KRS 163 were introduced from Thailand and nine clones *viz.*, PB 217, PB 235, PB 255, PB 260, PB 280, PB 310, PB 311, PB 312 and PB 314 were from Malaysia under the clone exchange programme. These clones are being evaluated in a large-scale trial employing randomized block design with five replications and seven plants per plot at the research farm of the Rubber Research Institute of India, Kottayam. The trial was laid out in 1989 and the trees were opened for tapping during 1997 in the 8th year after planting. The plants were under exploitation adopting 1/2S d/3 6d system of tapping.

Table 1. Details of the clones included in the study

Clone	Parentage	Country of origin
PB 217	PB 5/51 × PB 6/9	Malaysia
PB 235	PB 5/51 × PB S/ 78	Malaysia
PB 255	PB 5/51 × PB 32/36	Malaysia
PB 260	PB 5/51 × PB 49	Malaysia
PB 280	Primary clone	Malaysia
PB 310	PB 5/51 × RRIM 600	Malaysia
PB 311	RRIM 600 × PB 235	Malaysia
PB 312	RRIM 600 × PB 235	Malaysia
PB 314	RRIM 600 × PB 235	Malaysia
KRS 25	Primary clone	Thailand
KRS 128	PB 5/63 × KRS 13	Thailand
KRS 163	PB 5/63 × RRIM 501	Thailand
RRII 105	Tjir 1 × Gl 1	India

3.3. Yield and yield attributes

3.3.1. Girth

Girth at a height of 150 cm from the bud union was measured four times a year ie, in March, June, September and December from 1998 to 2001 using non streatchable measuring tape.

3.3.2. Girth increment rate on tapping (GI)

Increase in girth for the period under study was determined, from which the mean girth increment per year was worked out.

3.3.3. Dry rubber yield (DRY)

Dry rubber yield per tree per tapping was recorded once in every two weeks for a successive period of three years from March 1998 to February 2001. Yield was determined by coagulating the latex in the collection cup using one per cent formic acid. The coagula were dried in the smoke house for a month and their weights determined. Ten per cent of the weighed sample was deducted since the lumps retain moisture even after prolonged drying, to compensate for the residual moisture (Markose, 1984).

Mean values for annual yield, yield during stress (summer) period (February to May) and peak yielding season (October to January) for the study period was computed.

3.3.4. Latex yield (LY)

Total volume of latex from each tree per tapping was recorded at monthly intervals. Three trees per replication were selected. Mean values for annual, stress and peak period over two years were calculated separately.

3.3.5. Initial flow rate (IFR)

Initial rate of flow of latex for the first five minutes of tapping was recorded at monthly intervals. Rate of flow of latex per unit length of tapping cut was estimated as

Initial volume for the first five minutes (ml/min^{-5}) × 50 / length of tapping cut (cm).

Mean values for annual, stress and peak period were worked out separately.

3.3.6. Dry rubber content (DRC)

A known weight of latex samples from each experimental tree were collected and coagulated. The coagulum after passing through rollers, was dried at 55°C for one week. The percentage of dry rubber content for each sample was determined by the formula.

$$DRC = \frac{\text{Oven dried coagulum}}{\text{fresh wt. of latex sample colleted}} \times 100$$

Mean values for annual, stress and peak season over two years were collected separately.

3.3.7. Plugging Index (PI)

Plugging index was estimated at monthly intervals from March 1998 to February 2000 and PI was calculated by the formula proposed by Milford *et al.*, (1969).

$$\text{Plugging index} = \frac{\text{(mean initial flow rate (ml/min)}}{\text{total volume of latex (ml)}} \times 100$$

Average values for annual, stress and peak season were estimated separately.

3.3.8. Length of tapping panel (LTP)

Length of tapping panel was measured using measuring tape once in every six months.

3.3.9. Bark anatomy

Samples of virgin and renewed bark were collected (during 4th year of tapping) simultaneously to study the number of latex vessel rows, and thickness of the bark. Samples

were collected at 150 cm height from the bud union using a specially designed chisel. Radial longitudinal sections of 40 - 60 mm thickness were taken and stained using Sudan IV. Total thickness of bark, thickness of the hard and soft barks and the number of latex vessel rows including functional and nonfunctional were recorded from five sections for each sample. The observations for total thickness of bark, thickness of the soft and hard bark and the number of latex vessel rows were made for both virgin and renewed bark samples. The data was subjected to statistical analysis.

3.4. Latex and rubber properties

Latex samples of different clones were collected in different seasons for two consecutive years. Latex samples were collected from three replications. For each replications samples from 7 trees were mixed together to constitute one single composite. Latex from each clone was then processed into sheet rubber. Properties of field latex such as pH and non- rubber solids (NRS) were investigated as per IS standards. The dry rubber properties examined are those included in the Indian Standard Specification for raw natural rubber. Acetone extract and gel content of the rubber samples were measured as per IS 3660 and ASTM D-2765-84 respectively. Results are expressed as the mean of all the values obtained in each case.

3.5. Statistical techniques

3.5.1. Genetic variability

The mean data computed for each character from the five replications were subjected to statistical analysis. The estimates of mean, variance and standard error were worked out by adopting standard method suggest by Panse and Sukhatma (1985).

3.5.1.1. Analysis of variance [ANOVA]

Analysis of variance was carried out in order to:

1. Test whether there exist significant differences among clones, with respect to various traits.
2. Estimate the components of variance and covariance.
3. Compute the phenotypic and genotypic correlation coefficients.

The significance test was carried out by referring to the standard 'F' table given by Fisher and Yates (1963).

Analysis of variance was carried out for all the characters studied using the standard procedure. With regard to dry rubber yield, volume of latex, flow rate of latex, plugging index, and dry rubber content, the data for annual, stress and peak periods were analysed separately. The pooled data was analysed as suggested by Pearce (1953). The manifestation of genotypic (G) and environmental (E) effects on the observed total value of a character was partitioned by the standard method (Kempthrone, 1975).

$$V(_x) = V_G + V(E)$$

OR

$$\sigma^2 P(x) = \sigma^2 g(x) + \sigma^2 e(X)$$

Where $\sigma^2(x)$ is the phenotypic variance of character X, $\sigma^2 g$ is the genotypic variance of X, and $\sigma^2 e$ (X) is the variance due to environment.

3.5.2. Genetic parameters

3.5.2.1. Phenotypic and genotypic variance

Phenotypic and genotypic variance was estimated using the formula derived by Singh and Choudhary (1985).

$$\text{Genotypic variance } (\sigma^2 g) = \frac{\text{treatment mean square - error mean square}}{\text{number of replications}}$$

$$\text{Phenotypic variance } (\sigma^2 p) = \sigma^2 g + \sigma^2 e$$

Where $\sigma^2 g$ = Genotypic variance.

Where $\sigma^2 p$ = Phenotypic variance.

Where $\sigma^2 e$ = Error variance.

3.5.2.2. Coefficient of variation

Genotypic and phenotypic coefficients of variability were estimated as proposed by Burton and Devane (1953).

i. Genotypic coefficient of variability (GCV)

$$GCV = \text{Where } \frac{\sqrt{\sigma^2 g}}{X} \times 100$$

Where $\sigma^2 g$ is the genotypic variance x, the mean of the population.

ii. Phenotypic coefficient of variability (PCV)

$$PCV = \frac{\sqrt{\sigma^2 p}}{X} \times 100$$

Where $\sigma^2 P$ is the phenotypic variance and X is the mean of the population.

3.5.2.3. Heritability (H^2) - Broad sense

Heritability in broad sense was estimated (Jain, 1982). It is the fraction of the total variance that is heritable.

$$\text{Heritability } (H^2) = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

3.5.2.4. Genetic advance under selection (GA)

Genetic advance under selection was calculated employing the formula

$$\text{Genetic advance (GA)} = \frac{KH^2 \sigma^2 p\,(x)}{\bar{X}}$$

Where H^2 = Heritability estimate

P = Phenotypic standard deviation

K = Selection differential which is equal to 2.06 at 5% intensity of selection (Allard, 1960).

$\bar{X}$ = Mean of the character X

3.5.3. Association of characters

3.5.3.1. Simple correlation

Simple correlation coefficient was estimated as below

$$r_{xy} = \frac{\text{Cov}\,(xy)}{\sqrt{V(x)\,V(y)}}$$

Where, rxy = simple correlation coefficient between character x and y

Cov(xy) = Covariance of characters x and y

V(x) = Variance of character x

V(y) = Variance of character y

3.5.3.2. Genotypic and phenotypic correlation

Genotypic (r_g) and phenotypic (r_p) correlations coefficients among all the characters were estimated using variance and covariance components as suggested by Singh and Choudhary (1985). Mean values of different characters were utilised for the estimation of the correlation.

i. Genotypic correlation

Genotypic correlation coefficient ($r_{xy}(g)$)

$$= \frac{\text{Cov xy (g)}}{\sqrt{\sigma^2 x\,(g)\,\sigma^2 yg}}$$

Where Cov xy(g) = genotypic covariance of character x and y

σ^2 x(g) = genotypic variance of character X

σ^2y (g) = genotypic variance of character Y

ii. Phenotypic correlation

Phenotypic correlation coefficient (r_{xy} (p))

$$= \frac{\text{Cov xy (p)}}{\sqrt{\sigma^2 \times (p)\,\sigma^2 y\,(p)}}$$

Where Cov xy (p) = phenotypic covariance of character x and y

σ^2 x (p) = phenotypic variance of character X

σ^2y (p) = phenotypic variance of character Y

Significance of the correlation coefficients were tested according to Fisher and Yates (1963).

3.6. Classificatory analysis

3.6.1. D^2 Analysis

Genetic divergence of the 13 clones was estimated by D^2 Statistic as per Mahalanobis (1928, 1936) and computed as:

$$D_x^2 = \sum_i^p \sum_j^p (\lambda^{ij})\, di\, dj$$

Where, x = number of metric traits in point,

p = number of genotypes

di and dj = the differences between the mean values of two genotypes, for i^{th} and j^{th} characters respectively.

λ^{ij} = dispersion matrix reciprocal to the common dispersion matrix.

After computing the relative genetic distance between the clones, they were clustered into genetically divergent clusters as per iterative relocation algorithm suggested by Friedman and Rubin (1967) and modified by Suresh and Unnithan (1996). The mean intra cluster distances were computed using the formula

$$= \Sigma (d_{ij})^2 / n$$

where d_{ij}^2 is the distance between i^{th} and j^{th} genotypes in the same cluster.

n = number of values

The mean inter distances were worked out by using the distances between all possible combinations of the clusters obtained. For this purpose, the sum of distances between all possible combinations of the clones in a pair of clusters was taken. The sum of D^2 values divided by the product of the number of genotypes in each cluster gave the inter cluster distance between the particular pair of clusters. The mean inter and intra cluster distances were then tabulated.

3.7. Isozyme studies

Nine isozyme systems *viz.*, alcohol dehydrogenase (E.C.1.1.1.1), glutamate dehydrogenase (E.C.1.4.1.2), peroxidase (E.C.1.11.1.7), shikimate dehydrogenase (E.C.1.1.1.25), superoxide dismutase (E.C.1.15.1.1), aspartate aminotransferase (E.C.2.6.1.1), acid phosphatase (E.C.3.2.3.1), alkaline phosphatase (E.C.3.1.3.1) and aryl esterase (E.C.3.1.1.2) were studied. However, some of them, which are poorly resolved and are not giving any polymorphism among clones, were eliminated from the studies. Finally the four enzyme systems selected were aryl esterase, peroxidase, shikimate dehydrogenase and aspartate aminotransferase. Staining protocols were as defined by Tanksley and Orton, 1983. After staining the gels were fixed and photographed using a 'Fotodyne VariQuest 100' white light illuminator.

3.7.1. Enzyme extraction

One gram of young leaf tissue was ground to a fine powder with liquid nitrogen with a mortar and pestle. Two hundred milligram of insoluble polyvenylpolypyrrolidone was added to remove the phenolics. 1 ml of the extraction buffer (50 mM Tris, 10 mM cystein, pH 7.4 and 1 mM PMSF) was added to allow the ground tissue to thaw in the buffer. After thawing the samples were squeezed through 4 layers of cheeze cloth to a 1.5 ml microfuge tube. The tubes were centrifuged at 12, 000 rpm for 15 min. at 4^0C. The clear supernatant was recovered and its protein content was measured by Lowry's method (Lowry *et al.*, 1951). The samples were stored in -70^0C until use and this crude enzyme preparation was used for isozyme studies.

3.7.2. Electrophoresis

Proteins were separated in a 7.5 per cent native polyacrylamide gel. Electrophoresis was carried out at 8^0C in a refrigerator and the samples were run overnight at a constant voltage of 50V.

3.8. Molecular approaches

The plant materials were obtained from the bud wood nurseries of Rubber Research Institute of India. Plants were cut back and after one month the newly expanded leaves were collected for enzyme extraction. Leaf samples of the same age were collected at random from three replications of all clones.

3.8.1. Random amplified polymorphic DNA (RAPD) analysis

3.8.2. Preparation of Genomic DNA

Genomic DNA from fully expanded and disease free young leaves of selected clones was isolated and purified following the modified CTAB extraction procedure (Doyle and

Doyle 1990). One gram of fresh leaf tissue was ground to a fine powder under liquid nitrogen and homogenized in DNA isolation buffer (2% CTAB; hexadecyltriethylammonium bromide), 1.4 M NaCl, 20 mM EDTA (pH 8.0), 100 mM Tris-HCl (pH 8.0), 1% polyvinyl polypyrolidone (PVPP), 1% 2-mercaptoethanol). The homogenate was then incubated in a water bath at 65°C for 30 min with gentle mixing. The extracts were centrifuged for 15 min (12000 xg) and the supernatant was treated with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) and spun at 10,000 rpm for 10 min. The aqueous phase was carefully removed to new tubes and incubated at 37°C for 1 h after the addition of 10 µl of RNase A (10 mg/ml). The samples were extracted with chloroform and spun at 10,000 rpm for 5 min and re-extracted until a clear aqueous phase was obtained. The DNA was precipitated with an equal volume of isopropanol. After 15 min of centrifugation at 10,000 rpm, the DNA pellet was washed with 70 per cent ethanol, air-dried and dissolved in about 300µl of TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0). DNA quality was analysed by 0.8 per cent agarose gel electrophoresis and stored at –20°C until use for PCR amplification.

3.8.3. DNA amplification by PCR

PCR was carried out in a 20 µl reaction mixture containing 15 ng of template DNA, 250 nM of primer, 1.5 mM $MgCl_2$, 100 µM each of dATP, dGTP, dCTP and dTTP (Amersham-Pharmacia, UK), 0.5 unit of Taq DNA Polymerase enzyme and 1x reaction buffer. In order to avoid evaporation, the reaction mixture was overlaid with 25 µl of mineral oil (Sigma, USA). Amplification was performed in 0.5 ml tubes placed in a 48-well thermal cycler [Perkin-Elmer DNA Thermal Cycler 480, USA]. Tubes containing all the reaction components except DNA template were included as control for each primer used. The PCR programme included: a 4 min initial denaturation step at 94°C, 1 min denaturing at 94°C, 1.30 min at 38°C for

annealing and 2.0 min at 72°C for extension. Thirty-five cycles were performed and the last cycle was followed by 7 min at 72°C to ensure that primer extension reactions proceeded to completion. Eighty random oligonucleotide primers (OPA, OPB, OPC, OPD and OPE), each of 10 nucleotides long (Operon Technologies Inc., Alameda, CA, USA) were used as single primer for the amplification of genomic DNA. The primers, which produced clear banding pattern after PCR amplification were selected for further RAPD analysis of 13 clones. In order to confirm whether the amplified products are reproducible, amplification with each primer was repeated at least thrice.

3.8.4. Gel electrophoresis and photography

After PCR amplification, 6x loading buffer was added to the amplified products. The RAPD products were separated by electrophoresis in 1.5% agarose gels containing 0.5 µg/ml ethidium bromide in 0.5X TBE buffer (Sambrook *et al.*, 1989). Electrophoresis was performed at 50V for about 4 h until the bromophenol blue dye front had migrated to the bottom of the gel. The molecular standard used was the lambda DNA double digested by EcoRI/HindIII. The gels were visualized under UV-light and photographed with Canon Camera, Japan.

3.8.5. DNA blotting

Amplified RAPD products were electrophoresed on 1.5% agarose gel in TBE buffer (0.045 M Tris-borate and 0.001 M EDTA) at 25 V for 8 h. After depurination in 0.25 M HCl for 10 min, denaturation of the DNA in the gels was carried out in 1.5 M NaCl; 0.5 M NaOH for 30 min and then neutralized for 30 min in 1.5 M NaCl. 1.0 M Tris-HCl pH 7.4. The DNA was then transferred onto a nylon membrane (Hybond N^+, Amersham-Pharmacia, UK) in 10X SSC buffer (1X SSC is 0.15 M NaCl, 0.015 M trisodium citrate) for 18h (Sambrook *et al.*, 1989).

After DNA transfer, the nylon membranes were rinsed in 2X SSC buffer, UV-crosslinked and stored at 4°C until use.

3.8.6. DNA probe preparation, labelling and hybridization

The selected polymorphic band was cut out from the low-melting agarose gel and DNA was eluted (Sambrook *et al.*, 1989). They were then reextracted once with phenol:chloroform 1:1 and the DNA pellet was dissolved in sterile double distilled water and used for labelling. Radioactive probes were synthesized with α-^{32}PdATP (BARC, Trombay, Mumbai, India, 4000 Ci/mmol) using the random primer labeling kit (Amersham-Pharmacia, UK). The nylon membranes with DNA were placed in hybridization bottles and prehybridized for 4h (Hybridization buffer is 6X SSC, 5X Denhardt's, 0.5% SDS) at 65°C. After 4 h, the radio labeled DNA probe was added into the prehybridization buffer and hybridization was performed at 65°C for 20 h in a rotary hybridization oven (Amersham-Pharmacia, UK). After completion of hybridization, membranes were washed at low stringency at room temperature twice in 2X SSC + 0.1% SDS for 5 min and 1X SSC + 0.1% SDS for 15 min and high stringency at 65°C, twice in 0.5X SSC + 0.1% SDS for 30 min and 0.1X SSC + 0.1% SDS for 30 min, followed by radio active signal generation. The labelled blots were then exposed to X-ray film (X-Omat, Kodak) with intensifying screens at -80°C.

3.8.7. Data analysis

A conservative approach to scoring of the amplified fragments was adopted and only consensus bands were included for the final analysis. Individual amplified bands were indicated by the primer used and its size in bp. Data were scored for computer analysis on the basis of the presence or absence of the amplified product of a given length. If a product was present in a genotype it was designated "1"; if absent, it was designated "0". Pair-wise comparisons of genotypes, based on the presence or absence of unique and shared

polymorphic products were used to generate complementation of Jaccard's similarity coefficients. The matrix of dissimilarities was then used to construct a dendrogram according to the UPGMA (unweighed pair-group method with arithmetical average) using the TREECON programme (Van de Peer and De Wachter, 1994).

3.9. Performance Index

Performance index of the genotypes was computed based yield and major yield components *viz.*, dry rubber yield, latex yield, initial flow rate of latex, plugging index, dry rubber content, virgin bark thickness and latex vessel rows in virgin bark.

Application of discriminant function as a basis for making selection on several characters simultaneously is aimed at discriminating the desirable genotypes from undesirable ones on the basis of their phenotypic performance. Singh and Chaudhary (1985) defined the genetic worth (H) of an individual as:

$$H = a_1 G_1 + a_2 G_2 + \ldots\ldots\ldots + a_n G_n$$

Where, $G_1, G_2, \ldots\ldots\ldots\ldots G_n$ are the genotypic values on individual characters and $a_1, a_2, \ldots\ldots\ldots a_n$ signify their relative economic importance. Another function (I) based on the phenotypic performance of various characters, is defined as:

$$I = b_1 p_1 + b_2 p_2 + \ldots\ldots\ldots + b_n p_n$$

Where, $b_1, b_2, \ldots\ldots\ldots\ldots, b_n$ are to be estimated such that the correlation between H and I, ie, r (H, I) becomes maximum. Once such function is obtained, descrimination of good genotypes from the undesirable ones will be possible on the basis of phenotypic performance, ie, $P_1, P_2, \ldots\ldots\ldots\ldots, P_n$ directly.

The maximization of r (H, I) leads of a set of simultaneous equations which upon solving give the desired estimate of b_i values. Considering the 16 characters in this study, the simultaneous equations are as follows:

$$b_1 x_{11} + b_2 x_{12} + \ldots\ldots\ldots\ldots b_7 x_{17} = a_1 G_{11} + a_2 G_{12} + \ldots\ldots\ldots\ldots a_7 G_{17}$$

$$b_1 x_{21} + b_2 x_{22} + \ldots\ldots\ldots\ldots b_7 x_{27} = a_1 G_{21} + a_2 G_{22} + \ldots\ldots\ldots\ldots a_7 G_{27}$$

$$\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

$$b_1 x_{71} + b_2 x_{72} + \ldots\ldots\ldots\ldots b_7 x_{77} = a_1 G_{71} + a_2 G_{72} + \ldots\ldots\ldots\ldots a_7 G_{77}$$

which in matrix form become:

$$\begin{bmatrix} x_{11} & x_{12} & \cdots & x_{17} \\ x_{21} & x_{22} & \cdots & x_{27} \\ \cdots & \cdots & \cdots & \cdots \\ x_{71} & x_{72} & \cdots & x_{77} \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \\ \cdots \\ b_7 \end{bmatrix}$$

$$\begin{bmatrix} G_{11} & G_{12} & \cdots & G_{17} \\ G_{21} & G_{22} & \cdots & G_{27} \\ \cdots & \cdots & \cdots & \cdots \\ G_{71} & G_{72} & \cdots & G_{77} \end{bmatrix} \begin{bmatrix} a_1 \\ a_2 \\ \cdots \\ a_7 \end{bmatrix}$$

The solution of these equations give the estimates of b_i values in the following manner:

$$b = X^{-1} G a$$

where, b is the column vector X^{-1} is the inverse of the phenotypic variance and covariance matrix, G is the genotypic variance and covariance matrix and a is the column vector for economic weights. We take *a* as a unit vector assuming equal economic importance for all characters. The genotypes were ranked based on their performance indices.

3.10. Progeny analysis

Open pollinated seeds were collected from 12 introduced clones from Malaysia and Thailand along with the seeds of RRII 105. For each clone seeds were collected from trees covering all the five replications. Seedling progenies were planted in randomized block design with three replications Plate 5. Forty plants per progeny were raised in

replications in a spacing of 60 x 60 cm was adopted. Observations on juvenile yield, girth, height and number of whorls were taken from 16 plants grown in two central rows discarding the border ones.

Observations for morphological traits *viz.*, girth (at 10 cm from the ground level), height and number of leaf flushes were made at the first and second year after planting. Seedling height at the age of six months were also recorded. Juvenile yield was determined by the test tapping of the seedlings at the age of two years by the modified Hammaker Morris Mann method following a 1/2S d/3 system, at a height of 15 cm from the plant base. Latex from ten successive tappings was allowed to accumulate in collection cup, following which the cup lumps were oven dried and weighed to record yield. Analysis of variance was worked out to estimate variability among progenies with respect to all variables studied.

Performance index of the 13 progenies was estimated based on vegetative vigour and juvenile yield at the age of two years (Mydin, 1990). Considering the variables, plant height (x1), girth (x2) number of leaf flushes (x3) and juvenile rubber yield (x4).

Performance Index $= W1x1 + W2x2 + W3x3 + W4\,x4$

Where W1, W2, W3 and W4 = $1/\sigma_1^2$, $1/\sigma_2^2$, $1/\sigma_3^2$ and $1/\sigma_4^2$

denote weights attached to the traits X1, X2, X3 and X4 respectively and provide information on each trait. x1, x2, x3 and x4 represents the mean value of the traits X1, X2, X3 and X4.

Progenies were ranked on the basis of their performance indices. The percentage of seedling progenies which recorded above average mean yield of the total seedling population were also computed.

Plate 5. A view of seedling nursery under test tapping.
A Seedling under test tapping (inset)

Chapter 4

RESULTS

RESULTS

4.1. Clonal variability

4.1.1. Analysis of variance

The analysis of variance was carried out for all the characters in different seasons *viz.*, girth at opening, girth increment rate on tapping, girth at fifth year of tapping, dry rubber yield (annual, stress, peak), yield depression under stress, latex yield (annual, stress, peak), initial flow rate (annual, stress, peak), rubber content (annual, stress, peak), plugging index (annual, stress, peak), length of tapping panel, bark thickness, number of latex vessel rows, and the latex and rubber properties (Table 2).

4.1.2. Performance of clones in yield and associated characters

4.1.2.1. Girth at opening

Highly significant variation was observed for girth at opening among the clones studied. The girth of clones during the commencement of tapping is given in Table 3. Mean girth at opening for different clones was observed to be 55.66 cm. The highest girth at opening was recorded for the clone PB 235 (61.33 cm) followed by PB 280 (60.02), PB 314 (57.83) and PB 255 (57.39) while KRS 128 exhibited lowest girth at opening (50.96 cm). All the clones were found to be on par with RRII 105. Three clones *viz.*, PB 255, PB 314 and PB 280 were on par with PB 235 whereas, PB 235 and PB 280 were significantly superior to RRII 105.

Table 2. ANOVA for yield and associated characters

Sl.No.	Characters	F value	Significance
1.	Girth at opening	4.67	**
2.	Girth increment on tapping	1.83	NS
3.	Girth at 5th year of tapping	2.67	**
4.	Dry rubber yield (annual)	9.08	**
5.	Dry rubber yield (stress)	8.37	**
6.	Dry rubber yield (peak)	10.29	**
7.	Yield depression during stress	9.94	**
8.	Latex yield (annual)	6.42	**
9.	Latex yield (stress)	7.26	**
10.	Latex yield (peak)	8.26	**
11.	Initial flow rate (annual)	7.67	**
12.	Initial flow rate (stress)	5.67	**
13.	Initial flow rate (peak)	10.81	**
14.	Dry rubber content (annual)	13.46	**
15.	Dry rubber content (stress)	10.10	**
16.	Dry rubber content (peak)	12.39	**
17.	Plugging index (annual)	24.77	**
18.	Plugging index (stress)	11.92	**
19.	Plugging index (peak)	22.95	**
.20.	Length of tapping panel	2.34	*
21.	Virgin bark thickness	7.39	**
22.	Latex vessel rows in virgin bark	5.16	**
23.	Renewed bark thickness	4.65	**
24.	Latex vessel rows in renewed bark	7.43	**

* Significant at $P < 0.05$, ** Significant at $P < 0.01$, NS: Not significant

4.1.2.2. Girth increment rate on tapping

The mean girth increment on tapping for different clones was found to be non-significant. The range of variation in girth increment was from 1.58 cm for the clone RRII 105 to 2.98 cm for PB 255 (Table 3). The general mean was 2.16 cm per year. Two clones *viz.*, PB 255 and PB 310 were significantly superior to RRII 105 while five clones *viz.*, PB 260, PB 310, KRS 25, KRS 128 and KRS 163 recorded high girth increment rate and were on par with PB 255.

4.1.2.3. Girth at 4th year of tapping

The vigour of the clones in terms of girth at fifth year of tapping is shown in Table 3. The range of variability of girth was 57.88 cm for KRS 128 to 65.99 cm for PB 255 with a general mean of 60.82 cm. Three clones *viz.*, PB 310, PB 280 and PB 235 recorded high girth on par with PB 255 while PB 235, PB 255 and PB 280 were significantly superior to RRII 105.

4.1.2.4. Dry rubber yield

The annual mean dry rubber yield of the 13 clones is given in Table 3. The highest yield was observed for the clone PB 255 i.e., 73.52 g t^{-1} t^{-1} whereas, clone PB 217 exhibited the lowest yield (38.17 g t^{-1} t^{-1}) with a general mean of 57.26 g t^{-1} t^{-1}. Eight clones recorded yield above general mean. Of these, the clones PB 311, PB 312, KRS 163, PB 260, PB 280, PB 314 and PB 255, recorded above 60.00 g t^{-1} t^{-1}. Two clones *viz.*, PB 280 and PB 314, were on par with the highest yielding clone PB 255. Seven clones PB 255, PB 260, PB 280, PB 311, PB 312, PB 314 and KRS 163 were significantly superior to RRII 105.

During stress period, the clones PB 255 and PB 217 recorded the highest and lowest dry yield respectively. The highest value exhibited was 52.74 g t^{-1} t^{-1} and the lowest value was 26.29 g t^{-1} t^{-1} with a general mean of 36.30 g t^{-1} t^{-1}. Only one clone *viz.*, PB 314 was on par with PB 255 whereas, PB 255, PB 311, PB 312 and PB 314 were significantly superior to RRII 105.

During the peak period, the highest yield was recorded by the clone PB 280 (80.42 g t^{-1} t^{-1}) followed by PB 255 (76.22 g t^{-1} t^{-1}), the lowest dry rubber yield was exhibited by PB 217 (44.75 g t^{-1} t^{-1}) with a general mean of 62.89 g t^{-1} t^{-1}. Eight clones *viz.*, PB 235, PB 255, PB 260, PB 280, PB 311, PB 312, PB 314 and KRS 163 were significantly superior to RRII 105. Three clones *viz.*, PB 260, KRS 163 and PB 255 exhibited above 70 g t^{-1} t^{-1} and were on par with PB 280.

4.1.2.5. Yield depression under stress

Highly significant clonal variations were observed for this trait. Yield depression under stress ranged from 27.99 per cent (PB 255) to 49.87 per cent (PB 235) and is shown in Table 3. The mean yield depression of the clones recorded was 36.41 per cent. RRII 105 recorded 38.99 per cent yield depression during stress. Six clones viz., PB 217, PB 310, PB 311, PB 312, PB 314 and KRS 128 were on par with PB 255. PB 235, PB 260, PB 280, KRS 25, KRS 163 and RRII 105 exhibited the value above general mean. The clones PB 260, PB 280 and KRS 163 were on par with PB 235 while two clones PB 235, and KRS 163 were significantly superior to RRII 105.

Table 3. Growth and yield of different clones

Clone	Girth at opening (cm)	Girth increment on tapping (cm)	Girth at 4th year of tapping (cm)	Dry rubber yield ($g\ t^{-1}\ t^{-1}$)			
				Annual	Stress	Peak	Yield depression during stress (%)
PB 217	52.92 efg	2.09 bc	58.32 c	38.17 f	26.29 g	44.75 d	31.01 de
PB 235	61.33 a	2.15 bc	65.27 ab	57.45 bcd	28.99 fg	63.24 c	49.87 a
PB 255	57.39 abcd	2.98 a	65.99 a	73.52 a	52.74 a	76.22 ab	27.99 e
PB 260	53.86 defg	2.37 abc	59.35 c	63.20 b	35.66 cdef	74.62 ab	43.75 ab
PB 280	60.02 ab	1.80 c	64.74 ab	66.81 ab	37.12 cde	80.42 a	44.96 ab
PB 310	56.00 cde	2.73 ab	62.36 abc	43.61 ef	30.71 efg	46.93 d	28.24 e
PB 311	55.08 cdef	2.01 bc	59.56 c	60.33 bc	41.91 bcd	65.38 bc	30.88 de
PB 312	56.73 bcde	1.74 c	60.61 bc	62.13 b	42.92 bc	67.42 bc	30.56 de
PB 314	57.83 abc	1.87 c	60.95 bc	66.88 ab	47.30 ab	68.62 bc	28.69 e
KRS 25	52.00 fg	2.35 abc	58.45 c	48.08 de	30.96 efg	51.05 d	36.52 cd
KRS 128	50.96 g	2.19 abc	57.88 c	51.78 cde	34.95 def	52.01 d	33.14 cde
KRS 163	54.61 cdefg	2.18 abc	58.94 c	62.96 b	32.36 efg	75.24 ab	48.70 a
RRII 105	54.82 cdefg	1.58 c	58.27 c	49.50 de	30.02 efg	51.66 d	38.99 bc
Mean	55.66	2.16	60.82	57.26	36.30	62.89	36.41
CV (%)	5.59	30.14	6.45	13.36	16.7	13.59	5.67
CD (0.05)	3.96	0.82	4.99	9.72	7.71	10.86	7.21

Means followed by the same letters are not significanty different at 5% error

4.1.2.6. Latex yield

Highly significant clonal variation for latex yield was observed among 13 clones studied (Table 2). Mean values of latex yield of clones are given in Table 4. PB 312 exhibited highest latex yield (176.85 ml t^{-1} t^{-1}) while lowest latex yield was recorded for PB 217 (107.60 ml t^{-1} t^{-1}) with a general mean of 140.95 ml t^{-1} t^{-1}. PB 311, PB 255 and PB 314, were on par with the highest yielding clone PB 312 whereas, seven clones *viz.*, PB 312, PB 314, PB 255, PB 311, PB 260, PB 280 and KRS 163 were significantly superior to RRII 105. Of the 13 clones, PB 255, PB 311, PB 312, PB 314 and KRS 163 exhibited above 150 ml t^{-1} t^{-1}. Four clones KRS 25, PB 235, PB 280 and PB 260 recorded good latex yield above the mean yield.

PB 255 exhibited the highest latex yield during the stress period (115.26 ml t^{-1} t^{-1}) followed by PB 312 (114.22 ml t^{-1} t^{-1}) and PB 314 (112.99 ml t^{-1} t^{-1}), with a mean volume of 86.51 ml t^{-1} t^{-1}. The lowest volume yield was shown by PB 235 (58.31 ml t^{-1} t^{-1}). PB 311, PB 312 and PB 314 were on par with PB 255 while PB 255, PB 312, PB 314 and PB 311 were significantly superior to RRII 105. Four clones *viz.*, PB 255, PB 311, PB 312 and PB 314 yielded above 100 ml t^{-1} t^{-1} during stress period.

During peak season, the range of latex yield among the clones was from 117.27 ml t^{-1} t^{-1} to 193.17 ml t^{-1} t^{-1}. The highest latex yield was exhibited by clone PB 312, and the lowest by PB 217. Seven clones *viz..*, PB 312, PB 280, PB 255, PB 314, KRS 163, PB 260 and PB 311 were significantly superior to RRII 105 whereas, PB 255, PB 280 and PB 314 were on par with the PB 312. Seven clones *viz.*, PB 255, PB 260, PB 280, PB 311, PB 312, PB 314 and KRS 163 recorded volume yield above general mean.

4.1.2.7. Initial flow rate

Clonal variation for initial flow rate of latex was highly significant (Table 2). The mean values of rate of flow of latex of the 13 clones are depicted in Table 4. The highest

flow rate of latex was recorded for RRII 105 (29.88 ml min^{-5} cm^{-1}) and the lowest flow rate of latex was observed for PB 260 (19.60 ml min^{-5} cm^{-1}) with a general mean of 24.14 ml min^{-5} cm^{-1}. Three clones *viz.*, PB 255, KRS 25, and PB 280 were on par with RRII 105. Six clones recorded latex flow rate of above 25 ml min^{-5} cm^{-1}.

During the stress period, PB 255 represented the highest flow rate of latex i.e. 26.89 ml min^{-5} cm^{-1} whereas, PB 235 indicated the lowest flow rate of latex i.e. 16.51 ml min^{-5} cm^{-1} with a general mean of 22.52. Seven clones PB 314, RRII 105, KRS 25, PB 280, KRS 128, PB 312 and PB 311 were on par with PB 255.

The highest flow rate during the peak season was recorded for PB 280, (30.52 ml min^{-5} cm^{-1}) and PB 235 showed lowest flow rate of 20.39 ml min^{-5} cm^{-1}. The general mean was 25.02 ml min^{-5} cm^{-1}. Three clones RRII 105, PB 255 and KRS 25 were on par with PB 280 having the highest initial flow rate of latex during peak period.

4.1.2.8. Dry rubber content

Highly significant clonal variation for this trait was observed in Table 2. The range of variation in dry rubber content is depicted in Table 4. The highest annual DRC recorded was 44.84 per cent for the clone PB 280 followed by KRS 128 (43.70 %) with a general mean of 39.94 per cent. The lowest dry rubber content recorded by PB 312 (36.45 %). Two clones PB 280 and KRS 128 were significantly superior to RRII 105 whereas, two clones PB 255, KRS 128 were on par with the clone PB 280. Seven clones *viz.*, PB 260, PB 235, RRII 105, KRS 163, PB 255, KRS 128 and PB 280 recorded a high DRC value above 40 per cent.

The range of dry rubber content during stress season was from 38.22 per cent for PB 312 to 48.21 per cent for PB 280. The general mean was 42.21 per cent. Dry rubber content

during the stress period indicated a wide variation among different clones. Ten clones *viz.*, PB 314, PB 310, KRS 25, PB 260, RRII 105, PB 235, KRS 163, PB 255, KRS 128 and PB 280 exhibited a very high DRC value above 40. Three clones PB 217, PB 311 and PB 312 recorded relatively low DRC during stress period.

Dry rubber content recorded during peak period was highest for KRS 128 (44.49 %), and lowest dry rubber content was for PB 311 (35.93 %) with a general mean of 39.72 per cent. Seven clones *viz.*, PB 235, PB 255, PB 260, PB 280, KRS 128, KRS 163 and RRII 105 exhibited a high value of DRC of above 40 per cent. Three clones *viz.*, RRII 105, PB 280 and PB 255 were on par with KRS 128 having high DRC during the peak period. Five clones *viz.*, PB 311, PB 312, PB 310, PB 314 and PB 217 were observed to give comparatively low dry rubber content during this period.

4.1.2.9. Plugging index

Highly significant clonal variation was observed for plugging index (PI) (Table 2). The range of variation among 13 clones for PI was from 2.44 (PB 312) to 4.67 (RRII 105) with a general mean of 3.31 (Table 4). Three clones *viz.*, KRS 25, PB 280 and RRII 105 indicated a high PI value and PB 312, PB 311, PB 260, KRS 163 and PB 314 exhibited low values for plugging index.

During the stress period the PI value ranged from 3.72 (PB 312) to 6.28 (RRII 105) and the general mean was 4.94. Four clones *viz.*, KRS 128, KRS 25, PB 235 and PB 280 recorded high values of plugging, and these clones were on par with RRII 105, while two clones i.e. PB 312 and PB 311 registered low values for PI during the stress period.

A wide range of variation in PI values was evident during the peak season also. The lowest value was showed for the clone PB 311 (2.06) and highest value registered was 4.46 for the clone RRII 105 with a general mean of 2.90. Other four clones which recorded high values for PI were PB 217, PB 280, KRS 25 and KRS 128 and all other clones recorded relatively low PI values.

4.1.2.10. Length of tapping panel

The clonal variation with regard to the tapping panel length was observed to be significant (Table 2). The length of tapping cut of different clones is shown in Table 4. The clone KRS 163 represented the minimum value of 34.37 cm while PB 235 recorded the highest value of 38.96 cm with a general mean of 36.12 cm. Three clones *viz.*, PB 235, PB 255 and PB 280 were significantly superior to RRII 105 whereas, three clones were on par with the PB 235.

Table 4. Physiological components of yield of different clones

Clone	Latex yield (ml t^{-1} t^{-1})			Initial flow rate (ml min^{-5} cm^{-1})			Dry rubber content (%)			Plugging index			Length of tapping panel (cm)
	Annual	Stress	Peak	Annual	Stress	Peak	Annual	Stress	Peak	Annual	Stress	Peak	
PB 217	107.60 f	73.48 cd	117.27 d	22.35 def	19.74 bcd	23.45 efg	36.51 g	38.92 e	36.68 ef	3.44 c	4.35 bcd	3.12 cde	34.76 cd
PB 235	137.80 cde	58.31 d	140.56 cd	19.71 f	16.51 d	20.39 g	40.79 cde	43.49 bc	40.33 cd	3.46 c	6.13 a	2.77 ef	38.96 a
PB 255	164.80 ab	115.26 a	176.04 ab	27.77 abc	26.89 a	28.60 ab	42.77 abc	44.85 bc	43.74 ab	3.21 c	4.75 b	2.88 def	38.01 ab
PB 260	149.30 bcd	77.68 cd	163.92 bc	19.60 f	18.54 d	20.46 g	40.51 de	42.32 cd	40.39 cd	2.67 d	4.57 bc	2.12 h	36.37 abcd
PB 280	148.10 bcd	78.31 c	176.92 ab	28.88 ab	24.52 a	30.52 a	44.84 a	48.21 a	43.36 ab	4.09 b	6.26 a	3.23 cd	38.13 ab
PB 310	111.00 f	72.89 cd	119.01 d	20.02 f	18.97 cd	20.84 g	37.35 fg	40.66 de	36.22 f	3.15 c	4.30 bcd	2.94 def	35.74 bcd
PB 311	158.61 abc	104.53 ab	163.58 bc	22.62 def	22.91 abc	23.26 efg	36.74 g	38.79 e	35.93 f	2.48 d	3.87 cd	2.06 h	35.02 cd
PB 312	176.85 a	114.22 a	193.17 a	22.64 def	23.32 ab	24.21 def	36.45 g	38.22 e	36.08 f	2.44 d	3.72 d	2.11 h	37.28 abc
PB 314	164.96 ab	112.99 a	174.03 ab	25.81 bcd	26.29 a	27.32 bcd	37.50 fg	40.50 de	36.33 f	2.73 d	4.05 bcd	2.60 fg	34.88 cd
KRS 25	127.01 def	79.91 c	133.17 d	28.17 abc	25.94 a	28.34 abc	39.17 ef	40.79 de	39.04 de	4.07 b	6.01 a	3.51 bc	35.80 bcd
KRS 128	118.18 ef	85.93 bc	126.54 d	25.01 cde	24.32 a	25.42 cde	43.70 ab	45.43 b	44.49 a	3.92 b	5.60 a	3.65 b	35.75 bcd
KRS 163	150.69 bcd	73.94 cd	165.54 bc	21.32 ef	19.15 cd	22.17 fg	41.91 bcd	43.90 bc	41.83 bc	2.69 d	4.41 bcd	2.23 gh	34.37 d
RRII 105	117.48 ef	77.22 cd	121.97 d	29.88 a	25.60 a	30.22 ab	41.00 cde	42.59 cd	41.94 abc	4.67 a	6.28 a	4.46 a	34.50 cd
Mean	140.95	86.51	151.67	24.14	22.52	25.02	39.94	42.21	39.72	3.31	4.94	2.90	36.12
CV (%)	14.28	17.96	13.38	12.26	14.55	9.95	4.39	4.9	5.13	9.55	12.64	11.44	6.16
CD (0.05)	25.59	19.75	25.8	3.76	4.16	3.17	2.23	2.63	2.59	0.4	0.74	0.42	2.83

Means followed by the same letters are not significanty different at 5% error

4.1.2.11. Virgin bark thickness

Highly significant clonal difference was observed for virgin bark thickness among the clones studied (Table 2). The range of variability for this trait was 7.25 mm (PB 312) to 11.36 mm (KRS 128) with a general mean of 9.19 mm (Table 5). Eight clones *viz.*, PB 255, PB 260, PB 280, PB 314, KRS 25, KRS 163, KRS 128 and RRII 105, exhibited bark thickness above the general mean. Two clones PB 255 and KRS 128 were significantly superior to RRII 105 while PB 255 was on par with KRS 128. Three clones PB 312, PB 217 and PB 311 were exhibited low bark thickness.

4.1.2.12. Latex vessel rows in virgin bark

Highly significant variations among the clones were observed (Table 2). The range of latex vessel rows in virgin bark was 16.68 (PB 312) to 28.64 (PB 255) with a general mean of 21.56 (Table 5). Number of latex vessel rows in five clones was above the general mean. Two clones *viz.*, PB 255 and KRS 163 were significantly superior to RRII 105. Of these PB 260 and KRS 163 were on par with PB 255.

4.1.2.13. Renewed bark thickness

Variation for renewed bark thickness was highly significant and is given in the Table 2. The general mean of the bark thickness of different clones was 7.18 mm. Bark thickness varied from 6.01 mm (PB 310) to 8.79 mm (PB 255). Four clones *viz.*, KRS 25, PB 280, KRS 25 and PB 255 recorded a high value of renewed bark thickness and five clones recorded the value above the general mean (Table 5). PB 255 was significantly superior to RRII 105 while three clones *viz.*, KRS 25, PB 280 and KRS 128, were on par with PB 255.

4.1.2.14. Latex vessel rows in renewed bark

Highly significant clonal variation for latex vessel rows in renewed bark was observed (Table 2). Variability ranged from 15.56 (KRS 25) to 30.96 (PB 255) with a general mean of 19.12. PB 255 was significantly superior to RRII 105. Six clones recorded higher value for number of latex vessel rows than the general mean (Table 5).

Table 5. Bark anatomical traits of different clones (4th year of tapping)

Clone	Virgin bark thickness (mm)	LVR in virgin bark	Renewed bark thickness (mm)	LVR in renewed bark
PB 217	7.88 efg	18.72 cd	6.48 de	16.04 de
PB 235	8.70 cdef	20.04 cd	6.47 de	16.44 cde
PB 255	10.73 ab	28.64 a	8.79 a	30.96 a
PB 260	9.09 cde	24.76 ab	6.68 cde	21.00 b
PB 280	9.79 bc	19.72 cd	7.68 abc	17.04 bcde
PB 310	8.31 defg	21.48 bc	6.01 e	14.20 e
PB 311	7.76 fg	21.88 bc	6.14 e	19.16 bcd
PB 312	7.25 g	16.68 d	6.63 cde	19.00 bcd
PB 314	9.69 bc	21.60 bc	7.32 bcd	19.24 bcd
KRS 25	9.93 bc	18.80 cd	7.96 ab	15.56 de
KRS 128	11.36 a	19.84 cd	8.42 ab	20.52 bc
KRS 163	9.59 bc	26.92 a	7.33 bcd	20.76 bc
RRII 105	9.41 cd	21.16 bc	7.40 bcd	18.60 bcd
Mean	9.19	21.56	7.18	19.12
CV (%)	10.64	15.46	12.57	17.80
CD (0.05)	1.24	4.25	1.15	4.33

Means followed by the same letters are not significanty different at 5% error

4.1.3. Latex and rubber properties

Mean values of latex and rubber properties of different clones are given in Table 6. Clonal variations were highly significant. The pH of latex of RRII 105 was nearly neutral (7.03) and KRS 163 registered the highest pH (7.26). PB 235, PB 255, PB 260, PB 280, PB 311, PB 314 and KRS 128 were on par with KRS 163. Seven clones *viz.*, PB 235, PB 255, PB 260, PB 280, PB 311, PB 314 and KRS 163 were significantly superior to RRII 105.

The highest total solid content (TSC) was estimated for the clone KRS 128 (44.13 %) followed by RRII 105 (43.46 %). The lowest TSC was measured for PB 312 (35.32 %). Highly significant clonal variation was observed for this trait. Two clones *viz.*, PB 280 and RRII 105 were on par with KRS 128 and no clones were significantly superior to RRII 105. KRS 25 (3.53 %) and PB 235 (2.75 %) showed the highest and lowest content of non-rubber substances (NRS) respectively. PB 255 and PB 280 were on par with KRS 25 whereas, seven clones PB 217, PB 255, PB 280, PB 310, PB 311, KRS 25 and KRS 128 were significantly superior to RRII 105. Ash content varied from 0.14 to 0.26 per cent. PB 312 recorded highest ash content (0.26 %) of natural rubber among the clones studied whereas, PB 260 and KRS 163 recorded lowest value for ash (0.14 %). PB 217 and PB 312 were significantly superior to RRII 105 and PB 217 were on par with PB 312. Data of the clonal rubbers for the gel content showed that clone PB 255 exhibited the highest gel content (22.87 %) followed by RRII 105 (16.66 %) and clone KRS 163 recorded the lowest value (4.43 %). Clonal variation was significant. PB 255 was significantly superior to RRII 105. KRS 128 recorded the lowest content for acetone extract (2.60 %) whereas, PB 260 recorded highest (4.17 %), and the acetone extract content of RRII 105 was in the lower range. Three clones *viz.*, PB 217, PB 235 and KRS 163 were on par with PB 260 while seven clones *viz.*, PB 217, PB 235, PB 260, PB 280, PB 310, KRS 25 and KRS 163

were significantly superior to RRII 105. Mean values for the nitrogen level ranges from 0.38 to 0.49 per cent. The highest nitrogen content was recorded for PB 312 (0.49 %) and lowest was for PB 235 and KRS 163 (0.38 %). PB 311 and PB 314 were on par with PB 312, while three clones PB 311, PB 312 and PB 314 were significantly superior to RRII 105. Rubber obtained from three clones recorded a viscosity range i.e. 60 to 70 units almost close to the processable range of viscosity. Six clones produced medium to hard rubbers whose viscosity ranges were 70 to 80 units. The remaining four clones including RRII 105 produced hard rubbers having a viscosity higher than 80. PB 255 exhibited the highest Mooney viscosity (88.24 unit). Significant differences were observed between clones. Only one clone PB 255 was significantly superior to RRII 105. Plasticity values determined for the clones showed a range varying from 43 to 61 units. Clone KRS 163 (42.83 unit) exhibited the lowest P_0 and MV, whereas, the highest value was observed for the clone PB 255 (60.67). Two clones PB 217 and KRS 128 were on par with PB 255 whereas, PB 255 was significantly superior to RRII 105. PRI determinations of the natural rubber from 13 clones ranged from 80 to 88 per cent. The highest and lowest PRI was recorded for PB 280 (88.11 %) and PB 217 (79.94 %) respectively. PB 260, PB 310, PB 314, KRS 25 and KRS 163 were on par with PB 280 whereas, one clone *viz.*, PB 280 was significantly superior to RRII 105.

Table 6. Clonal variation in latex and rubber properties

Clone	pH	TSC (%)	NRS (%)	AC (%)	GC (%)	AE (%)	N_2 (%)	MV	P_0	PRI (%)
PB 217	7.08 cde	37.24 fg	3.21 cd	0.25 ab	13.39 cd	4.08 a	0.41 cde	83.19 b	58.11 ab	79.95 d
PB 235	7.19 abc	40.92 cd	2.75 e	0.16 def	6.13 gh	3.96 a	0.38 e	76.47 c	52.50 cd	80.39 cd
PB 255	7.17 abc	43.21 ab	3.48 ab	0.19 cde	22.87 a	2.93 de	0.39 de	88.24 a	60.67 a	81.89 bcd
PB 260	7.24 ab	39.57 de	3.08 cd	0.14 ef	4.78 h	4.17 a	0.41 cde	64.41 e	43.83 gh	86.44 ab
PB 280	7.24 ab	42.87 ab	3.31 abc	0.21 bcd	7.82 fg	3.50 b	0.41 cde	72.18 d	48.28 e	88.11 a
PB 310	7.13 bcde	36.17 gh	3.24 bc	0.22 abc	12.34 cde	3.29 bc	0.45 abcd	71.46 d	47.67 ef	85.22 abc
PB 311	7.20 abc	37.60 fg	3.29 abc	0.22 abc	14.12 bc	3.02 cde	0.48 ab	70.08 d	46.22 efgh	82.17 bcd
PB 312	7.04 de	35.32 h	2.81 e	0.26 a	10.33 ef	2.93 de	0.49 a	66.11 e	44.22 fgh	82.17 bcd
PB 314	7.25 ab	37.51 fg	3.17 cd	0.22 abc	11.94 cde	2.94 de	0.48 ab	70.27 d	46.78 efg	83.44 abcd
KRS 25	7.11 cde	38.76 ef	3.53 a	0.20 cd	10.52 def	3.14 cd	0.44 bcd	73.29 cd	49.22 de	85.61 ab
KRS 128	7.15 abcd	44.13 a	3.29 abc	0.20 bcd	10.42 ef	2.60 f	0.39 de	82.63 b	57.39 ab	82.28 bcd
KRS 163	7.26 a	41.56 bc	2.80 e	0.14 f	4.43 h	4.07 a	0.38 e	63.47 e	42.83h	86.89 ab
RRII 105	7.03 e	43.46 a	2.95 de	0.20 cd	16.66 b	2.80 ef	0.42 cde	81.68 b	54.67 bc	81.89 bcd
Mean	7.16	39.87	3.15	0.2	11.21	3.34	0.43	74.11	50.18	83.57
CV (%)	0.97	2.61	4.49	11.55	15.63	5.19	5.74	3.03	4.4	3.57
CD (0.05)	0.12	1.75	0.24	0.04	2.95	0.29	0.04	3.78	3.72	5.02

Means followed by the same letters are not significanty different at 5% error

ABBREVIATIONS

TSC - Total Solid Content
NRS - Non Rubber Substances
AC - Ash Content
GC - Gel Content
AE - Acetone Extract
N_2 - Nitrogen Content
MV - Mooney Viscosity
P_0 - Initial Wallace Plasticity
PRI - Plasticity Retention Index

4.1.4. Association of dry rubber yield with latex and rubber properties

Of the various latex and rubber properties studied pH, total solid contents (TSC), gel contents (GC) and plasticity retention index (PRI) showed positive association with dry rubber yield, however non-rubber substances (NRS) acetone extract (AE), nitrogen content (N_2), mooney viscosity (MV), initial plasticity (P_0) and ash content (AC) indicated negative association with rubber yield (Table 7). Among these variables pH showed significant positive relation (0.583) with yield whereas, the association of ash with rubber yield was high and negative (-0.348) but it was non-significant. This correlation was followed by correlation of yield with PRI (0.271), TSC (0.266), P_0 (-0.251) and MV (-0.238). All other variables showed low association with yield.

Table 7. Association of dry rubber yield with latex and rubber properties

	Dry rubber yield	pH	TSC	NRS	AE	N_2	GC	MV	P_0	PRI	AC
Dry rubber yield											
pH	0.583*										
TSC	0.266	0.195									
NRS	-0.056	0.026	0.080								
AE	-0.093	0.393	-0.119	-0.370							
N2	-0.013	-0.247	-0.761	0.111	-0.455						
GC	0.004	-0.449	0.091	0.500	-0.604	0.173					
MV	-0.238	-0.398	0.475	0.402	-0.342	-0.387	0.714				
P0	-0.251	-0.370	0.478	0.368	-0.262	-0.452	0.649	0.992			
PRI	0.271	0.504	0.095	0.153	0.230	-0.037	-0.496	-0.609	-0.616		
AC	-0.348	-0.596	-0.533	0.253	-0.452	0.665	0.458	0.231	0.194	-0.401	

*Significant at $p<0.05$

ABBREVIATIONS

TSC - Total solid content
NRS - Non rubber substances
AE - Acetone extract
N_2 - Nitrogen content
GC - Gel content
MV - Mooney viscosity
P_0 - Initial Wallace plasticity
PRI - Plasticity retention index
AC - Ash content

4.2. Genetic parameters

The range and estimates of genetic parameters such as phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H^2) and genetic advance at 5 per cent intensity of selection as percentage of means are given in Table 8. Latex yield during peak season showed the highest range of 117.27 ml t^{-1} t^{-1} to 193.17 ml t^{-1} t^{-1}. It was followed by annual latex yield of 107.60 ml t^{-1} t^{-1} to 176.85 ml t^{-1} t^{-1}. Latex yield in stress season was 58.31 ml t^{-1} t^{-1} to 115.26 ml t^{-1} t^{-1}. Dry rubber yield during peak season exhibited a range of 44.75 per cent to 80.42 per cent. It was followed by annual dry rubber yield i.e. 38.17 per cent to 73.52 per cent, dry rubber yield during stress season i.e. 26.29 per cent to 52.74 per cent. Yield depression during stress exhibited a range of 27.99 per cent to 49.87 per cent. The range of latex vessel rows in renewed bark was 14.20 to 30.96 followed by latex vessel rows in virgin bark of 16.68 to 28.64. Girth at opening registered the range of 50.96 cm to 61.33 cm. Dry rubber content in stress season recorded a range of 38.22 per cent to 48.21 per cent. It was followed by dry rubber content in peak period that ranged from 35.93 per cent to 44.99 per cent. Annual rubber content showed a range of 36.45 per cent to 44.84 per cent. Virgin bark thickness registered a low range of 7.25 mm. to 11.26 mm. It was followed by renewed bark thickness i.e. 6.01 mm to 8.79 mm. The lowest range was exhibited for girth increment on tapping that ranged from 1.58 cm to 2.98 cm. Histogram representing clonal variability for all these traits are presented in Fig. 1 to 8.

4.2.1. Phenotypic coefficient of variation

Among the phenotypic coefficient of variation girth increment rate recorded the highest value (32.56 %) and a minimum for girth at opening (7.36 %) (Table 8). The girth increment rate was followed by latex yield in stress period (26.95 %), dry rubber yield in

stress season (26.26 %), yield depression under stress (26.01 %), latex vessel rows in renewed bark (26.94 %), rubber yield during peak period (22.97 %), annual rubber yield (21.60 %), latex yield during peak period (20.96 %), latex vessel rows in virgin bark (20.92 %) and mean annual latex yield (20.61 %). All these characters represented a relatively high value for phenotypic coefficient of variation whereas, renewed bark thickness (16.53 %) and virgin bark thickness (16.07 %) showed a moderately high value. Dry rubber content in peak period (9.29 %), dry rubber content during stress period (8.23 %) and mean annual dry rubber content (8.21 %), recorded low phenotypic coefficient of variation (Figure 8).

4.2.2. Genotypic coefficient of variation

Genotypic coefficient of variation (GCV) ranged from 4.78 per cent to 20.84 per cent for the variables studied (Table 8). The highest GCV was recorded for yield depression during stress followed by dry rubber yield during stress (20.27 %), latex vessel rows in renewed bark (20.19 %), latex yield during stress period (20.09 %), dry rubber yield in peak season (18.52 %), mean annual rubber yield (16.98 %), and latex yield in peak period (13.38 %) all of which exhibited relatively high GCV value of above 15 per cent. Annual latex yield (14.87 %), latex vessel rows in virgin bark (14.10 %), girth increment rate under tapping (12.31), virgin bark thickness (12.04 %) and renewed bark thickness (10.74 %) recorded moderately high GCV value above 10 per cent. Dry rubber content in peak period, (7.74 %), mean annual dry rubber content (6.94 %), dry rubber content during stress period (6.61 %), and girth at opening (4.78 %), indicated a low GCV of below 10 per cent (Figure 8).

4.2.3. Heritability

The broad sense heritability (H^2) ranged from 14.28 per cent to 71.39 per cent for yield and the associated characters studied (Table 8). High heritability expressed by a high value of above 60 per cent, was observed for most of the characters. The highest broad

sense heritability was observed for annual dry rubber content followed by dry rubber content during peak period (69.50 %), dry rubber yield in peak period (65.00 %), dry rubber content during stress period (64.55 %), yield depression during stress (64.15 %), and annual dry rubber yield (61.77 %). The heritability value for latex vessel rows in virgin bark and renewed bark thickness was 45.43 and 42.10 respectively.

Latex vessel rows in renewed bark (37.02 %), girth at opening (42.31 %), and girth increment under tapping (14.29 %) exhibited a heritability value of less than 50 per cent whereas, annual latex yield (52.03 %), latex yield during stress period (55.58 %), virgin bark thickness (56.11 %), latex yield during peak period (59.22 %), and yield during stress period (59.58 %) recorded by a moderately high heritability of above 50 per cent (Figure 8).

4.2.4. Genetic advance

The genetic advance under selection (GA) was highest for yield depression during stress (34.37 %) followed by rubber yield during stress period (32.23 %), latex vessel rows in renewed bark (31.21 %), latex yield in stress period (30.85 %) and rubber yield in peak season (30.76 %). All these characters showed a high genetic advance of the value above 30 per cent, whereas, annual rubber yield, latex yield during peak period (25.56 %), annual latex yield (22.09 %), latex vessel rows in virgin bark (19.57 %), virgin bark thickness (18.57 %) and latex vessel rows in renewed bark (17.68 %) depicted moderate values for genetic advance. A relatively low genetic advance of below 15 per cent was recorded for other variables like renewed bark thickness, dry rubber content during peak period, annual mean dry rubber content, dry rubber content in stress period, girth increment, and girth at opening (Table 8, Figure 8).

Table 8. Genetic parameters of yield and associated characters

Character	Range		PCV (%)	GCV (%)	H^2 (%)	GA (%)
	Min	Max				
Girth at opening (cm)	50.96	61.33	7.36	4.78	42.31	6.41
Girth increment on tapping (cm)	1.58	2.98	32.56	12.31	14.29	9.58
Dry rubber yield (g t^{-1} t^{-1})- (annual)	38.17	73.52	21.60	16.98	61.77	27.49
Dry rubber yield (g t^{-1} t^{-1})-(stress)	26.29	52.74	26.26	20.27	59.58	32.23
Dry rubber yield (g t^{-1} t^{-1})-(peak)	44.75	80.42	22.97	18.52	65.00	30.76
Yield depression during stress (%)	27.99	49.87	26.01	20.84	64.14	34.37
Latex yield (ml t^{-1} t^{-1})-(annual)	107.60	176.85	20.61	14.87	52.03	22.09
Latex yield (ml t^{-1} t^{-1})(-stress)	58.31	115.26	26.95	20.09	55.58	30.85
Latex yield (ml t^{-1} t^{-1})-(peak)	117.27	193.17	20.96	13.38	59.22	25.56
Dry rubber content (%)-(annual)	36.45	44.84	8.21	6.94	71.39	12.08
Dry rubber content (%)-(stress)	38.22	48.21	8.23	6.61	64.55	10.94
Dry rubber content (%)-(peak)	35.93	44.99	9.29	7.74	69.50	13.30
Virgin bark thickness (mm)	7.25	11.36	15.99	11.97	55.76	18.57
Renewed bark thickness (mm)	6.01	8.79	16.53	10.74	42.10	14.37
No. of LVR in virgin bark	16.68	28.64	20.92	14.10	45.43	19.57
No. of LVR in renewed bark	14.20	30.96	26.94	20.19	56.25	31.21

ABBREVIATIONS

GCV - Genotypic coefficient of variation

PCV - Phenotypic coefficient of variation

GA - Genetic advance

H^2 - Heritability

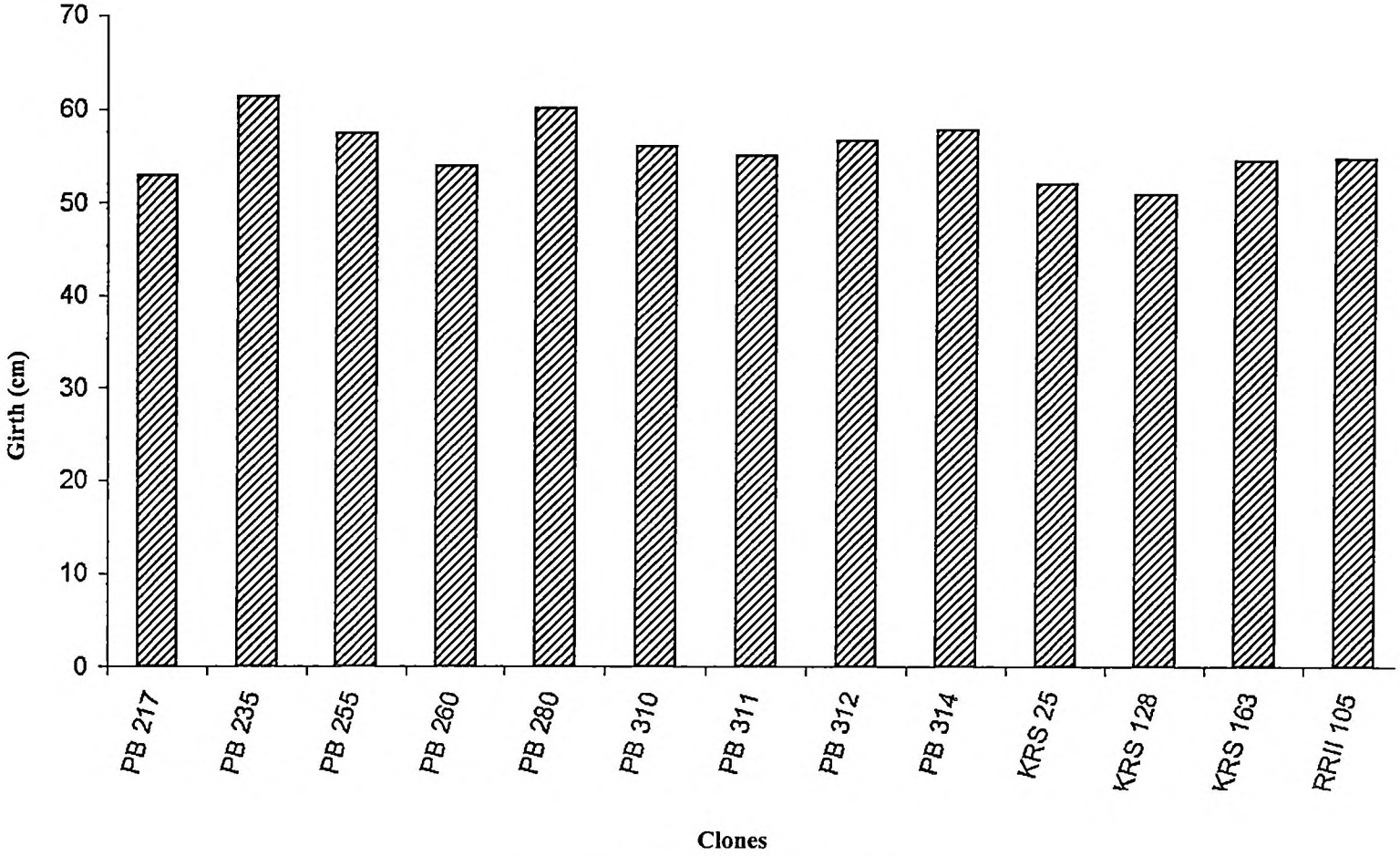


Fig. 1 Clonal variation for girth at opening

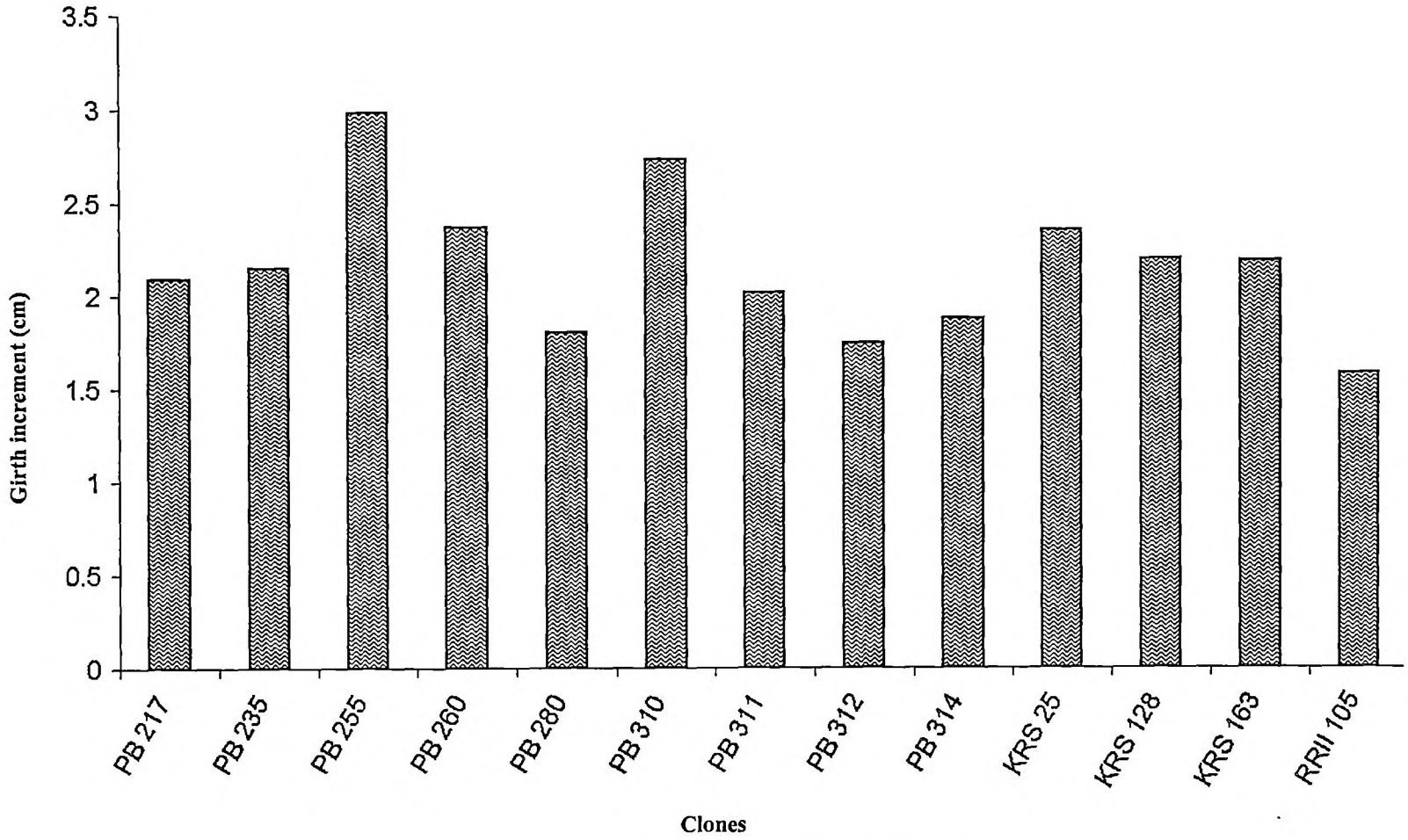


Fig. 2 Clonal variation for girth increment on tapping

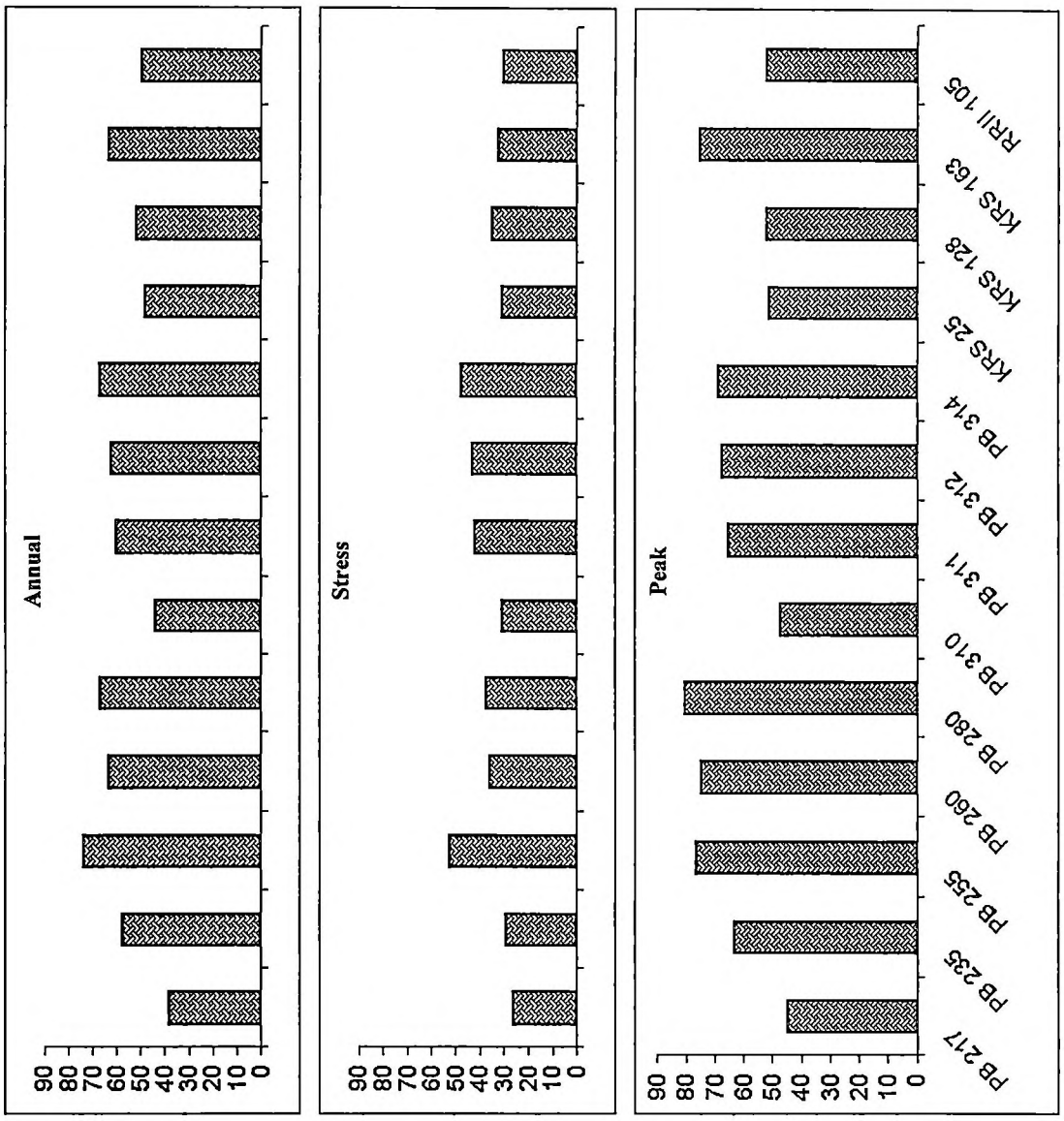


Fig. 3 Clonal variation for dry rubber yield

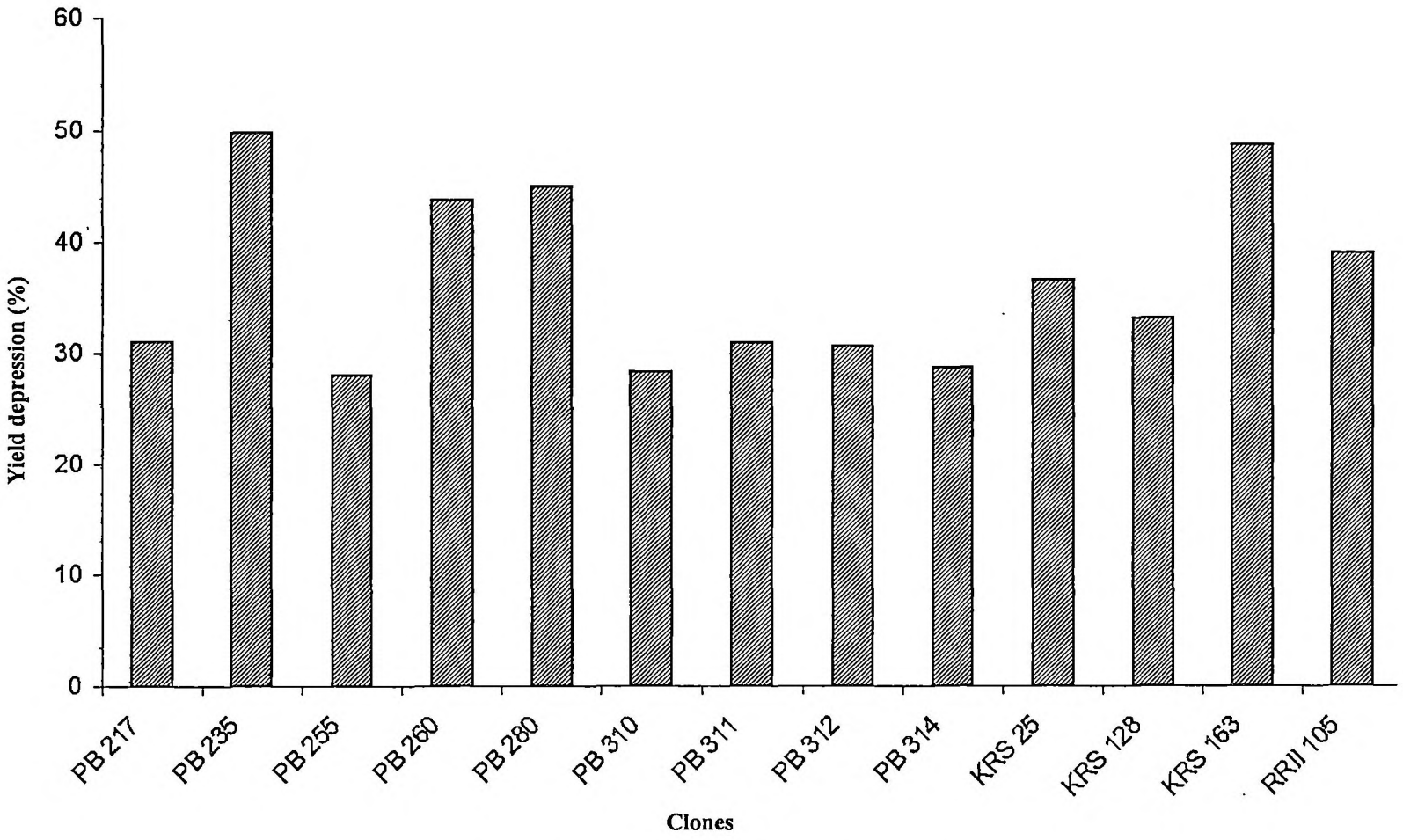


Fig. 4 Clonal variation for yield depression under stress

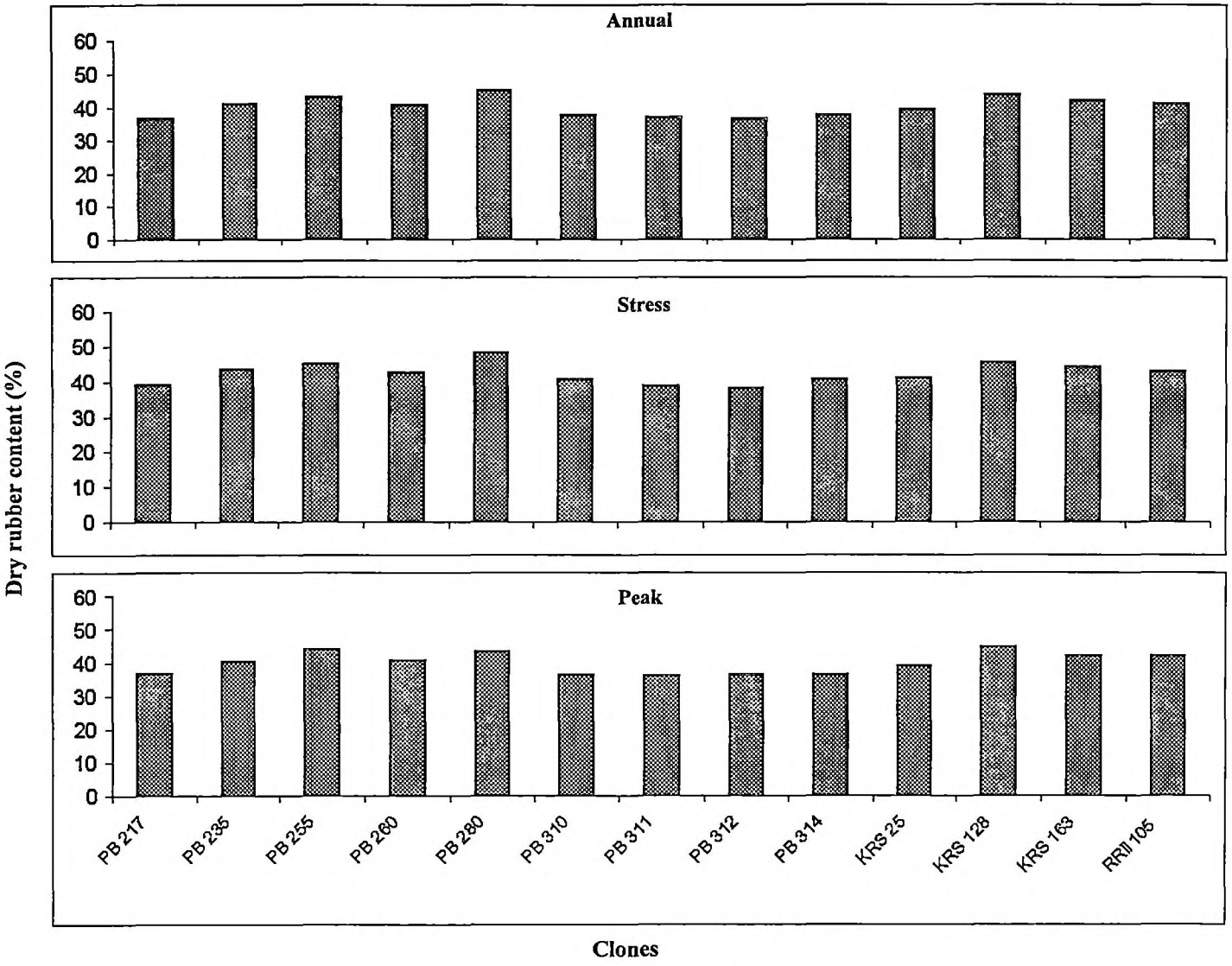


Fig. 5 Clonal variation for dry rubber content

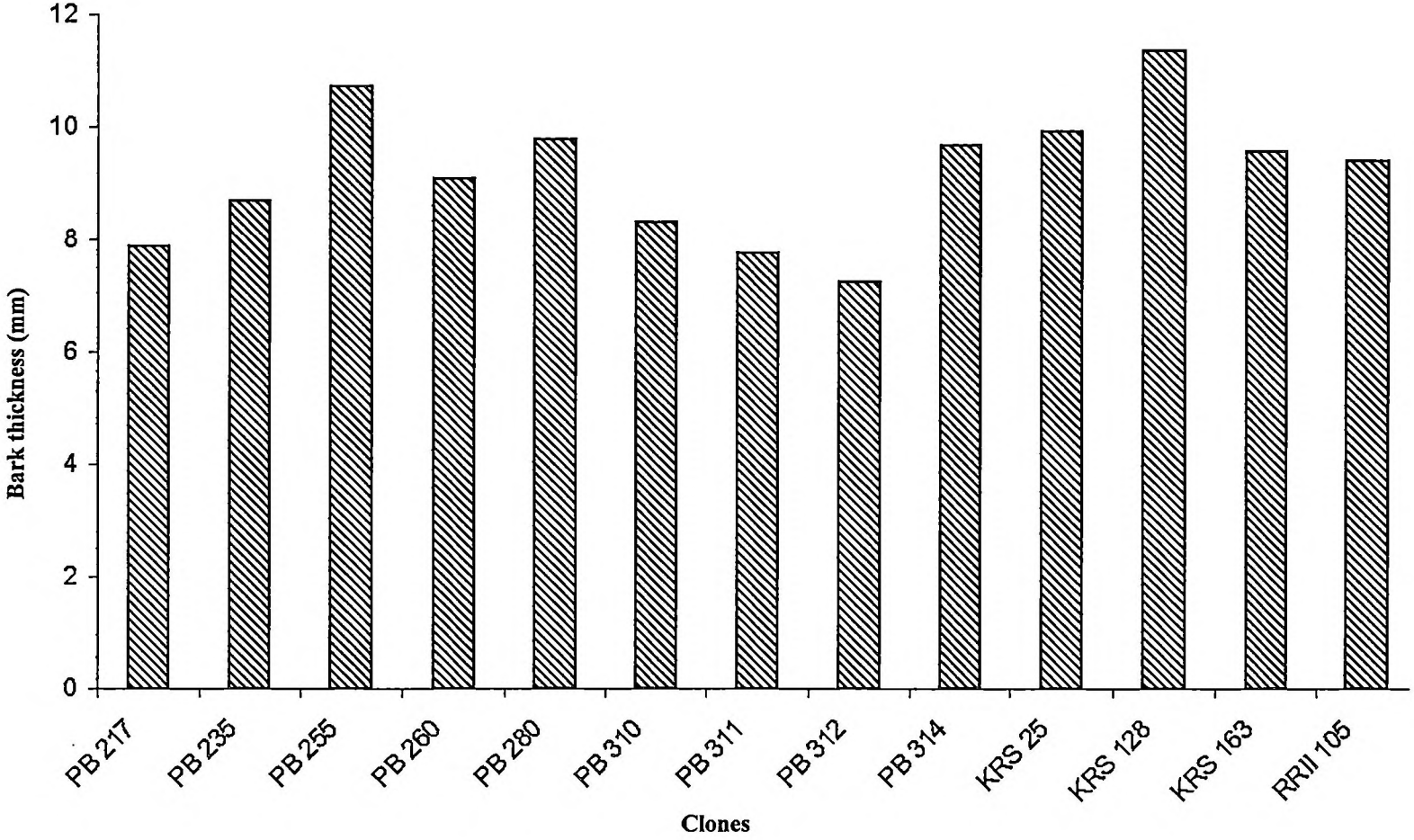


Fig. 6 Clonal variation for virgin bark thickness

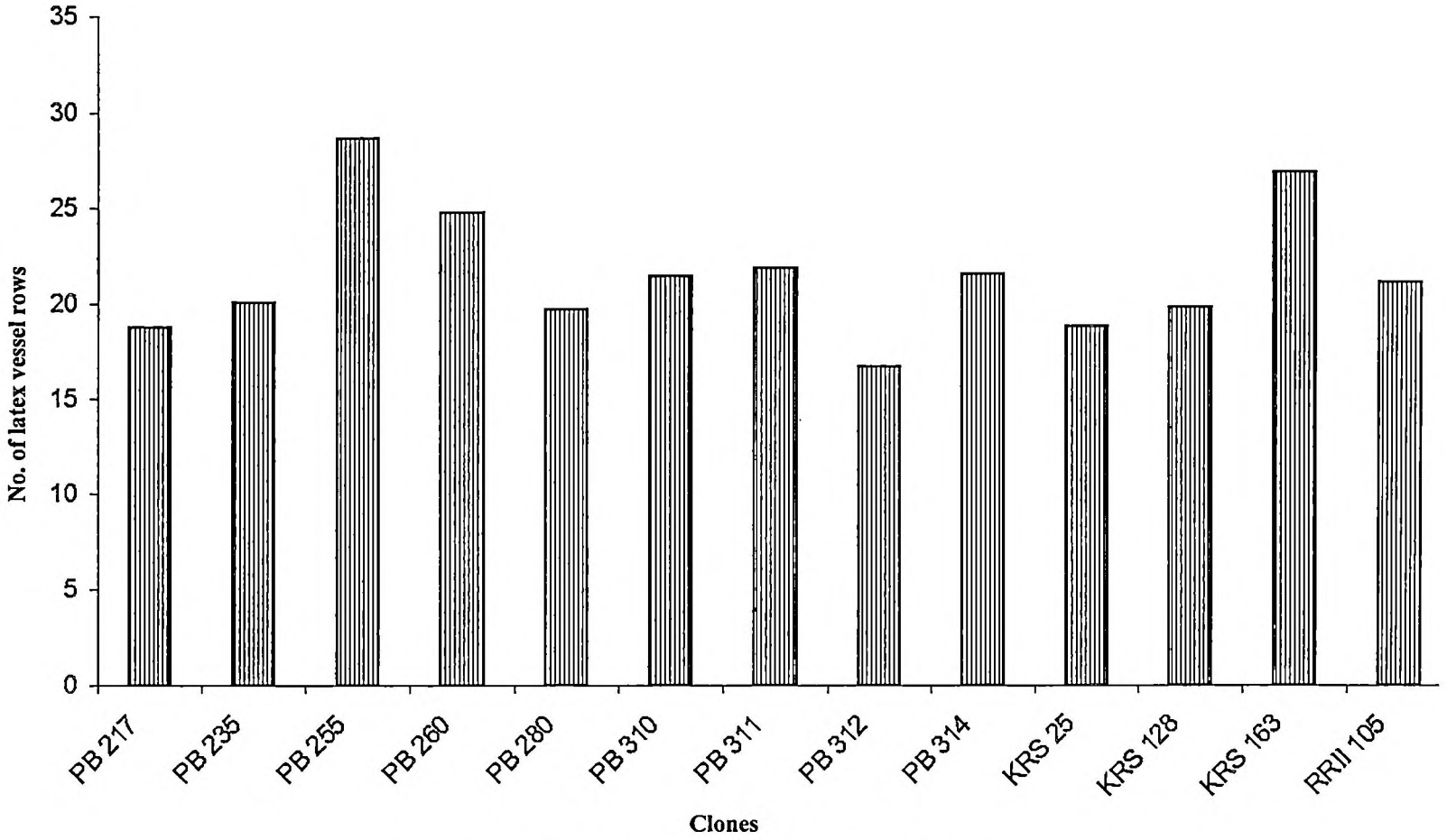


Fig. 7 Clonal variation for latex vessel rows in virgin bark

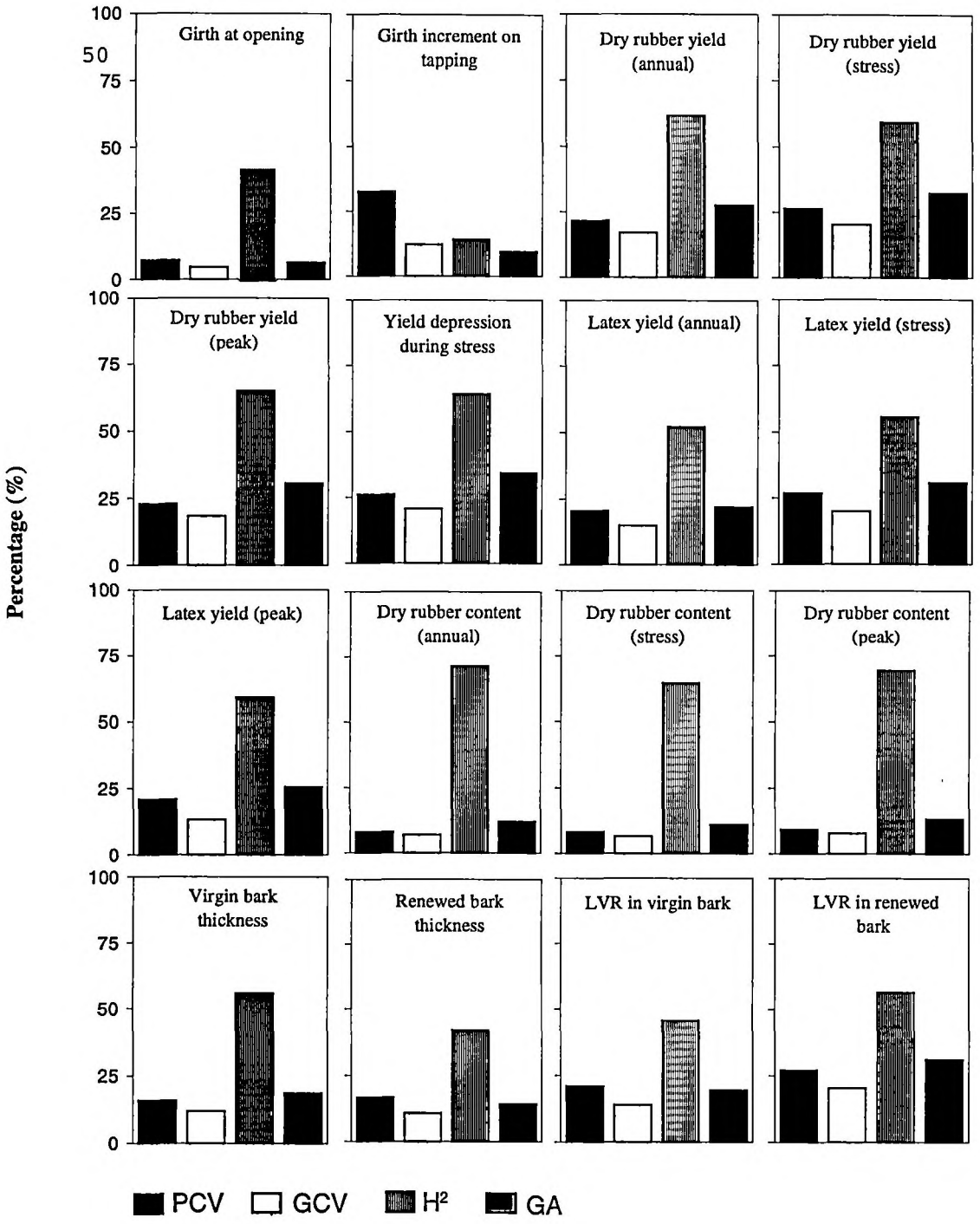


Fig. 8 Genetic parameters of yield and associated characters

4.3. Association of characters

4.3.1. Correlation between yield and growth

Annual rubber yield recorded positive genotypic correlation with girth (0.540) and the association of rubber yield in peak season with girth was also positive. Rubber yield in stress season exhibited favourable high positive association with girth increment (0.704) whereas, yield depression during stress recorded high but negative correlation with girth increment (-0.527). Girth showed positive genotypic correlation with dry rubber yield during stress (0.406) and peak period (0.511).

At phenotypic level, annual rubber yield showed positive association with girth (0.517), whereas, the association of rubber yield with girth increment on tapping was low (0.097). The association of rubber yield during different periods exhibited low relationship with girth increment on tapping.

4.3.2. Correlation between yield and physiological components

The values for genotypic association of different characters are given in Table 9. At genotypic level annual dry rubber yield showed high positive relationship with dry rubber yield in stress and peak periods (0.796, 0.945). Rubber yield in stress and peak season, annual latex yield and latex yield during stress and peak periods showed high favorable positive relationship with rubber yield. The correlation of annual rubber yield with annual plugging index, PI during stress and peak periods were low and negative. The highest correlation was recorded for annual dry rubber yield with yield in peak season (0.945) followed by latex yield in peak season (0.914), annual latex yield (0.893) and rubber yield during stress period (0.796). Among the physiological variables, rubber yield during stress showed a high correlation with annual latex yield (0.817) and latex yield during stress and

peak periods (0.916, 0.798). The rubber yield in peak period exhibited high genotypic correlation with mean annual latex yield (0.835) and latex yield in peak season (0.898). Genotypic correlation between yield depression and other physiological components indicated by low value except for annual dry rubber content (0.557), dry rubber content in stress (0.554) and peak season (0.491), latex yield during stress (-0.690), flow rate of latex during stress (-0.510) and plugging index in stress period (0.547).

At the phenotypic level annual yield showed a highly significant positive correlation with dry rubber yield during stress (0.808) and peak periods (0.938) (Table 10). It also indicated a highly significant favorable association with annual latex yield (0.875), latex yield during stress (0.570) and peak periods (0.844). Rubber yield during stress was observed to have a highly significant positive correlation with rubber yield in peak period (0.612), annual latex yield (0.786) latex yield in stress (0.898) and peak periods (0.682). Rubber yield during stress and peak season recorded highly significant correlation with annual latex yield (0.786, 0.803) and latex yield in peak periods (0.682, 0.854). Rubber content exhibited low and negative association with PI at phenotypic level. The correlation of rubber yield with flow rate and rubber content was comparatively low.

4.3.3. Correlation of yield with structural components

At genotypic level, annual dry rubber yield showed a positive correlation with virgin bark thickness (0.287), number of latex vessel rows in virgin bark (0.608), renewed bark thickness (0.437), and number of latex vessel rows in renewed bark (0.817). Dry rubber yield during stress and peak periods also showed positive genotypic correlation with virgin bark thickness (0.191, 0.169), latex vessel rows in virgin bark (0.463, 0.591) renewed bark thickness (0.457, 0.278) and number of latex vessel rows in renewed bark (0.844, 0.672).

At phenotypic level, annual dry rubber yield showed positive correlation with growth and structural components. The phenotypic correlation of annual dry rubber yield with virgin bark thickness (0.236), latex vessel rows in virgin bark (0.337), renewed bark thickness (0.186), latex vessel rows in renewed bark (0.519). Rubber yield in stress and peak periods also showed positive correlation at phenotypic level with growth and structural components of yield. The phenotypic correlation for stress and peak rubber yield with virgin bark thickness (0.217, 0.142), latex vessel rows in virgin bark (0.211, 0.333), renewed bark thickness (0.198, 0.097) and latex vessel rows in renewed bark (0.462, 0.438) were positive but low.

4.3.4. Interrelationship among growth, physiological and structural parameters

Genotypic correlation showed that annual latex yield exhibited a high positive correlation with latex yield in stress season (0.718) and peak seasons (0.992) while its relationship to rubber content was negative and low. Annual latex yield was inversely related to PI and rubber content. Latex yield during stress period indicated a negative genotypic correlation with dry rubber content and plugging index and its relationship to initial flow rate of latex during stress period was high (0.700).

Annual dry rubber content indicated a positive relationship with rubber content in stress (0.994) and peak periods (0.975), initial flow rate of latex (0.464) and annual plugging index (0.549). The genotypic correlation of annual mean dry rubber content with PI was high during stress period (0.751). It was also observed that the association of rubber content in stress and peak seasons with annual initial flow rate of latex (0.430, 0.490) and annual PI (0.533, 0.593) and PI in stress (0.727, 0.752) was also high.

Annual flow rate of latex represented a strong positive genotypic correlation with latex flow during stress and peak seasons (0.900, 1.014). It also exhibited a strong positive correlation with annual PI (0.716) and PI during stress (0.560) and peak (0.438) periods. The correlation with flow rate of latex during stress and annual PI (0.644) and also with PI during stress (0.652) was also high. Annual PI indicated a strong positive relationship with PI in stress (0.898) and peak periods (0.971).

Girth increment indicated negative relationship with yield depression during stress (-0.527) at genotypic level. All other growth and structural components exhibited positive relationship with yield depression under stress. Annual latex yield exhibited high correlation with latex vessel rows in renewed bark (0.630) while it's relation to virgin bark thickness was low and negative (-0.125). Latex yield during stress period showed high positive relationship with latex vessel rows in renewed bark (0.696). Annual rubber content and rubber content in all seasons exhibited high positive correlation with virgin bark thickness and renewed bark thickness. Annual initial flow rate of latex and flow rate during stress and peak season showed high positive association with renewed bark thickness (0.866, 0.832, 0.841). Flow rate of latex in stress season showed low and negative correlation with girth (-0.025). In case of initial flow rate of latex during peak season, a very low and negative relationship was noticed for latex vessel rows in virgin bark (-0.066) while virgin bark thickness showed strong positive association with annual rubber content (0.862), rubber content in stress (0.820), peak seasons (0.891).

At genotypic level, girth exhibited positive correlation with most of the structural components of yield. The highest relationship could be observed for girth and length of

tapping panel cut (1.055), all other variables showed comparatively low correlation with girth. Annual girth increment indicated a high positive correlation with of latex vessel rows in virgin bark (0.851). Virgin bark thickness registered a very high positive association with renewed bark thickness (1.012) and its relationship with latex vessels in renewed bark was also high and positive (0.533). Latex vessel rows in virgin bark showed a high positive relation with latex vessel rows in renewed bark (0.793) and also a high relationship was exhibited between renewed bark thickness and with latex vessel rows in renewed bark (0.663).

At phenotypic level, annual latex yield showed highly significant positive correlation with latex yield in stress (0.690) and peak seasons (0.921) whereas, it had a significant negative association with mean annual PI (-0.574) and PI in peak period (-0.624). Latex yield during stress period showed a highly significant positive correlation with initial flow rate of latex during stress period (0.580) and a significantly negative relationship with PI in stress period (-0.553) while latex yield during peak season indicated a highly significant positive association with PI in peak season (0.620). Phenotypic correlation coefficient indicated that mean annual rubber content showed highly significant positive association with rubber content in stress (0.929) and peak periods (0.961) and rubber content during stress season exhibited a positive correlation with rubber content in peak season (0.840). At phenotypic level annual initial flow rate of latex showed highly significant positive correlation with initial flow rate during stress (0.870) and peak season (0.938). Initial flow rate of latex during stress exhibited highly significant positive relationship with flow rate of latex in peak season (0.790). Annual PI showed positive association with PI during stress (0.869) and PI in peak period (0.908). PI during stress season indicated a highly significant positive correlation with PI during peak season (0.634) at the phenotypic level.

Phenotypic correlation coefficient among morphological structural and physiological components of yield showed that virgin bark thickness exhibited a highly significant positive association with annual rubber content (0.596), rubber content in stress (0.560) and peak seasons (0.587) and all other morphological and structural components showed negligible relationship with physiological components of yield. Annual PI and PI in all the periods registered a negative correlation with number of latex vessels in virgin and renewed bark whereas, it showed a high positive relationship with virgin and renewed bark thickness.

Girth showed a highly significant positive correlation with length of tapping panel (0.608) while it exhibited a very low and inverse correlation with virgin bark thickness (-0.003) and renewed bark thickness (-0.115), all other morphological and structural components showed low but positive correlation with girth. Annual girth increment exhibited a very low correlation with length of tapping panel cut (0.009), number of latex vessel rows in virgin (0.186) and renewed bark (0.208). Length of tapping panel cut registered a very low relationship with all the structural variables studied. Virgin bark thickness showed highly significant positive correlation with renewed bark thickness (0.673). All other variables exhibited very low but positive association among the structural components of yield studied.

Table 9. Genotypic correlations among yield and associated characters

Variables	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)
Girth (1)		0.415	0.540	0.406	0.511	0.096	0.365	0.009	0.424	0.022	-0.025	0.038	0.360	0.549	0.208	-0.020	0.277	-0.179	1.055	0.129	0.363	0.265	0.194
GI (2)			0.416	0.704	0.132	-0.527	0.436	0.654	0.171	0.168	0.341	0.108	-0.073	-0.126	0.129	-0.215	-0.351	0.032	0.147	0.018	0.851	0.247	0.853
DRY-Annual (3)				0.796	0.945	0.168	0.893	0.543	0.914	0.131	0.314	0.206	0.374	0.386	0.294	-0.392	-0.117	-0.474	0.440	0.287	0.608	0.437	0.817
DRY-stress (4)					0.607	-0.460	0.817	0.916	0.798	0.271	0.634	0.339	0.009	0.010	-0.014	-0.439	-0.400	-0.389	0.199	0.191	0.463	0.457	0.844
DRY-Peak (5)						0.374	0.835	0.334	0.898	0.003	0.086	0.084	0.414	0.440	0.303	-0.411	-0.108	-0.547	0.445	0.169	0.591	0.278	0.672
RY-dpres (6)							-0.026	-0.690	0.020	-0.187	-0.510	-0.203	0.557	0.554	0.491	0.194	0.547	-0.009	0.328	0.162	0.130	-0.029	-0.134
LY-annual (7)								0.718	0.992	-0.088	0.226	0.023	-0.064	-0.085	-0.129	-0.667	-0.454	-0.705	0.289	-0.125	0.340	0.107	0.630
LY-stress (8)									0.693	0.285	0.700	0.351	-0.281	-0.307	-0.262	-0.453	-0.555	-0.337	-0.124	-0.002	0.179	0.340	0.696
LY-peak (9)										-0.019	0.235	0.094	0.067	0.066	-0.017	-0.609	-0.420	-0.663	0.390	-0.079	0.315	0.130	0.593
IFR-Annual (10)											0.900	1.014	0.464	0.430	0.490	0.716	0.560	0.738	0.039	0.640	-0.033	0.866	0.204
IFR-Stres (11)												0.942	0.237	0.192	0.273	0.354	0.152	0.461	-0.009	0.576	0.012	0.832	0.448
IFR-Peak (12)													0.450	0.431	0.455	0.644	0.496	0.652	0.022	0.581	-0.066	0.841	0.198
DRC-Annual (13)														0.994	0.975	0.549	0.751	0.403	0.447	0.862	0.417	0.838	0.456
DRC-stres (14)															0.934	0.533	0.727	0.383	0.475	0.820	0.388	0.751	0.314
DRC-peak (15)																0.593	0.752	0.479	0.356	0.898	0.469	0.934	0.588
PI-Annual (16)																	0.898	0.971	0.100	0.540	-0.322	0.524	-0.312
PI-Stres (17)																		0.788	0.477	0.599	-0.229	0.494	-0.258
PI-Peak (18)																			-0.125	0.520	-0.298	0.540	-0.257
LTP (19)																				0.071	-0.143	0.296	0.059
VBT (20)																					0.416	1.012	0.533
LVR-VB (21)																						0.308	0.793
RBT (22)																							0.663
LVR-RB (23)																							

ABBREVIATIONS

GI (2) - Girth increment on tapping
DRY-Annual (3) - Annual dry rubber yield
DRY-stress (4) - Dry rubber yield during stress period
DRY-Peak (5) - Dry rubber yield during peak period
DRY-dpres (6) - Yield depression during stress period
LY-annual (7) - Annual latex yield
LY-stress (8) - Latex yield during stress period
LY-peak (9) - Latex yield during peak period
IFR-Annual (10) - Initial flow rate of latex
IFR-Stres (11) - Initial flow rate of latex during stress period
IFR-Peak (12) - Initial flow rate of latex during peak period
DRC-Annual (13) - Annual dry rubber content
DRC-stres (14) - Dry rubber content during stress period
DRC-peak (15) - Dry rubber content during peak period
PI-Annual - Annual Plugging Index
PI-Stress - Plugging Index during stress period
PI-Peak - Plugging Index during peak period
LTP - Length of tapping panel
VBT - Virgin bark thickness
RBT - Renewed bark thickness
LVR-VB - Latex vessel rows in virgin bark
LVR-RB - Latex vessel rows in renewed bark

Table 10. Phenotypic correlations among yield and associated characters

Variables	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)
Girth (1)		0.213	0.517	0.396	0.481	0.107	0.351	0.204	0.331	0.036	-0.047	0.141	0.237	0.261	0.205	-0.103	-0.037	-0.146	0.608[a]	-0.003	0.084	-0.115	0.095
GI (2)			0.097	0.099	0.040	-0.019	0.016	0.052	0.065	-0.009	-0.019	0.031	-0.056	-0.080	-0.023	0.000	-0.020	0.051	0.009	-0.024	0.186	-0.031	0.208
DRY-Annual (3)				0.808[b]	0.938[b]	0.119	0.875[b]	0.570[a]	0.844[b]	0.199	0.231	0.207	0.405	0.390	0.353	-0.413	-0.213	-0.473	0.455	0.236	0.337	0.186	0.519
DRY-stress (4)					0.612[a]	-0.480	0.786[b]	0.898[b]	0.682[a]	0.257	0.460	0.269	0.103	0.106	0.112	-0.467	-0.459	-0.375	0.323	0.217	0.211	0.198	0.462
DRY-Peak (5)						0.341	0.803[b]	0.355	0.854[b]	0.105	0.071	0.132	0.426	0.424	0.346	-0.401	-0.157	-0.540	0.434	0.142	0.333	0.097	0.438
RY-dpres (6)							-0.025	-0.651	0.083	-0.108	-0.416	-0.109	0.433	0.396	0.354	0.211	0.497	-0.034	0.150	-0.004	0.113	-0.044	-0.023
LY-annual (7)								0.690[b]	0.921[b]	0.193	0.288	0.176	0.049	0.067	0.006	-0.574[a]	-0.378	-0.624[a]	0.396	-0.043	0.149	0.054	0.443
LY-stress (8)									0.539	0.306	0.580[a]	0.311	-0.155	-0.195	-0.097	-0.446	-0.553[a]	-0.292	0.152	0.048	0.030	0.121	0.340
LY-peak (9)										0.186	0.251	0.205	0.107	0.137	0.021	-0.496	-0.278	0.620[a]	0.360	-0.038	0.118	0.116	0.407
IFR-Annual (10)											0.870[b]	0.938[b]	0.239	0.201	0.257	0.534	0.394	0.504	-0.106	0.290	-0.107	0.428	0.132
IFR-Stres (11)												0.790[b]	0.029	-0.022	0.065	0.275	0.148	0.289	-0.195	0.257	-0.096	0.437	0.174
IFR-Peak (12)													0.200	0.183	0.214	0.521	0.366	0.516	-0.001	0.283	-0.098	0.396	0.151
DRC-Annual (13)														0.929[b]	0.961[b]	0.360	0.437	0.269	0.298	0.596[a]	0.267	0.413	0.295
DRC-stres (14)															0.840[b]	0.329	0.442	0.215	0.384	0.560[a]	0.254	0.367	0.286
DRC-peak (15)																0.370	0.391	0.334	0.247	0.587[a]	0.259	0.394	0.308
PI-Annual (16)																	0.869[b]	0.908[b]	-0.043	0.300	-0.153	0.308	-0.195
PI-Stres (17)																		0.634[a]	0.118	0.296	-0.062	0.323	-0.116
PI-Peak (18)																			-0.162	0.335	-0.146	0.261	-0.156
LTP (19)																				0.063	0.059	-0.027	0.167
VBT (20)																					0.368	0.673[a]	0.331
LVR-VB (21)																						0.306	0.593[a]
RBT (22)																							0.415
LVR-RB (23)																							

[a]Signifcant at p<0.05 [b]Signifcant at p<0.01

ABBREVIATIONS

GI (2) - Girth increment on tapping
DRY-Annual (3) - Annual dry rubber yield
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LY-stress (8) - Latex yield during stress period
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IFR-Annual (10) - Initial flow rate of latex
IFR-Stres (11) - Initial flow rate of latex during stress period
IFR-Peak (12) - Initial flow rate of latex during peak period
DRC-Annual (13) - Annual Dry rubber content
DRC-stres (14) - Dry Rubber content during stress period
DRC-peak (15) - Dry Rubber content during peak period
PI-Annual - Annual Plugging Index
PI-Stress - Plugging Index during stress period
PI-Peak - Plugging Index during peak period
LTP - Length of tapping panel
VBT - Virgin bark thickness
RBT - Renewed bark thickness
LVR-VB - Latex vessel rows in virgin bark
LVR-RB - Latex vessel rows in renewed bark

4.4. D^2 analysis

The D^2 value ranged from 1.79 to 159.55 showing that genetic variability exist among the 13 clones based on yield and yield components studied (Table 11). The highest genetic divergence was observed for PB 280 from PB 312 (159.55) followed by PB 311 (157.43) and the minimum between PB 260 and KRS 163 (1.79) followed by PB 217 and PB 310 (5.83).

Cluster analysis based on D^2 values showed that two major groups existed in 13 clones studied (Fig. 9). The first group consisted of eight clones ie, PB 260, KRS 163, PB 310, PB 217, PB 235, PB 311, PB 312 and PB 314. The second major cluster consisted of five clones *viz.*, PB 255, KRS 128, RRII 105, KRS 25 and PB 280. These two groups were further divided into four sub groups. The first major group consisted of two clusters. PB 260, KRS 163, PB 310, PB 217 and PB 235 were grouped into one whereas PB 311, PB 312 and PB 314 were isolated into another cluster. The second major group consisted of two clusters. KRS 128, RRII 105, KRS 25 and PB 280 were grouped into one however, PB 255 separated from all other clones with a genetic divergence of 50.60 per cent.

The two major groups of clones were clustered irrespective of their country of origin. In cluster I, KRS 163 (Thailand clone) was incorporated and the rest of the clones in this group were of Malaysian origin. In the second cluster, two Thailand clones i.e., KRS 128, KRS 25 and the Indian clone RRII 105 were included along with other three Malaysian clones. The country of origin of clones in cluster II was diverse. As far as the yield trend is concerned PB 255 and PB 280 are high yielding clones and the other clones in this group are either superior or comparable in yield with the Indian clone RRII 105.

The inter cluster distance between cluster I and II was 78.11. The intra cluster distance in group I was 24.65 and that of group II was 34.14. The average intra cluster distance was 28.60. The inter cluster distance (D^2 value) showed considerable genetic divergence among the clones studied.

Table 11. D^2 value among 13 clones - Genetic distance matrix based on yield and major yield components

	PB 260	KRS 163	PB 255	PB 310	KRS 128	RRII 105	PB 311	KRS 25	PB 217	PB 310	PB 314	PB 235	PB 280
PB 260	0	1.79	33.03	18.58	86.67	99.4	18.81	65.77	27.00	29.09	19.78	27.54	98.86
KRS 163		0	25.97	25.54	80.87	96.01	29.96	67.27	35.41	43.6	27.38	28.76	90.64
PB 255			0	41.11	50.76	52.94	72.01	39.44	48.26	88.98	42.71	32.01	37.71
PB 310				0	83.64	83.38	24.34	50.31	5.83	38.18	23.10	29.22	92.69
KRS 128					0	17.93	134.19	16.00	79.32	137.5	78.33	50.75	27.69
RRII 105						0	141.24	10.06	69.87	143.5	90.85	48.74	18.34
PB 311							0	92.07	34.76	7.27	13.94	63.09	157.43
KRS 25								0	39.57	92.47	48.29	34.27	28.13
PB 217									0	44.03	32.24	31.13	81.73
PB 312										0	16.95	60.14	159.55
PB 314											0	40.48	106.52
PB 235												0	47.29
PB 280													0

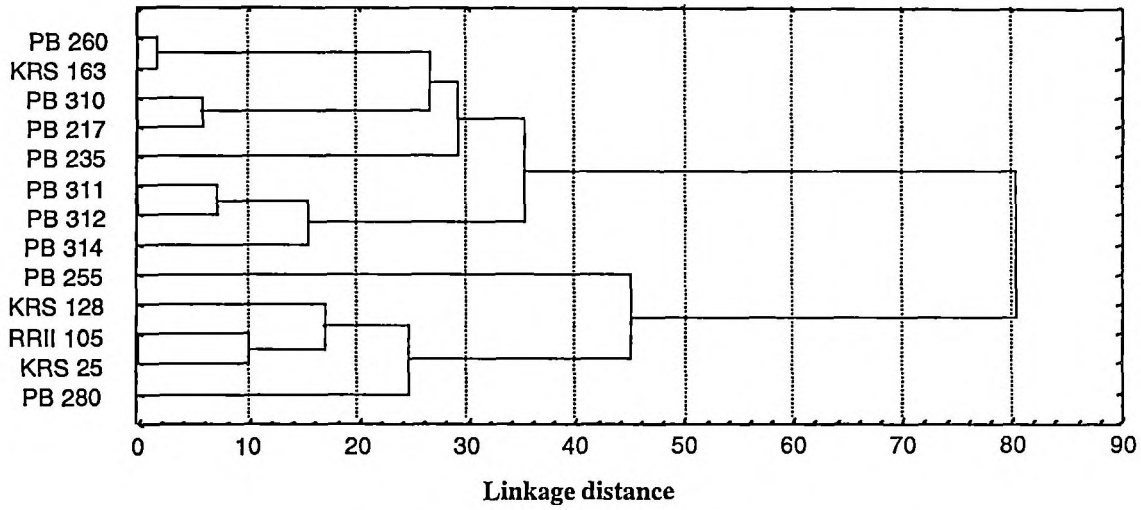


Fig. 9. UPGMA Clustering pattern of the clones based on Jaccard's dissimilarity coefficient using yield and major yield component

4.5. Isozymes

Out of the nine isozymes *viz.*, alcohol dehydrogenase, glutamate dehydrogenase, peroxidase, shikimate dehydrogenase, superoxide dismutase, aspartate aminotransferase, acid phosphatase, alkaline phosphatase and aryl esterase were studied, five were poorly resolved and were eliminated later. The enzyme system which provided polymorphic bands and fully resolved were peroxidase, shikimate dehydrogenase, aspartate aminotransferase and aryl esterase (Plate 6, Fig. 10 - 13). These four isozymes were used to characterize *Hevea* clones.

Of the four-isozyme systems studied, a combined total of 22 bands were scorable and a mean of 15 bands per clone could be observed. The number of isozyme bands in different clones ranged from 11 to 19. The highest number of bands was observed for PB 312 whereas, PB 255 registered the lowest number of bands.

4.5.1. Aryl esterase

The number of bands were highest for aryl esterase (7 bands), among the four isozymes studied that ranged from 3 - 7 in different cultivars. PB 255 recorded the lowest number of bands whereas, seven clones *viz.*, RRI 105, PB 217, PB 260, PB 280, PB 312, PB 314 and KRS 163 exhibited highest number of bands. For all clones 2^{nd}, 4^{nd}, 5^{nd} and 6^{nd} locus was common. Clones having highest number of bands showed a common zymogram however, cultivars having six number of bands *viz.*, PB 235, PB 310, PB 311, KRS 25 and KRS 128 were exhibited characteristic band pattern, the 7^{th} locus was absent in this case. The clone PB 255 was characterised by the presence of 2^{nd}, 4^{nd} and 5^{nd} locus whereas, all other bands were absent in this case.

4.5.2. Peroxidase

A total number of eight peroxidase isozyme loci was observed that ranged from 2 - 6 in various clones studied. The lowest number of bands *viz.*, 2 was observed in two clones PB 217 and PB 255 and these two clones were characterised by similar zymogramme. Six clones *viz.*, RRII 105, PB 235, PB 280, PB 311, KRS 25 and KRS 163 were observed by the presence of 3 bands but these clones differentiated from each other by the different banding patterns. Of these PB 280, PB 311 and KRS 25 showed common bands at 3rd, 6th and 7th locus. The clones RRII 105, PB 235 and KRS 163 exhibited a common band in the 7th locus whereas, in PB 235 one band at the 6th locus was absent but it was observed in the other two clones. The 5th peroxidase band was present in RRII 105 and PB 235 but it was not observed in KRS 163. The next highest number of 4 bands were observed in PB 260, PB 314 and KRS 128 varieties, however, they were differentiated from each other by polymorphic bands. The 6th and 7th peroxidase bands were common for these three clones but they were separated from each other by the remain polymorphic bands. In KRS 128, 8th band was characteristic, whereas, it was absent in other two clones. In PB 260 the 5th band was characteristic but they were not present in PB 314 and KRS 128 while in PB 314 and KRS 128 4th locus was observed that was not identified in PB 260. The two varieties PB 260 and PB 314 were characterised by the presence of 3rd peroxidase locus whereas, it was not visible in KRS 128. The clone PB 310 was characterised by the presence of five isozyme loci. The highest number of 6 bands were noticed for PB 312.

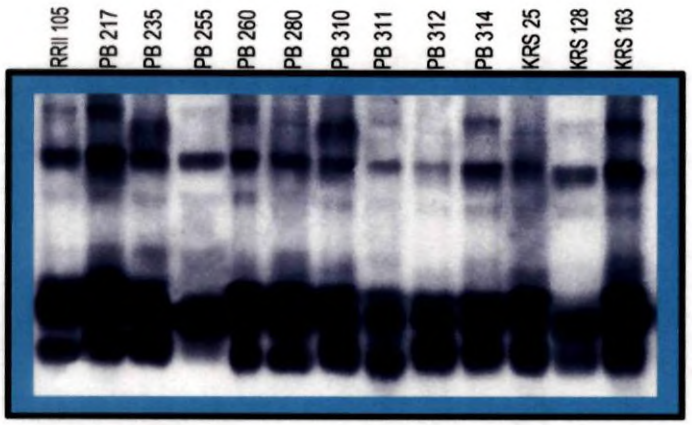
4.5.3. Aspartate aminotransferase

A total of four aspartate aminotransferase bands could be observed that ranged from three to four in different clones. The majority of clones depicted lowest number of

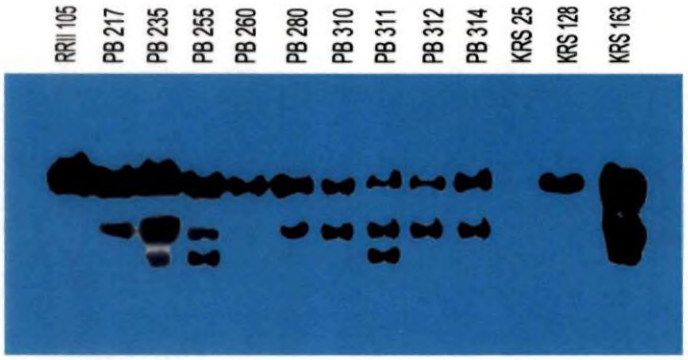
three bands which were common for 1st, 2nd, and 4th locus for all these clones. The clones characteristic of this type of banding pattern were RRII 105, PB 235, PB 255, PB 310, PB 311, PB 314, KRS 25, KRS 128 and KRS 163, so that characterization among these clones were difficult by means of aspartate aminotrasferase alone. Another set of clones characterized by the presence of an additional band at the 3rd locus. So that a total number of four isozyme band position could be noticed. The zymogramme patterns in these four varieties were identical, so discrimination was tedious among these varieties. Here 1st, 2nd and 4th band position were common to all the varieties studied, whereas, the third locus could be possible to discriminate the cultivars.

4.5.4. Shikimate dehydrogenase

The numbers of bands for shikimate dehydrogenase in different clones were rather limited. A total number of 3 bands observed and that ranged from zero to three in different clones. No bands were identified in the clone KRS 25, while RRII 105, PB 260, and KRS 128 were characterized by the presence of a single band at the 3th position. These were common for all the three clones hence characterization among these was tedious only by shikimate dehydrogenase enzyme. The next highest number of bands observed was two, which was noticed for PB 217, PB 280, PB 310, PB 312 and PB 314. The isozyme band pattern was similar in these clones and was noticed in the 2nd and 3rd locus. Another set of clones was characterized by the presence of an additional band in the 1st position. The clones characterized by this band was PB 235, PB 255, PB 311 and KRS 163. The banding pattern was also similar for these clones.



(a)



(b)

Plate 6. Zymogram of (a) aryl esterase (b) shikimate dehydrogenase isozyme profile showing polymorphism in 13 clones of *Hevea* brasiliensis.

No. of bands	RRII 105	PB 217	PB 235	PB 255	PB 260	PB 280	PB 310	PB 311	PB 312	PB 314	KRS 25	KRS 128	KRS 163
7	—	—			—	—			—	—		—	—
6	—	—	—		—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—	—
3	—	—	—		—	—	—	—	—	—	—		—
2	—	—	—	—	—	—	—	—	—	—	—	—	—
1	—	—	—		—	—	—	—	—	—	—	—	—

Fig. 10. Diagramatic representation of aryl esterase isozyme polymorphism in 13 clones of *Hevea brasiliensis*.

No. of bands	RRII 105	PB 217	PB 235	PB 255	PB 260	PB 280	PB 310	PB 311	PB 312	PB 314	KRS 25	KRS 128	KRS 163
4	—	—	—	—	—	—	—	—	—	—	—	—	—
3		—			—	—			—				
2	—	—	—	—	—	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—	—	—	—	—	—

Fig. 11. Diagramatic representation of aspartate amino transferase isozyme polymorphism in 13 clones of *Hevea brasiliensis*.

No. of bands	RRII 105	PB 217	PB 235	PB 255	PB 260	PB 280	PB 310	PB 311	PB 312	PB 314	KRS 25	KRS 128	KRS 163
3	-	-	-	-	-	-	-	-	-	-		-	-
2		-	-	-		-	-	-	-	-			-
1			-	-				-					-

Fig. 12. Diagramatic representation of shikimate dehydrogenase isozyme polymorphism in 13 clones of *Hevea brasiliensis*.

No. of bands	RRII 105	PB 217	PB 235	PB 255	PB 260	PB 280	PB 310	PB 311	PB 312	PB 314	KRS 25	KRS 128	KRS 163
8												-	
7	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-		-	-	-	-	-	-	-	-	-	-
5	-		-		-								
4			-						-	-		-	-
3					-	-	-	-	-	-	-		
2							-		-				
1							-		-				

Fig. 13. Diagramatic representation of peroxidase isozyme polymorphism in 13 clones of *Hevea brasiliensis*.

4.6. RAPD analysis

The 13 cultivated *Hevea* clones selected for the present study represent a wide spectrum of variation for several phenotypic traits, and in their origin. The 13 clones showed greater diversity for yield, disease reaction, drought, tapping panel dryness (TPD) etc. DNA from the 13 clones was studied with random oligonucleotide primers for RAPD assay. In total, 9 primers produced clear and readable bands, 43 primers poorly amplified and the remainder failed to amplify or generated only a smeared pattern. Primer OPC-11 produced monomorphic bands among the different clones, and therefore it was excluded from further analysis since it was not informative. The 8 remaining primers were selected as most informative, which produced RAPD profiles in all the clones used to measure genetic relationships among them. The nucleotide sequences of these primers are illustrated in Table 12. Easily detectable, well-resolved bands were those, which were reproducible over repeated runs with sufficient intensities to determine presence or absence in samples with the same relative intensity. The agarose gels showing polymorphisms observed with six primers OPA-04, OPC-05 and OPA-07, OPB-12, OPA-16 and OPA-17 are illustrated in Plate 7a, b, c and 8a, b, c respectively. Primers varied greatly in the ability to resolve variability among the clones. Some primers (e.g. OPA-17 and OPC-05) generated several markers and were able to show high genetic diversity, others (e.g. OPA-04) generated fewer markers and showed less variability. Out of the total of 82 bands from 13 clones using 8 primers, 35 were polymorphic. The total number of bands produced per primer varied from 5 to 21, although only 7 to 10 of these was polymorphic. The size of bands ranged from 300 to 4000 base pairs. Dissimilarity between clones varied from 15 to 60 per cent indicating high degree of genetic diversity (Table 13). This molecular information concurs with the reported high morphological variability in *Hevea*.

For further molecular confirmation of the presence of a specific polymorphic band in the RAPD profile, the amplification product indicated in Plate 8 b was excised, labeled with a^{32} P- dATP and used to probe Southern blotts of the PCR amplification products of 13 clones Plate 9 (a) demonstrates that the labeled product hybridizes to the corresponding amplified bands frommt he following clones namely PB 235, PB 311, PB 312, PB 314 and KRS 25 but not with other clones. In addition, genomic DNA was digested with Hind III restriction enzyme and the selected polymorphic band was used as probe for hybridization. The southern results confirmed that the amplified polymorphic band is a part of genomic DNA and nnot any artifact or contamination Plates 9 (b).

The dendrogram based on RAPD showed that 13 clones were clustered into two major groups. Group I comprised nine PB clones along with RRII 105 and group II included three KRS clones. These major clusters were further divided into four sub groups. It was observed that all the Malaysian and Thailand clones were clustered into separate groups. The phenogram showed that the popular clone RRII 105 (India) is separated from the other clones with a genetic distance of 44.84 per cent, since both of its parents are different from the others. It is interesting to note that most of the Malaysian clones have been put into two clusters. Three clones belonging to Thailand have been put into the same cluster. The first major groups were further separated into three sub groups. In this PB 255, PB 235, PB 280, PB 260 and PB 217 were isolated into one group. PB 310, PB 311, PB 314 and PB 312 were grouped as another set.

RAPD analysis clearly distinguished all the 13 *Hevea* clones from each other. KRS clones are clustered together to a major group (Fig. 14). KRS 25, KRS 128 and KRS 163 are

grouped together as they were separated from the remaining clones with 30.80 per cent dissimilarity. The clones PB 312 and PB 314 were grouped together with a maximum dissimilarity of 15.20 per cent followed by PB 280, PB 260, and PB 217. The two clones PB 312 and PB 314 appear genetically very close since both clones originated from the same series. In the present study 59.60 per cent of the RAPD were polymorphic among 13 cultivated clones of *Hevea* studied. Table 13 shows the dissimilarity matrix developed on the basis of RAPD data. The Indian clone RRII 105 separated from the other clones originated from Malaysia and Thailand, since pedigree of RRII 105 is different from all other cultivars. It could be observed that all the Malaysian clones are clustered into a single major group since pedigree of them are common for at least one of it's parent. Among this major cluster two sub groups could be identified. The two clones PB 312 and PB 314 are found to be genetically very close and are separated by 15.20 per cent dissimilarity from the other varieties. The highest dissimilarity was noticed between KRS 163 and RRII 105 which is represented by a dissimilarity index of 59.60 per cent followed by the clone PB255. In most cases clones with a common pedigree such as PB 312, PB 314, PB 311 and PB 310 were clustered together. In PB 217, PB 260, PB 280, PB 235 and PB 255, one of the parents in their ancestory are common could be clustered together to form a single group. Phenetic relationship among KRS clones was close which is depicted in the dendrogram based on the RAPD data. Thailand clones were clustered together and were separated from the Malaysian and Indian clones.

Table 12. Number of amplification products generated and the nucleotide sequence of RAPD primers that showed DNA polymorphism with the 8 random oligonucleotide primers in 13 selected *Hevea* clones.

Primer code	Primer Sequence (5' — 3')	Number of bands	
		Total	Polymorphic
OPA-01	CAGGCCCTTC	16	7
OPA-04	AATCGGGCTG	21	7
OPA-07	GAAACGGGTG	19	9
OPA-16	AGCCAGCGAA	13	8
OPA-17	GACCGCTTGT	15	11
OPA-18	AGGTGACCGT	16	7
OPB-12	CCTTGACGCA	14	9
OPC-05	GATGACCGCC	18	10
	Total	132	68

Table 13. Jaccard's dissimilarity matrix of the clones based on RAPD

	RRII 105	PB 312	PB 314	KRS 25	KRS 128	KRS 163	PB 217	PB 235	PB 255	PB 260	PB 280	PB 310	PB 311
RRII 105		49.00	45.80	47.7 0	37.5 0	59.6 0	36.4 0	40.00	45.50	47.90	45.70	39.50	43.50
PB 312			15.20	40.80	41.70	44.40	40.40	40.40	45.10	41.50	42.30	33.30	30.60
PB 314				43.80	41.30	52.70	43.10	43.10	44.90	44.20	42.00	32.60	29.80
KRS 25					30.80	35.60	47.90	44.70	53.20	52.00	46.80	47.80	44.70
KRS 128						46.80	48.90	45.70	51.10	50.00	47.80	41.90	45.70
KRS 163							50.90	50.90	55.80	46.20	47.10	51.00	45.10
PB 217								30.40	35.60	20.50	20.90	33.30	34.00
PB 235									31.80	31.90	32.60	40.40	34.00
PB 255										40.40	30.20	42.20	31.80
PB 260											18.60	31.10	38.80
PB 280												35.60	32.60
PB 310													40.40
PB 311													

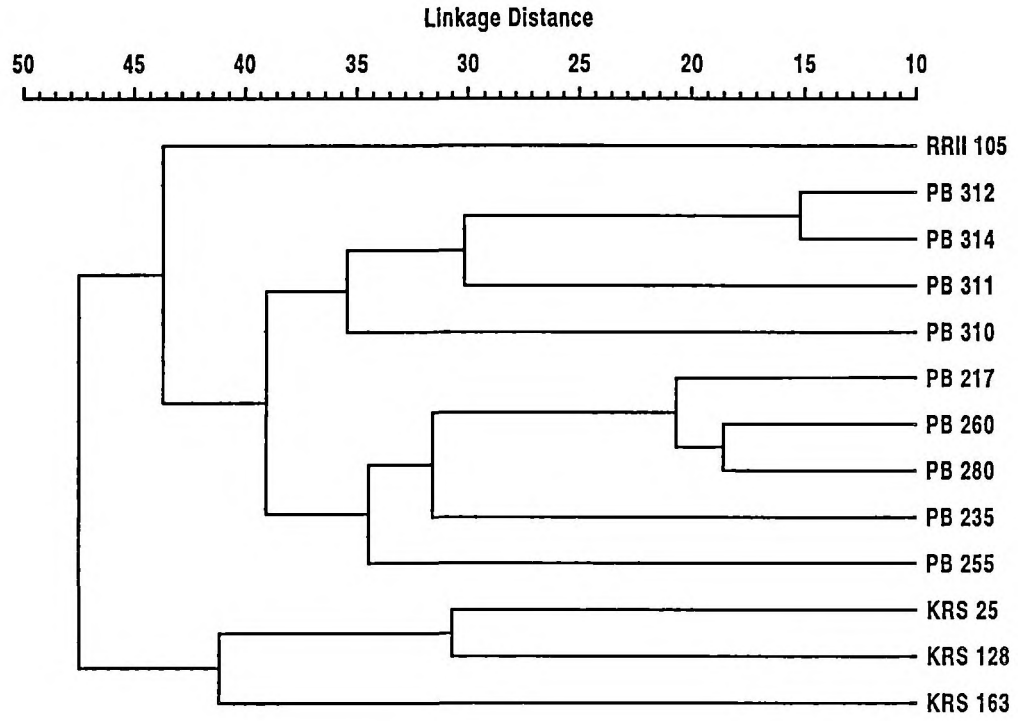


Fig 14. UPGMA clustering pattern of the clones based on Jaccard's dissimilarity coefficients using RAPD.

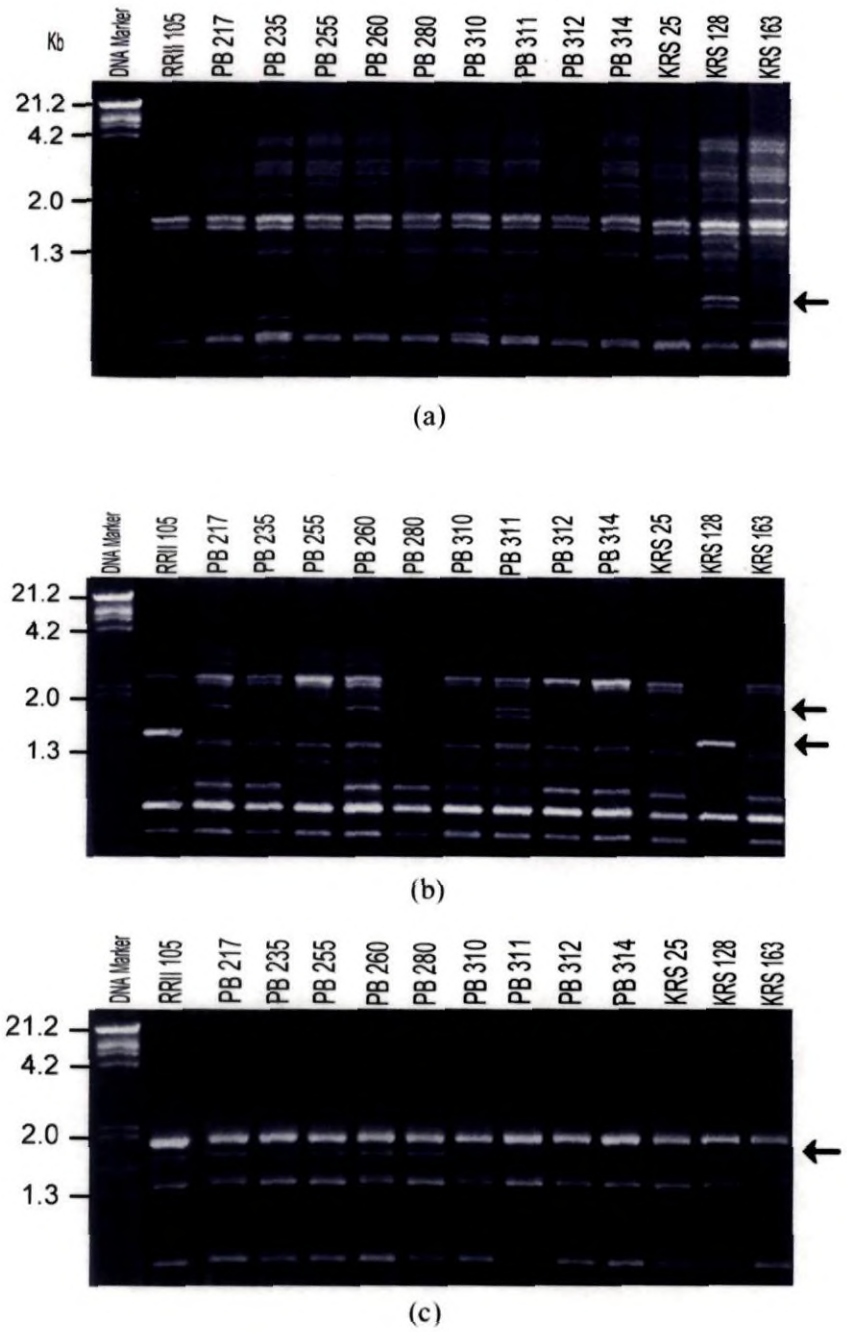


Plate 7. (a) RAPD profile generated by OPA-04 oligonucleotide primers in 13 *Hevea* clones showing DNA polymorphism (see arrow).

(b) RAPD profile generated by OPC-05 oligonucleotide primers in 13 *Hevea* clones showing DNA polymorphism (see arrow).

(c) RAPD profile generated by OPA-07 oligonucleotide primers in 13 *Hevea* clones showing DNA polymorphism (see arrow).

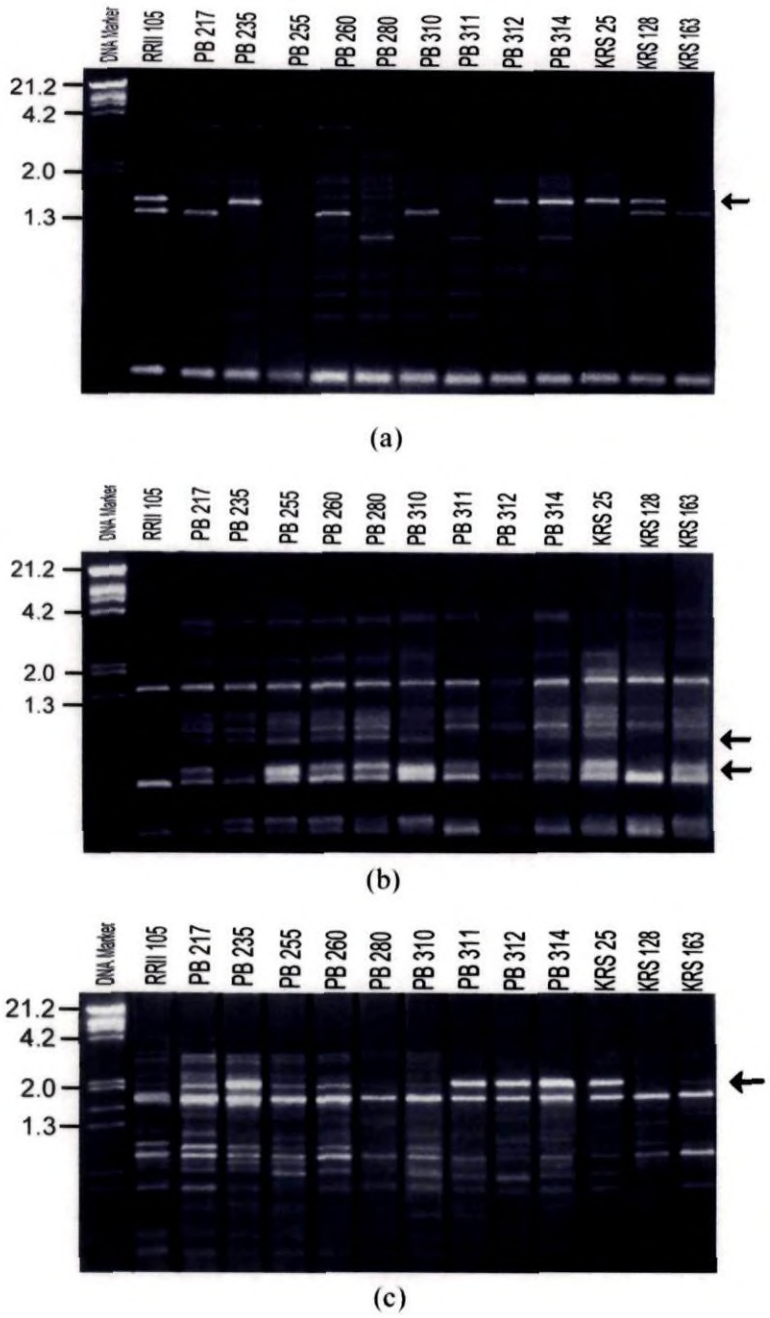


Plate 8. (a) RAPD profile generated by OPA-12 oligonucleotide primers in 13 *Hevea* clones showing DNA polymorphism (see arrow).

(b) RAPD profile generated by OPA-16 oligonucleotide primers in 13 *Hevea* clones showing DNA polymorphism (see arrow).

(c) RAPD profile generated by OPA-17 oligonucleotide primers in 13 *Hevea* clones showing DNA polymorphism (see arrow).

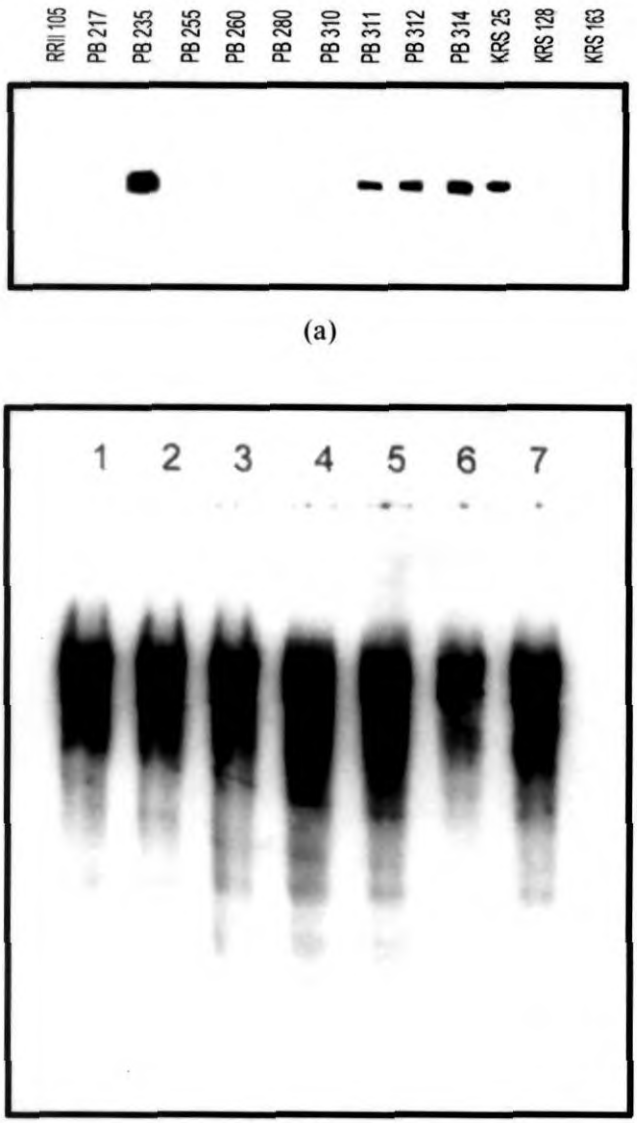


Plate 9. (a) The polymorphic band (2.0 kb) is arrowed. The arrrowed band used to probe a southern blot of amplified DNA fragments generated by primer OPA-17. Hybridization of band is arrowed.

(b) Genomic Southern blot hybridization analaysis of *Hevea* clones; The radio labelled probe used was a selected polymorphic RAPD band (2.0 kb) identified with OPA-17 primer. Lane 1 - 7 genomic DNA samples from different *Hevea* clones digested with Hind III restriction enzyme.

4.7. Performance index

Performance index was worked out for the 13 clones based on yield and major yield components *viz.*, girth, latex flow rate, plugging index, dry rubber content, virgin bark thickness and number of latex vessel rows in the virgin bark. The clones are ranked based on their performance and are given in Table 14. Five genotypes were found to have an index value greater than the mean index value of 284.24 and these clones were PB 280, PB 255, KRS 128, RRII 105 and KRS 163. The Indian clone RRII 105 ranked fourth among the clones studied and three clones *viz.*, PB 280, PB 255 and KRS 128, indicated better performance than RRII 105 based on yield and major yield components.

Table 14. Performance index of the clones based on yield and associated characters

Clone	Index	Rank
PB 280	325.6	1
PB 255	317.5	2
KRS 128	301.9	3
RRII 105	294.9	4
KRS 163	293.5	5
PB 260	283.4	6
PB 314	282.6	7
KRS 25	281.4	8
PB 235	280.0	9
PB 312	267.4	10
PB 311	267.1	11
PB 310	251.7	12
PB 217	248.1	13
Mean	284.24	

4.8. Progeny analysis

The mean values for height, girth and number of whorls of leaves for the progenies during the first year are presented in Table 15. Analysis of variance indicated that there is no significant variation among seedling progenies with respect to height and girth at one year after planting. The mean values for girth ranged from 2.22 cm for RRII 105 to 3.20 cm for PB 255 with a general mean of 2.69 cm. In case of height of progenies during the first year after growth, the mean value ranged from 73.64 cm for RRII 105 to 116.70 cm for PB 217. The general mean was 93.85 cm. The range of mean values for the number of whorls was from 2.78 (RRII 105) to 4.30 (PB 312).

Performance of the superior and inferior progenies of the clones *viz.*, PB 255 and PB 217 is visible Plate 10 a and b. Mean values for yield, height, girth and number of whorls of leaves for the progenies of 13 clones at the age of two years are depicted in Table 16. Highly significant variation could be observed for the traits studied at the age of two year of planting (Table 17). Juvenile yield ranged from 2.13 g plant^{-1} 10 t^{-1} (KRS 163) to 6.24 g plant^{-1} 10 t^{-1} (PB 311) while the progenies of RRII 105 recorded 4.20 g plant^{-1} 10 t^{-1}. The mean progeny yield was recorded was 4.14 g plant^{-1} 10 t^{-1}. Progenies of clones *viz.*, PB 235, PB 255, PB 260, PB 311, PB 312, PB 314 and RRII 105 exhibited above mean progeny yield (4.14 g plant^{-1} 10 t^{-1}). The highest girth was recorded for PB311 (10.07 cm) whereas, PB 235 exhibited the lowest value of 4.90 cm with the general mean was 7.67 cm. Progenies of RRII 105 recorded 8.63 cm of girth. Height of the progenies varied from 2.30 m. for PB 217 to 3.66 m. for PB 255 with a general mean of 3.11 m. The number of whorls of leaves showed a range of values from 10.42 for PB 310 to 8.17 for PB 314. The general mean was 9.08.

Correlations among plant height, girth, number of leaf flushes produced and juvenile yield at the age of 2 years are given in Table 18. Significant positive correlation was observed for all the traits studied. The highest correlation observed between juvenile yield and the vigour of seedlings in terms of height (r = 0.669) followed by girth (r = 0.578) while the lowest relationship was established between yield and number of leaf whorls produced, the correlation was only at 5 per cent level (r = 0.315) and between girth and the number of leaf whorls (r = 0.344). Correlation between seedling girth, and height was also significantly high at one per cent level (r = 0.441). The relationship between seedling height and number of whorls were also very high at 1per cent level (r = 0.557). To assess the performance of progenies, performance indices were estimated based on morphological traits and juvenile yield. Performance indices ranged from 88.46 (PB 217) to 125.57 (PB 311) with a general mean of 105.62. The performance indices of the progenies of the 13 clones are given in Table 19. Based on the performance indices, four clones exhibited highest ranks above RRII 105 (112.46), while progenies of the clones *viz.*, PB 255, PB 260, PB 310, PB 311, PB 314 and RRII 105 showed the mean value of above 105.62. The progenies of PB 311 and PB 255 exhibited highest index value of 125.57 and 124.24 respectively.

The percentage of seedling which exhibited progeny yield above the mean yield are represented in the Table 19. Thirty per cent to sixty eight per cent of progenies showed above average progeny yield. Progenies of clone PB 255 recorded highest (68 %) and that of PB 280 (30.23 %) recovered the lowest percentage of superior seedlings in terms of juvenile yield. The progenies of PB 255, PB 260, PB 310, PB 311, PB 312, PB 314 and RRII 105 recorded high percentage of seedlings with above mean progeny yield.

Table 15. Performance of progenies during the first year of establishment

Progeny	Height (cm)	Girth (cm)	Number of whorls (m)
PB 217	116.78 a	3.10 ab	3.53 bc
PB 235	100.29 abc	2.72 abcd	3.67 abc
PB 255	102.67 ab	3.20 a	3.82 ab
PB 260	99.58 abc	2.70 abcd	3.60 bc
PB 280	112.60 ab	2.99 abc	3.16 bcd
PB 310	109.84 ab	2.95 abc	3.70 abc
PB 311	103.88 ab	2.60 bcd	3.52 bc
PB 312	103.69 ab	2.57 bcd	4.30 a
PB 314	86.79 bc	2.54 bcd	3.47 bc
KRS 25	87.11 bc	2.52 bcd	3.04 cd
KRS 128	87.35 bc	2.45 cd	3.47 bc
KRS 163	94.39 abc	2.48 cd	3.61 bc
RRII 105	73.64 c	2.22 d	2.78 d
General Mean	98.35	2.69	3.51
C.V. (%)	16.58	12.87	11.40
C.D. (0.05)	27.43	0.58	0.65

Means followed by the same letters are not significantly different at 5 % error

Table 16. Performance of progenies during the second year of establishment

Progeny	Yield (g plant^{-1} 10 t^{-1})	Girth (cm)	Height (m)	Number of whorls
PB 217	3.52 cde	6.70 e	2.30 e	8.28 c
PB 235	4.50 abcd	4.90 f	3.11 bcd	9.49 ab
PB 255	5.81 ab	9.57 a	3.66 a	9.35 b
PB 260	4.23 bcd	8.17 b	3.49 abc	9.80 ab
PB 280	3.36 cde	6.70 e	3.03 cd	8.23 c
PB 310	4.11 bcd	8.23 b	3.41 abc	10.42 a
PB 311	6.24 a	10.07 a	3.40 abc	9.77 ab
PB 312	4.18 bcd	7.10 de	3.28 abc	9.00 bc
PB 314	4.89 abc	7.43 cd	3.57 ab	8.17 c
KRS 25	2.71 de	7.00 de	2.40 e	8.21 c
KRS 128	3.58 cde	8.13 bc	2.68 de	8.24 c
KRS 163	2.13 e	7.13 de	3.03 cd	9.64 ab
RRII 105	4.60 abcd	8.63 b	3.10 bcd	9.50 ab
General Mean	4.14	7.67	3.11	9.08
C.V.(%)	27.96	5.53	9.65	6.61
C.D. (0.05)	1.95	0.71	0.50	1.01

Means followed by the same letters are not significantly different at 5 % error

Table 17. ANOVA for juvenile traits during the second year of establishment

Characters	Range	Mean	F value	Significant
Juvenile yield	2.13-6.24	4.14	2.90	**
Girth	4.90-0.07	7.67	30.29	**
Height	2.30-3.66	3.11	5.95	**
Number of whorls	8.17-10.42	9.08	4.93	**

**Significant at P = 0.01

Table 18. Correlations among juvenile yield and growth characters

	YIELD	GIRTH	HEIGHT
GIRTH	0.579 **		
HEIGHT	0.669 **	0.441 **	
WHORLS	0.315 *	0.344 *	0.557 **

Table 19. Performance index and percentage of superior progenies after second year of growth

Progeny	Performance Index	% of seedlings recorded above mean progeny yield
PB 217	88.46	43.57
PB 235	91.59	40.00
PB 255	124.24	68.00
PB 260	114.55	58.00
PB 280	96.28	30.23
PB 310	115.70	61.11
PB 311	125.57	60.00
PB 312	104.07	56.00
PB 314	107.37	60.00
KRS 25	90.43	31.60
KRS 128	100.58	43.48
KRS 163	101.77	33.30
RRII 105	112.46	54.50
Mean	105.62	49.22

(a)

(b)

Plate 10. Seedling progenies of the clones (a) PB 255 and (b) PB 217

Chapter 5

DISCUSSION

DISCUSSION

5.1. Analysis of Variance

5.1.1. Performance of Clones

Analysis of variance revealed highly significant clonal variation for all the characters studied except for girth increment rate under tapping. The results showed that genetic variation exists in the population which would enhance selection programme wherein selection pressure can be profitably exerted on these characters. The result of the present study is supported by the findings of earlier workers like Mydin (1992), Premakumari (1992), Licy (1997), Licy *et al.*, (2002) and John *et al.*, (2003). Eight clones recorded above average annual yield and these clones were identified as high yielding clones. Of these seven clones yielded significantly higher than that of the high yielding clone RRII 105. Among these clones, PB 255 recorded the highest mean yield of 73.52 g t^{-1} t^{-1} over the three years of tapping followed by PB 314 (66.88 g t^{-1} t^{-1}), PB 280 (66.81 g t^{-1} t^{-1}), PB 260 (63.20 g t^{-1} t^{-1}), KRS 163 (62.96 g t^{-1} t^{-1}), PB 312 (62.13 g t^{-1} t^{-1}), PB 311 (60.33 g t^{-1} t^{-1}), PB 235 (57.45 g t^{-1} t^{-1}), KRS 128 (51.78 g t^{-1} t^{-1}), RRII 105 (49.50 g t^{-1} t^{-1}). Nga and Subramaniam (1974) and Gilbert *et al.*, (1973) have also reported a high genetic variability for yield, which is in agreement with the result of the present study.

H. brasiliensis is a perennial tree crop and shed their leaves annually known as v n as 'wintering'. In South India 'wintering' usually takes place during December to February and by that time all the leaves are shedded. It has been observed that the productivity of the trees dropped down during this period. Dijkman (1951), Wimalaratna and Pathiratna (1974) and Sethuraj (1977) reported the drop in yield soon after wintering. Latex yield is observed

to be low during the dry season of February to May, when the soil water availability is least and the rainfall is about zero. Mass and Bokma (1950), Polhamus (1962), Ninane (1967) and Edgar (1987) reported that summer months are lean in terms of crop production. All the clones have shown reduction in yield during stress period. Highly significant clonal variation was recorded in the yield drop in present study. Yield depression during stress was comparatively less for PB 255 followed by PB 310, PB 314, PB 312, PB 311, PB 217 and KRS 128 and were considered in having stability in yield whereas, PB 235 followed by KRS 163 and PB 280 showed high yield depression during stress. Webster and Paardekooper (1989) reported marked variation among clones in yield depression during the period of refoliation. The result of the present study is in conformity with the reports of the earlier workers.

Ranges of variation was high for yield and all yield components, however panel length, girth increment on tapping, plugging index and bark thickness exhibited relatively low values. Saraswathyamma and Sethuraj (1975) and Licy (1997) reported low values for latex flow characters which is in agreement with the results of present study. Six clones recorded high girth of above 60 cm. The highest girth was recorded for PB 255 (65.99 cm) followed by PB 235 (65.27 cm), PB 280 (64.74 cm), PB 310 (62.36 cm), PB 314 (60.95 cm) and PB 312 (60.61 cm). Highly significant clonal variation was recorded for all the components of yield. It implies that genetic variation exist in the population and there is scope for selection based on these characters. There are four clones *viz.*, PB 255, PB 280, PB 312 and PB 314 showing high vigour in terms of bole girth and high yield and were identified as latex timber clones. All these clones exhibited trunk girth above 60 cm and yield above 60 g t^{-1} t^{-1}. Latex-timber clones are gaining importance in the changing global scenario where rubber wood is viewed as an alternate source of timber.

5.1.2. Latex and rubber properties

Important properties like pH, total solid content, non rubber substances, ash, gel, acetone extract, nitrogen, Mooney viscosity, plasticity and plasticity retention index, which relate to the qualities of latex and rubber were studied for 13 clones of *Hevea brasiliensis.* The impact of clonal variations on these parameters was observed to be significant. A major source of variability within and between NR grades is probably is dependance of the property on the clones from which the latex is collected (Fuller, 1988). Different clones have different characteristic and give rubber with different properties. The colour and composition of the latex and the plasticity of the rubber tend to be uniform within a clone and different for different clones (Martin, 1961).

The latex pH of the clones showed that the clone KRS 163 recorded highest pH value followed by PB 314, PB 260, PB 280 and PB 311. Clone RRII 105 exhibited neutral latex pH (7.03). Brozozowska *et al.,* (1979) and Coupe and Lambert, (1977) reported rubber production has been shown to be correlated positively with cytosolic pH and transtonoplastic pH gradient (cytosol-lutoids) in the latex. Total solid content is the total fraction of solid particles in natural rubber including dry rubber content. The highest mean value for total solid content recorded for KRS 128 followed by RRII 105, PB 255, PB 280 and PB 235. Viscosity of latex depends on the total solids present. A high total solid content may limit yield by hindering flow (Milford *et al.,* 1969; Buttery and Boatman, 1976 and Brozozowska *et al.,* 1979). On other hand low total solid contents is indication of weak latex regeneration in *situ* (Eschback *et al.,* 1984; Prevot *et al.,* 1984). The non-rubber constituents in natural rubber have a profound effect on the vulcanization of the hydrocarbon and the physical properties of the resulting vulcanizates. The include proteins, acids, ash and water (Bengtsson and Stenberg, 1996).

These impurities influence the rubber in different ways. The soluble non rubber materials influence mainly the time variation of the relaxation modulus of row NR at longer relaxation time the increases rate of relaxation quite markedly (Campbell and Fuller, 1984). Clone KRS 25 showed highest non rubber substances (3.53 %) followed by PB 255 (3.48 %), PB 311 (3.29 %), KRS 128 (3.29 %) and PB 310 (3.24 %). Clone RRII 105 exhibited 2.95 per cent of NRS.

The ash content represents the amount of mineral matter present in the rubber, such as carbonates and phosphates of potassium, magnesium, calcium, sodium and other trace elements. The highest ash content was noticed for PB 312 (0.26 %) whereas, the lowest for PB 260 and KRS 163 (0.14 %). A high ash content in rubber could also result from contamination during latex collection or processing. Copper and manganese are the two trace elements in ash. These materials are very powerful catalysts for the oxidation of NR by oxygen in the air (Cole, 1958). The presence of inorganic compounds can increase the tendency of vulcanized articles to swell in water (Stagracznski and Kunst, 1993).

Gel is the insoluble fraction of the material when the rubber is dissolved in the solvent. Two types of gel exist in NR, micro gel and macro gel. Micro gel consists of sub micron size particles, which are cross-linked latex particles. Macro gel appears to be a secondary bonded network incorporating micro gel and most of the proteinaceous materials (Allen and Bristow, 1963; Fuller, 1988). Clone PB 255 exhibited highest gel content (22.87 %) followed by RRII 105 (16.66 %) and PB 311 (14.22 %). However, KRS 163 recorded lowest value 4.43 per cent. The gel has a marked influence on the relaxation behaviour of NR. It has predominantly stiffening effect. It produces a comparatively slight decreases in the rate of relaxation. The macrogel phase is an extremely stiff, almost non relaxing material. The

macrogel particles also have a stiffening effect, but they also significantly reduce the rate of relaxation (Campbell and Fuller, 1984).

Acetone extract was highest for PB 260 (4.17 %) followed by PB 217 (4.08 %), KRS 163 (4.07 %) and PB 235 (3.96 %). However, RRII 105 showed 2.80 per cent of acetone extract. Esah (1990) reported that is property has not be extensively studied. It has been shown to increase after yield stimulation using 2, 4, 5 - trichlorophenoxyacetic acid (2, 4, 5 - T) and to decrease with the age of a tree (Moris and Sekhar, 1959). The acetone extract of NR contains naturally occurring non- rubber constituents such as lipids, fatty acids, quebrachitol, sterols and esters. In addition, acetone will extract the degraded rubber, if the rubber has been exposed to oxidative influences such as strong sun light (Rubber Research Institute of Malaysia, 1992). Lipids are responsible for the stability of the rubber particles (Ho *et al.*, 1976). The sterols and esters are believed to contain the antioxidant which is effective in preserving the raw rubber against oxidation and softening during storage (Bengtsson and Stenberg, 1996). Fatty acids influence strongly the rate of vulcanization with certain accelerator system (Ebi and Kolawole, 1992). Generally acetone extract varies between 2 to 5 per cent in dry rubber (Esah, 1990) and for all the clones, the values are with in the limit.

It is known that NR when it leaves the tree contains definite proportion of nitrogen as integral part of macro molecule (Bengtsson and Stenberg, 1996). Clone PB 312 registered highest value for nitrogen content (0.49 %) whereas, RRII 105 showed 0.42 per cent for nitrogen content. The higest nitrogen for PB 312 was followed by PB 311 (0.48 %), PB 314 (0.48 %) and PB 310 (0.45 %). The nitrogen content of dry rubber is reported to be the proteinaceous material either tenaciously held or chemically bonded to the rubber (Burfield *et al.*, 1976). Tata 1980 reported that about 30 per cent of these materials are present in the rubber

hydrocarbon and about 70 per cent in the non rubber phase. Most of these have been shown to play an important role in the stability of *Hevea* latex. Certain proteinaceous materials had been shown to exert various effects on the technological properties of rubber (Alias and Hasma, 1988).

Mooney viscosity gives an indication of the quantum of mechanical work required on the raw rubber to give mixes with consistent rheological properties after standard mastication, compounding and mixing. This means that a rubber with very high Mooney viscosity may require longer premastication time or need expensive peptisers to obtain a product of a workable and consistent viscosity, whereas the rubbers with comparatively low Mooney viscosity require lesser mastication (Esah, 1990). Mooney viscosity was highest for PB 255 (88.24 unit) followed by PB 217 (83.19 units), KRS 128 (82.63 unit) and RRII 105 (81.68 unit), whereas, KRS 163 exhibited lowest value (63.47 unit) for Mooney viscosity. Besides Mooney viscosity, the important property of bulk viscosity of rubber is also measured by the Wallace plasticity and the plasticity values determined for the clones. Initial Wallace plasticity was recorded highest for PB 255 (60.67) followed by PB 217 (58.11), KRS 128 (57.39) and RRII 105 (54.67).

Plasticity retention index is a measure of the resistance of rubber to molecular breakdown by heat. It is assessed by the percentage change of the original plasticity when the rubber is heated at 140^{0}C for 30 minute. High values correspond to good heat resistance. Clone PB 280 exhibited highest PRI value (88.11 %) followed by KRS 163 (86.89 %), PB 260 (86.44 %) and KRS 25 (85.61 %). RRII 105 exhibited 81.89 per cent for PRI.

In terms of plasticity, most of the clones gave medium to hard rubbers. Clone RRII 105 could be graded to the higher viscosity range. KRS 163 had the lowest P_0, Mooney

viscosity and gel content, where as the highest values were observed for clone PB 255. Ash content and plasticity retention index had lesser effect on clone. The data generated could provide a comparative assessment of the latices of clones, though some variations could be expected on changing the soil and environmental conditions.

Hevea latex as obtained from the tree consists not only of rubber hydrocarbon particles, but also non-rubber substances, which include lipids, proteins, carbohydrates, acids, amines and some inorganic constituents. It is generally known that some of these non rubbers can affect the properties of latex concentrates and bulk rubber derived from the field latex. As all the rubbers were prepared in the same manner using the same procedure, any variation observed in the properties studied could be considered as mainly due to differences between the clones.

5.1.3. Association of dry rubber yield with latex and rubber properties

Among the various latex and rubber properties viz., pH, total solid content (TSC), non-rubber substances (NRS), acetone extract (AE), nitrogen content (N_2), gel content (GC), mooney viscosity (MV), initial plasticity (P_0), plasticity retention index (PRI) and ash content (AC) of *Hevea* studied, pH of latex indicated significant correlation with rubber yield (0.583). It shows that pH of latex may have the influence on the rubber yield of *Hevea*. pH, TSC, GC and PRI showed positive association with rubber yield, whereas, NRS, AE, N_2, MV, P_0 and AC registered a negative association with yield. The highest correlation of rubber yield with pH of latex is followed by yield × ash content (-0.348), yield × PRI (0.271), yield × TSC (0.266), yield × P_0 (-0.251) and yield × MV (-0.238) was noticed. One of the most important factors affecting the coagulation of latex is its pH value. Rubber production has been correlated positively with the cytosolic pH of the latex and the transtonoplastic (cytosol/lutoids) pH gradient

(Brzozowska – Hanower *et al.*, 1979). The result of the present study is in conformity with the findings of Chrestin and Gidrol (1985) indicated that the positive correlation between rubber production and cytosolic pH of the latex and the transtonoplastic (Cytosol/lutoids) pH gradient is due to the reactivity of Mg dependent AT pase. The pH showed apparent correlation with yield, if it is confirmed to be of general occurrence it will be of importance in explaining the mechanism of yielding rubber in *Hevea.*

5.2. Genetic parameters

Highly significant clonal variation was recorded for all the yield components studied. Wide range of variations was recorded for girth at opening (50.96-61.33 cm.), annual dry rubber yield (38.17 - 73.52 g t^{-1} t^{-1}), rubber yield in stress season (26.29 - 52.74 g t^{-1} t^{-1}) and peak season (44.75 - 80.42 g t^{-1} t^{-1}), yield depression during stress (27.99 % - 49.87 %), latex yield (107.60 ml t^{-1} t^{-1} - 176.85 ml t^{-1} t^{-1}), latex yield during stress (58.31 ml t^{-1} t^{-1} - 115.26 ml t^{-1} t^{-1}) and peak season (117.27 ml t^{-1} t^{-1} - 193.17 ml t^{-1} t^{-1}), latex vessel rows in virgin (16.68 - 28.64) and renewed bark (14.20 - 30.96). However, low range of mean was recorded for girth increment, rubber content, in different seasons and also for bark thickness.

5.2.1. Phenotypic and genotypic coefficient of variation

The present study indicated substantial differences in phenotypic and genotypic coefficient of variation for the characters studied. The highest value of phenotypic coefficient of variation (PCV) was exhibited by mean girth increment (32.56 %) followed by latex yield during stress period (26.95 %), latex vessel rows in renewed bark (26.94 %), rubber yield in stress period (26.26 %) and yield depression under stress (26.01 %), rubber yield in peak period (22.91 %), annual rubber yield (21.60 %), latex yield in peak period (20.96 %), latex vessel rows in virgin bark (20.92 %) and annual latex yield (20.61 %). Girth at opening and

rubber content showed lowest phenotypic coefficient of variation. Moderate PCV was recorded for virgin and renewed bark thickness among the variables studied.

The highest GCV was observed for yield depression under stress (20.84 %) followed by dry rubber yield in stress season (20.27 %), latex vessel in renewed bark (20.19 %), latex yield in stress period (20.09 %), rubber yield in peak period (18.52 %) and annual rubber yield (16.98 %). Low genotypic coefficient of variation was observed for girth at opening, annual rubber content and rubber content in different seasons whereas, virgin and renewed bark thickness, girth increment, latex vessel rows in virgin bark and annual latex yield showed moderate GCV among the characters. Genotypic coefficient of variation (GCV) for yield and yield components showed the existence of substantial genetic variability among the clones studied.

The high value of GCV observed for rubber yield is in agreement with the reports of Whitby (1919), Simmonds (1969), Gilbert *et al.*, (1973), Nga and Subramaniam (1974), Markose and George (1980), and Hamazh and Gomez (1982). Licy (1997) reported a high degree of GCV and PCV for dry rubber yield, latex yield and number of latex vessel rows as observed in the present study. Premakumari (1992) reported a moderate GCV and PCV for rubber yield, latex yield and low values for girth and rubber content, which are in conformity with the present study.

GCV was lower than PCV for all the characters studied. However, the magnitude of difference between GCV and PCV estimate was high for girth increment on tapping, indicating environmental factors influencing the character. It was low for all other characters indicating that genetic factors were predominantly responsible for these characters. Markose (1984) and Mydin (1992) reported genotypic coefficient of variation was lower than the phenotypic

coefficient of variation for all the characters studied which indicates the influence of environment on the genotype in the expression of these characters. Moderate to high GCV and PCV was observed for girth increment on tapping, annual dry rubber yield, dry rubber yield in two seasons, yield depression during stress, annual latex yield and latex yield in two seasons, virgin and renewed bark thickness and latex vessel rows in virgin and renewed bark indicating that selection based on these characters would be advantageous, since there is the predominance of additive gene action in the expression these characters. Low value of genotypic coefficient of variation and phenotypic coefficient of variation for girth and rubber content are in conformity with the findings of Markose (1984). Alika and Onokpise (1982), Mydin (1992), Chandrasekar *et al.* (1995) and Licy (1997), reported low GCV and PCV for girth.

5.2.2. Heritability

Heritability is the proportion of the total variance of an observable characteristic that may be accounted for by genetic factors. It can also be stated as the fraction of total phenotypic variance that remains after exclusion of the variance due to environmental effects. Burton (1952) suggested that genotypic coefficient of variation together with heritability estimates would give a better idea of selection advance to be expected. Selection acts on genetic differences and gains from selection for a specific character depends largely on heritability of the character (Allard, 1960 and 1999). A high heritability expressed by a high value of above 60 per cent was observed for most of the characters. Broad sense heritability was highest for annual rubber content (71.39 %) followed by rubber content during peak period (69.50 %), rubber yield in peak period (65.00 %), rubber content during stress period (64.55 %), yield depression during stress (64.15 %) and annual rubber yield (61.77 %)

indicating that observed variability for these traits are heritable. Breeder can expect additive genetic variance to be available for selection in progeny generation for these characters. Simmonds (1989) explains that in rubber, heritability of economic characters are high. Low heritability was observed for girth at opening and girth increment rate on tapping. Low heritability coupled with low GCV for girth at opening confirms that marked improvement may not be achievable in such characters through selection. The relatively high value of heritability observed for the major yield components explains large proportion of variability observed for these characters is heritable with negligible influence of environment. The low heritability observed for girth may be due to that the observations were conducted during the early years of production phase of the plants. Liang *et al.*, (1980), Alika (1982) reported similar findings. The moderate heritability observed for virigin bark thickness and renewed bark thickness are in agreement with Tan *et al.*, (1975), and Alika and Onokpise (1982). Relatively high heritability for yield and girth was reported by Nga and Subramaniam (1974), Liang *et al.*, (1980), Markose and George (1980) and Markose (1984). However, Mydin (1992) and Licy (1997) reported low heritability for girth which was in agreement with the results of the present study. In general, high heritability for rubber content, latex yield, and annual dry rubber yield indicates that observed variability for the trait is often heritable.

5.2.3. Genetic advance

Genetic advance is the improvement in the mean genotypic value of selected families over the base population. Genetic advance under selection depends up on several factors like 1) the genetic variability among different plants or families in the base population 2) the heritability of character under selection and 3) the intensity of selection i.e., the population of plants or families selected. High genetic advance recorded for yield

depression during stress (34.37 %), dry rubber yield in stress season (32.23 %), latex vessel rows in renewed bark (31.21 %), latex yield in stress period (30.85 %), dry rubber yield in peak period (30.76 %), whereas, annual dry rubber yield (27.49 %), latex yield in peak seasons (25.56 %), annual latex yield (22.09 %), latex vessel rows in renewed bark (19.57 %), virgin bark thickness (18.57 %), registered moderate genetic advance under selection. Renewed bark thickness (14.37 %), rubber content in peak season (13.30 %), rubber content in stress period (10.94 %), girth increment on tapping (9.58 %) and girth at opening (6.41 %) showed genetic advance under selection. In the present study, moderate to high heritability associated with high genetic advance for yield depression during stress, rubber yield in stress period, latex vessel rows in renewed bark, latex yield in stress period and rubber yield during peak season have been observed, which indicated additive gene action in the inheritance of these traits and implies scope for improvement of these traits through selection. Ramanujam and Thirumalachar (1967) opined that broad sense heritability accompanied by high genetic advance is more reliable. Mydin (1992) reported a low to moderate genetic advance for renewed bark thickness, virgin bark thickness, rubber content in peak period, annual rubber content, latex yield during peak period, annual latex yield and rubber yield during peak season which are in conformity with the present study. Licy (1997) reported low genetic advance for rubber content, renewed bark thickness, virgin bark thickness, and number of latex vessel rows in virgin bark. Virgin bark thickness, annual latex yield and latex yield in peak season showed moderate heritability associated with moderate genetic advance indicating comparatively less influence of environment on these parameters. Girth at opening, girth increment, renewed bark thickness observed to have a low heritability estimate associated with low genetic advance under selection is due

to the high influence of the environmental factors in the expression of these traits and presume no improvement through selection. Among yield components rubber content had high heritability and low genetic advance. Since broad sense heritability includes both additive and epistatic effects, it will be reliable only when accompanied by high genetic advance. Panse (1957) indicated that moderate to high estimate of heritability with low genetic advance could be attributed to non-additive gene effects which includes epistasis and dominance.

5.3. Association of characters

Correlation coefficient analysis measures the mutual relationship between various plant characters and determines the component characters on which selection can be based for improvement in yield. In *Hevea* correlation studies provide information about the nature and magnitude of relationship between yield and its components. Correlation coefficients at both genotypic and phenotypic levels depicted that annual dry rubber yield and rubber yield in two seasons exhibited positive correlation with latex yield, flow rate of latex in all seasons, girth, girth increment rate on tapping, length of tapping panel, virgin and renewed bark thickness, number of latex vessel rows in virgin and renewed bark. Stress yield and peak yield also exhibited positive association with annual mean dry yield. The present results are in agreement with the findings of Dijkman and Ostendorf (1929), Narayanan *et al.*, (1973), Tan *et al.*, (1975), Mydin (1992) and Licy (1997). Girth and girth increment rate on tapping recorded a positive correlation with dry rubber yield in all the three seasons. Premakumari *et al.* (1989) reported high correlation between girth increment on tapping and yield increase on tapping. Dijkman, (1951), Templeton, (1969) and Sethuraj, (1985), reported a sustainable yield on tapping is highly dependant on girth increment on tapping. Narayanan *et al.*, (1974), Tan and

Subramaniam (1976), Liu (1980) reported a positive correlation of yield with girth while Wycherley (1969), Markose (1984), Premakumari (1992) and Abraham (2000) indicated a negative correlation between girth and yield. Ho *et al.*, (1973) and Ho (1976) reported a positive association of yield with girth in early years with gradual decreases in subsequent years of tapping and assumed that girth has a lesser importance in determining yield. Their assumption is that plant assimilates are partitioned in favour of latex formation rather than growth, especially in the case of high yielding clones leading to a negative association of girth and girth increment with rubber yield. The results of the present study indicated a positive association of girth and girth increment on tapping may be due to the early years of yielding of the trees.

Genotypic correlation coefficient were in general, was higher than the phenotypic correlation coefficients for the majority of characters studied. It could be the masking or modifying effect of the environment in genetic associations between characters (Johnson *et al.*, 1955, Oraon *et al.*, 1977) but it is explained that this could occur when genes governing two traits are similar but the environmental factors pertaining to the expression of the trait have a small effect. Information on the relationship of yield and the components controlling yield has an immense value in the crop improvement programme. Coefficient of correlation indicates the relationship between two variables however it provides no information regarding the extent of change in one variable resulting from change in another variable. In genetic studies it is common to find a correlation between two or more characters. Genotypic correlation between two or more characters may result from pleiotropic effects of genes or linkage of genes governing inheritance of two or more characters (Falconer, 1981 and 1989).

Sethuraj (1981) reported a relationship between yield and major yield components of rubber. According to him the yield of a rubber tree per tapping is proportional to the initial flow rate of latex, length of tapping cut, rubber content of latex and inversely proportional to the plugging index.

Among yield and yield components annual rubber yield vs. rubber yield in stress and peak period, annual latex yield, latex yield in stress and peak period and latex vessel rows in renewed bark; yield depression during stress vs. latex yield in stress and PI in stress period; annual latex yield vs. latex yield in stress and peak period, annual PI and PI in peak period; annual flow rate of latex vs. flow rate in stress and peak season, annual PI and PI in peak season; annual rubber contents vs. rubber content in stress and peak season, virgin bark thickness; annual plugging index vs. PI in stress and peak period; virgin bark thickness vs. renewed bark thickness; latex vessel rows in virgin bark vs. latex vessel rows in renewed bark; girth vs. annual rubber yield and length of tapping panel, showed high association with each other at genotypic and phenotypic level. Desirable genotypic and phenotypic level would be possible for simultaneous improvement of these characters under selection.

The relationship between pairs of characters i.e., annual rubber yield, vs. volume of latex in stress period, vs. rubber content, vs. rubber content during stress and peak season, vs. flow rate of latex showed a lower value at genotypic level than that of the phenotypic level showed the influence of environment to the expression of these traits.

Generally phenotypic and genotypic correlations varying in magnitude and not the direction. But in the present study it is revealed that rubber yield in stress season vs. rubber content in peak period; yield depression during stress vs. virgin bark thickness; annual latex yield vs. annual rubber content and rubber content during different seasons

and annual initial flow rate of latex; latex yield during stress vs. panel length and virgin bark thickness; latex yield in peak seasons vs. rubber content in peak seasons, annual initial flow rate, and plugging index in peak period; rubber content during stress period vs. initial flow rate of latex in stress seasons; rubber contents in peak seasons vs. girth increment on tapping; annual initial flow rate vs. panel length and girth increment on tapping; initial flow rate of latex during stress vs. girth increment on tapping and latex vessel rows in virgin bark; initial flow rate of latex during peak seasons vs. panel length; annual plugging index vs. panel length; PI during stress period vs. girth; girth vs. virgin bark thickness; girth increment on tapping vs. virgin and renewed bark thickness; panel length vs. latex vessels in virgin bark and renewed bark thickness indicated difference in sign at genotypic and phenotypic level. It may be due to the environmental characters through different physiological mechanism. Licy (1997) reported similar observation in a study of forty clones to estimate the correlation between different yield components.

Phenotypic and genotypic correlation exhibited a negative relationship of dry rubber yield in all three periods with plugging index during three seasons. Milford *et al.*, (1969), Sethuraj *et al.*, (1974), Mydin (1992) and Licy (1997) reported a negative correlation of plugging index with dry rubber yield. It was reported that the flow rate of latex is positively correlated to dry rubber yield (Paardekooper and Samosorn 1969; Sethuraj *et al.*, 1974). The result of the present study indicates that the correlation between dry rubber yield and initial flow rate was positive but not pronounced.

At phenotypic level, the correlation between annual flow rate of latex and latex flow in peak season, girth vs girth increment, virgin bark thickness vs. renewed bark thickness showed highest values of correlation among the major yield components studied.

It is probably due to the fact that the environmental factors have a major role in the gene governing two traits. At phenotypic level, annual plugging index vs. girth increment indicated lowest correlation.

The dry rubber yield showed high positive association with rubber yield in stress and peak period, annual latex yield, latex yield during stress and peak seasons, girth, latex vessel rows. These associations showed that yield is not totally independent and have complex association with other parameters. Among yield components all characters showed positive association with yield except plugging index. It implies that favourable phenotypic and genotypic association among characters would be possible for the simultaneous improvement of these traits.

5.4. D^2 analysis

Genetic improvement for quantitative traits depends up on the nature and amount of genetic diversity present in the base material as well as the extent to which the desirable traits are heritable. The concept of genetic divergence provides an idea about the genetic diversity among the parents and has a vital utility in determining diversity among the parents.

Heterosis breeding is an added advantage for obtaining quantum jumps in the production and productivity of *Hevea* clones. The exploitation of heterosis to raise the yield levels has been tried by several workers (Mydin, 1992, Licy, 1997). The level of heterosis as well as selection advance in segregating generations depends up on the genetic diversity among the parents rather than geographic diversity. Therefore, the choice of diverse parents with good combining ability is the prerequisite for efficient hybridization programme.

All the clones were clustered in to two major groups. The first group consisted of eight clones and that of the second one included five clones. Clustering of clones was irrespective of their country was origin. Each of the two major clusters were separated into two subgroups. The inter cluster distance between cluster I and II was 78.11. The intra cluster distance in group I was 24.65 and that of groups II was 34.14. The average intra clusters distance was 28.60. The inter cluster distance showed considerable genetic divergence exists among the clones.

In the present study, the highest genetic diversity was estimated between PB 280 and PB 312 (159.55) followed by PB 280 and PB 311 (157.43). It implies that high heterotic progenies could be obtained if these genetically most divergent parents would be used in hybridization.

5.5. Isozyme

As a perennial crop with a long breeding and maturation cycle, conventional practises for evaluation of the genetic variability like raising the F_2 progenies is rather difficult and time consuming. To supplement with, molecular markers linked to a particular trait also offer great scope for improving the efficiency of conventional plant breeding.

Isozymes offer most reliable single gene markers and they are often co-dominant in inheritance Arulsekar and Parfitt, (1986). In *Hevea* also isozymes were successfully used in estimating the genetic diversity (Chevallier, 1988; Sreelatha *et al.*, 1993; Yeang *et al.*, 1998). In *Hevea* it is used for the genetic variability studies (Chevallier, 1988) and to identify field plantings or in source bush nurseries (Leconate *et al.*, 1994). Several enzyme gene loci have been identified in *Hevea* for genetic variability studies in germplasm materials by isozyme analysis (Chevallier, 1988). Out of the nine isozyme systems

analysed, four enzymes showed polymorphism. The number of isozymes bands in different clones was ranged from 11 to 19. Out of the four isozyme systems studied, a combined total of 22 isozyme bands were scorable and a mean of 15 bands per clone could be observed. Yeang *et al.,* 1995 observed that out of the seven isozymes system a combined total of sixteen isozyme bands were consistently scorable and a mean of 11.41 bands could be scored per clone among a total of 60 *Hevea* genotypes studied. The esterase allele loci in 2^{nd}, 4^{th}, 5^{th} and 6^{th} positions are common to all the 13 clones studied and discrimination among these clones could be possible by esterase polymorphic bands at other loci. In peroxidase one band i.e., at the 7^{th} position, which is common to all the clones studied while the other locus could provide useful information to discriminate from one another. Whereas, in aspartate aminotransferase, 1^{st}, 2^{nd} and 4^{th} isozyme band positions were common to all the varieties studied, while the third locus could be possible to discriminate the cultivars. The number of bands were limited for shikimate dehydrogenase. So based on results of the present study it is clearly demonstrated that, characterization of cultivated clones of *Hevea* is possible using isozyme zymogram. Isozyme analysis clearly distinguished all the 13 clones from one another.

5.6. RAPD

The RAPD technique can generate polymorphic data more efficiently and less expensively. Most importantly, the application of RAPDs does not need any prior knowledge of genomic nucleotide sequences (Williams *et al.,* 1990). In the present study 59.60 per cent of the RAPD were polymorphic among 13 cultivated clones of *Hevea* studied. This genetic polymorphism found to be high compared to the reports of other RAPD studies in *Hevea* (Varghese *et al.,* 1997). The observed DNA polymorphism could be attributed

for the selection of clones with diverse characteristics including geographic origin, as well as to specificity of primers used in the RAPD analysis. These genotypes will be useful for developing new hybrid lines as well as mapping population for future breeding programmes.

This study indicated the presence of DNA polymorphism within the cultivated *Hevea* clones using RAPD analysis. Among the different clones tested, KRS 163 displayed the maximum and highest average genetic distance from the other clones followed by PB 255, KRS 128 and PB 314. This is supported by the view that PB clones and KRS clones are originated from divergent places and their pedigree is entirely different. This result suggests that the specified clones could be used as a potential parent in future breeding programmes.

Genetic distance estimated by RAPD markers revealed that there were three major groups among the thirteen cultivated clones of *Hevea* studied. The clones belonging to these three major groups originated geographically distinct places, as the places of origin of KRS, PB and RRII series are Thailand, Malaysia and India respectively. Similar reports were also available in *Hevea* germplasm collected from different geographic areas. The dendrogram based on RAPD data depicts that PB 312 and PB 314 are genetically close and separated by 15.2% of dissimilarity since it may be due to one of its parents common in their ancestry. Among the second group KRS 25, KRS 128 and KRS 163 are grouped together and are separated from the remaining clones with 30.8 per cent dissimilarity. The Indian clone RRII 105 is separated from the rest of the clones as the pedigree of it is different from that of other clones studied. All the Malaysian clones are clustered together as one of its parentage is common for most of these clones and this may be the fact that these clones are clustered into a single major group. The dendrogram based on RAPD analysis indicates that KRS 163 and RRII 105 are the two genetically

most divergent clones that could be utilized for the hybridization programme to raise superior progenies in future.

A comparative analysis of genetic divergence based on Mahalanobis' D^2 and RAPD analysis showed that PB 311 and PB 312 were clustered together in one group. It is interesting to observe that these clones originated from same series. Similarly PB 260, PB 217, KRS 25 and KRS 128 were clustered in one group in D^2 and RAPD analysis. It may be due to that at least one of its common parents for both clones in their ancestry. However in RAPD clustering pattern was different and all the Malaysian and Thailand clones were grouped independent of each other from RRII 105.

5.7. Performance index

The result of performance index analysis based on the pooled data of yield and major yield components indicate that the Malaysian clone PB 280 ranked first among the clones studied followed by PB 255. The Thailand clone KRS 128 positioned the third rank followed by the outstanding clone RRII 105. (Mydin, 1992) reported similar ranking based on the test tap yield, girth, number of latex vessel rows and number of flushes among 20 progenies of *Hevea.* Abraham (2000) estimated rank among 80 wild genotypes according to the performance index based on 16 different variables and 38 wild genotypes were ranked as per their performance index higher than the average index value of 247.10. Performance indices were also worked out in young Wickham clones where it was found that in general, high yielders recorded higher values in comparison to medium and low yielders (Varghese *et al.,* 1993). Planting recommendations of the Rubber Research Institute of Malaysia, for 1998 - 2000 included PB 280 and PB 260 in category I. PB 280 was under latex - timber clones. Clone PB 217 was included in category I, PB 255 in category II, PB 310, PB 311, PB 312 and PB 314 in category III

of the planting material recommendation for 1992 - 1994. In India PB 260 is included in category I, PB 217 in category II, PB 255, PB 311, PB 312, PB 314, KRS 25, KRS 128 and KRS 163 in category III of the clones recommended for planting.

5.8. Progeny analysis

Seedling progeny analysis or estimation of prepotency through seedling progenies is the assessment genetic potentiality of a female parent to produce superior offspring irrespective of the nature of the male parent. The prepotent ability of a clone to produce high quality seedlings could be determined by systematic and planned experiments like seedling progeny analysis (Mydin, 1990). Such multi parent first generation synthetic varieties of rubber (Simmonds, 1986) have been recommended in category 1 for wide scale planting in Malaysia. The timber yield from such trees is high because of their high vigour and girthing.

Polycross or synthetic seedling populations of polyclonal seed gardens of good clones have been successfully used as planting materials. The seedling population has special agricultural merits in maintaining the genetic variability and adaptability of the population (Mydin *et al.*, 1990; Varghese, 1992). The present investigation was undertaken to evaluate genetically superior mother parents through seedling progeny analysis for the production of quality seeds in the seed gardens. Seedlings, though not comparable with high yielding clones in production potential, have special agricultural merits that there is a need for superior polycross progeny from special polyclonal seed gardens as planting material.

Mydin *et al.*, (2002) reported that in a study of 11 clones, progenies of 5 clones *viz.*, PB 255, RRII 203, RRII 105, PB 260 and GT 1 were identified as likely prepotent with a high performance index and high recovery of superior and elite seedlings in their progeny. This

result of the present study is in conformity with the earlier report, as it is evident from the high performance index value for the progenies of the clones PB 255 and PB 260.

Prepotent parent clones by way of their high GCA are best used as components in polyclonal seed gardens for producing good quality poly cross seeds. The open pollinated progeny of such clones also comprises superior base population for selection and cloning of the best individuals as is the practice in *Hevea* breeding procedures (Simmonds, 1989; Tan, 1998). This could supplement ortet selection programmes as a means of evolving primary clones (Mydin, *et al.,* 2002).

Juvenile characters at the age after one year did not show significant difference among the progenies. However, highly significant variation was noticed for all the characters after two years of growth of progenies. Significant positive correlation was observed for seedling height, girth, number of leaf flushes and juvenile yield at the age of two years was observed. The highest correlation observed between juvenile yield and the vigour of seedlings in terms of height (r = 0.669) followed by girth (r = 0.578) while the lowest relationship was established between yield and number of leaf whorls produced, the correlation was only at 5% level (r = 0.314) and between girth and the number of leaf whorls (r = 0.340). Correlation between seedling girth, and height was also significantly high at 1 per cent level (r = 0.440). The relationship between seedlings height and number of whorls were also very high at 1 per cent level (r = 0.557). 30.23 - 68.00 per cent of progenies of the clones showed above average progeny yield. The progenies of PB 255, PB 260, PB 310, PB 311, PB 312, PB 314 and RRII 105 recorded high percentage of seedlings showing above mean progeny yield and seedling progenies of PB 255 recorded highest (68 %) and KRS 25 (29.58 %) recovered the lowest percentage of superior seedlings in terms of juvenile yield.

In the present study, out of the 13 clones evaluated, 7 clones were identified as likely prepotent with high performance index value and high percentage of the recovery of superior seedlings. Mydin, *et al.,* (2002) evaluated five clones as likely prepotents with a high performance index and high recovery of superior and elite seedlings in the progeny. High performance of progenies of a clone coupled with a high proportion of superior seedlings within the progeny is indicative of the ability of a parent to transmit superior traits to its offspring (Mydin *et al.,* 1996). Seven clones *viz.*, PB 312, PB 314, RRII 105, PB 260, PB 310, PB 255 and PB 311 exhibited high performance indices coupled with a high percentage of recovery of superior seedlings considered as prepotents.

Chapter 6

SUMMARY

SUMMARY

Genetic studies on yield and certain yield components in *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. were undertaken with respect to twelve exotic and one indigenous clone. The objectives of the present study were to assess the genetic variability for yield and yield components and evaluate the performance of clones in the local agroclimatic condition in comparison to RRII 105, the outstanding high yielder. The study also envisages to examine the major factors contributing to yield and identifying genetically divergent genotypes as well as prepotent clones. Biochemical and molecular approaches for assessment of genetic variability was also attempted. Observation were recorded for three consecutive years from 1998 - 2001 and data analysis was carried out separately for annual mean, peak yielding season (October - January) and stress (summer) period (February - May).

The highest annual mean dry rubber yield was estimated for the clone PB 255 (73.52 g t^{-1} t^{-1}) and lowest for PB 217 (38.17 g t^{-1} t^{-1}). Yield of seven clones viz., PB 255 (73.52 g t^{-1} t^{-1}), PB 314 (66.88 g t^{-1} t^{-1}), PB 280 (66.81 g t^{-1} t^{-1}), PB 260 (63.20 g t^{-1} t^{-1}), KRS 163 (62.96 g t^{-1} t^{-1}), PB 312 (62.13 g t^{-1} t^{-1}) and PB 311 (60.33 g t^{-1} t^{-1}) was significantly superior to RRII 105 (49.50 g t^{-1} t^{-1}). Significant difference in yield was noted among clones during different seasons also. The yield depression estimated was minimum for PB 255 (27.99 %) while the clone PB 235 (49.87 %) recorded the highest yield depression during stress. This showed the consistency of the clone PB 255, in yield potential during the entire period of the year.

Based on the performance of high yield and bole girth four clones *viz.*, PB 255, PB 280, PB 312 and PB 314 were identified as latex-timber clones.

Highly significant clonal variations were recorded for all the yield components studied. Wide range of variations were recorded for girth at opening (50.96 to 61.33 cm.), annual dry rubber yield (38.17 to 73.52 g t^{-1} t^{-1}), rubber yield in stress season (26.29 to 52.74 g t^{-1} t^{-1}) and peak season (44.75 to 80.42 g t^{-1} t^{-1}), yield depression during stress (27.99 to 49.87 %), latex yield (107.60 ml t^{-1} t^{-1} to 176.85 ml t^{-1} t^{-1}), latex yield during stress (58.31 ml t^{-1} t^{-1} to 115.26 ml t^{-1} t^{-1}) and peak season (117.27 ml t^{-1} t^{-1} to 193.17 ml t^{-1} t^{-1}), latex vessel rows in virgin (16.68 to 28.64) and renewed bark (14.20 to 30.96). However, low range was recorded for girth increment, rubber content, in different seasons and also for bark thickness.

Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the characters studied. However, it was closer for most of the characters, which implies the lesser influence of environment in the expression of these characters. High PCV and GCV was estimated for annual mean dry rubber yield, rubber yield in stress and peak seasons, yield depression, latex yield during stress season and latex vessel rows in renewed bark. High genetic advance coupled with high heritability was recorded for yield depression, rubber yield in stress period, latex yield during stress period and rubber yield during peak season.

Among the 23 characters studied, most of the correlations were found to be in positive direction. At both phenotypic and genotypic levels rubber yield during stress and peak period, annual latex yield, latex yield in stress and peak season, latex vessel rows in renewed bark exhibited high and positive association with annual dry yield. However, annual plugging index (PI), PI during stress and peak periods was low and negative with

annual dry yield. The high positive association implies the scope for simultaneous improvement of these traits by selection that in turn will improve yield as well.

Performance of the 13 clones based on the pooled data of yield and major yield components showed that PB 280 was ranked first followed by PB 255 and KRS 128. The Indian clone RRII 105 ranked fourth among the clones studied. Hence it is noted that in general, high yielders recorded higher values for these traits in comparison with medium and low yielders.

D^2 analysis based on yield and major yield components *viz.*, girth, dry rubber content, latex yield, plugging index, bark thickness and number of latex vessel rows showed that the cultivars were grouped into two major clusters. The inter cluster distance was 78.11. The intracluster distance in cluster I was 24.65 while that of cluster II was 34.14. The D^2 value ranged from 1.79 to 159.55 showing considerable genetic variability existing in the population. The highest genetic distance was recorded between PB 312 and PB 280 (159.55) and the lowest was between PB 260 and KRS 163 (1.79).

The present study also envisages isozyme and random amplified polymorphic DNA (RAPD) marker analysis to determine genetic variability and relatedness in a set of cultivated *Hevea* clones and to select genetically divergent genotypes for setting up hybridization aimed at achieving high heterosis for yield and vigour in progenies. Out of the nine isozyme systems studied, a total of four *viz.*, aryl esterase, peroxidase, aspartate aminotransferase and shikimate dehydrogenase showed polymorphism. A combined total of 22 isozyme bands were scorable and a mean of 15 bands per clone was observed. The number of bands in different clones ranged from 11 to 19. Isozyme analysis clearly distinguished all the 13 clones from one another.

Genetic studies using DNA based molecular markers are limited in *Hevea brasiliensis.* In the present study 55.80 per cent of the RAPD were polymorphic among the 13 cultivated clones of *Hevea* based on RAPD. The genetic polymorphism was found to be reasonably good according to the earlier reports. Among the different clones tested PB 255 displayed the maximum average genetic distance followed by KRS 163, KRS 128 and PB 314. Genetic distance estimated by RAPD markers reveals that there are three major groups among the 13 clones.

A comparative analysis of genetic distance estimated markers reveals that DNA based RAPD markers are more reliable than that of isozymes as there are limitation in utilising them are genetic markers. Genetic distance based on D^2 analysis revealed that all the clones were clustered irrespective of their country of origin. However, in RAPD 13 clones were grouped into three clusters as they originated from three different countries. In some cases clones were clustered together, perhaps it may be due to their common parentage in their ancestry.

Important properties that related to latex and rubber qualities for the 13 clones showed significant clonal variation.

To identify genetically superior mother parents for the production of quality seeds in seed gardens, progeny analysis was carried out. Correlation among juvenile characters elucidated highly significant positive association. The open pollinated progenies of PB 255, PB 260, PB 310, PB 311, PB 312, PB 314 and RRII 105 recorded more percentage of seedling showing above mean progeny yield (4.14 g $plant^{-1}$ 10 t^{-1}). The progenies of PB 255 recorded the highest percentage (68.00 %) and KRS 25 exhibited the lowest (29.58 %). Of the 13 clones evaluated, these seven clones were identified as likely prepotents with high performance

index value based on juvenile characters i.e., yield, girth, height and number of whorls. High proportion of superior seedlings was recovered based on juvenile yield. The high performance index and high percentage of superior seedlings are the indication of the ability of a parent to transmit superior traits to its offspring.

Based on the performance of clones at different stages of evaluation with respect to yield and secondary characters they are being included in different categories of planting materials recommended for growers. Clones included in category I are those approved for large scale planting. Merits and demerits of these clones are studied thoroughly. Category II comprises clones, which are suitable for moderate scale planting. Category III clones are recommended only for experimental planting in a limited scale. In the present study, PB 255, PB 260, PB 280, PB 312, PB 314 and KRS 163 are comparatively superior to RRII 105 with respect to yield. Since a single clone-RRII 105 occupies the major portion of rubber growing area, these clones can be used for multiclone planting. The clones having high yield and vigour can be considered as latex - timber clones. PB 255, PB 280, PB 312 and PB 314 possess both high yield and vigour. The output of timber from vigourous clone is comparatively more.

The results of present investigations show that genetic variation exist in the population and selection based on these characters can provide better genotypes for further breeding programmes. Among the 13 clones studied, 7 clones (PB 255, PB 260, PB 280, PB 311, PB 312, PB 314 and KRS 163) recorded significantly higher yield than RRII 105. These clones hold promise for further planting in growers sector.

SALIENT FINDINGS

1. *Seven clones viz., PB 255, PB 260, PB 280, PB 311, PB 312, PB 314 and KRS 163 were significantly superior in yield to the outstanding Indian clone RRII 105. The highest yield was recorded by PB 255 followed by PB 314 and PB 280.*
2. *Eight clones were classified as high yielding clones since all of them recorded above average annual yield.*
3. *Four clones viz., PB 255, PB 280, PB 312 and PB 314 were identified as latex-timber clones based on high vigour in terms of bole girth and high yield.*
4. *PB 255 exhibited lowest yield depression under stress and it was highest for PB 235 while RRII 105 exhibited medium yield depression.*
5. *The highest dry rubber content (DRC) was recorded for PB 280 followed by KRS 128 and PB 255.*
6. *Performance analysis showed PB 280 performed better followed by PB 255, KRS 128 and RRII 105.*
7. *D^2 analysis showed that thirteen genotypes were grouped in to two clusters irrespective of their country of origin and all other geographical barriers.*
8. *Isozyme studies showed that genetic polymorphism exists among Hevea clones.*
9. *RAPD analysis clearly distinguished all the clones from each other and revealed that clones with a common pedigree were clustered together*

10. *Seven clones were identified as likely prepotents with high performance index and high percent of the recovery of better progenies, so that they can be utilize as components in polycross seed gardens for the production of good quality seeds.*

11. *Latex and rubber properties showed significant clonal variation.*

12. *Highly significant variation for yield and major yield components showed that sufficient genetic variation exists in the population and it can be utilized as selection criteria in choosing genotypes for Hevea breeding programme to generate superior progenies.*

REFERENCES

Abraham, S.T. (2000). Genetic parameters and divergence in certain wild genotypes of *Hevea brasiliensis* (Willd. ex. Adr. de Juss.) Muell. Arg. P.h.D. Thesis, Mahatma Gandhi University, Kottayam, Kerala, 203 p.

Abraham, S.T., Reghu, C.P., George, P.J. and Nair, R.B. (1997). Studies on genetic divergence in *Hevea* brasiliensis. In: *Symposium on Tropical Crop Research and Development, India-International,* 9 - 12 September 1997, Trichur, Kerala.

Adams, W. T. (1983). Application of isozymes in tree breding. In: *Isozymes in Plant Genetics and Breeding*. (Eds. S.D. Tanksley and T. J. Orton). Elsevier, Amsterdam, pp. 381.

Alias, B.O. and Hasma, H. (1988). Influence of *Hevea* proteins and amino-acids on the properties of natural rubber. *Proceedings of International Rubber Technology Conference*, 1988, Penang, 166 p.

Alika, J.E. (1980). Estimates of repeatability and coefficient of variation in rubber. *Silvae Genetica,* **29**(3 & 4): 161 - 162.

Alika, J.E. (1982). Preliminary assessment of some hand pollinated progenies of rubber in Nigeria. *Indian Journal of Agricultural Sciences*, **52**(6): 367 - 369.

Alika, J.E. and Onokpise, O.U. (1982). Estimation of heritability parameter for yield, girth and bark thickness in rubber from a single pair mating design. *Journal of Plantation Crops*, **10**(2): 102 - 108.

Allard, R.W. (1960). *Principles of Plant breeding.* John Wiley and Sons, New York, 485 p.

Allard, R.W. (1990). Genetic changes associated with the evolution of adaptedness in cultivated plants and their wild relatives. *Journal of Heredity*, **79**: 225 - 238.

Allard R.W. (1999). *Principles of Plant Breeding.* John Wiley and Sons, Inc., New York, 254 p.

Allen, P. W. (1984). Fresh germplasm for natural rubber. *Span*, **27**(1): 7 - 8.

Allen, P.W. and Bloomfield, G. (1963). Natural rubber hydrocarbon. In: *The Chemistry and Physics of Rubber-like substances.* (Ed. L. Bateman) Maclaren, London, Chapter 1, pp. 1 - 17.

Arulsekar, S. and Parfitt, D.E. (1986). Isozyme analysis procedurers for stone fruits, almond, walnut, pistachio and fig. *Hort Science.* **21**: 928 - 933.

Arus, P. (1983). Genetic purity of commercial seeds lots. In: *Isozymes in Plant Genetics and Breeding*. Part A. (Eds. S.D. Tanksley and. T.J. Orton). Elsevier, Amsterdam, pp. 415 - 423.

Bengtsson, A. and Stenberg, B. (1996). Characterization of natural rubber from different producers. *Progress in Rubber and Plastics Technology*, **12** (1): 1 - 29.

Bergmann, F. (1987). Characterisation of multiclonal aspen cultivars using isozyme electrophoresis. *Forest Ecology and Management*, **22**: 167 - 172.

Besse, P., Seguin, M., Lebrun, P., Chevallier, M. H., Nicolas, D. and Lanaud, C. (1994). Genetic diversity among wild, cultivated populations of *Hevea brasiliensis* assessed by nuclear RFLP analysis. *Theoretical and Applied Genetics,* **88**: 199 - 217.

Besse, P., Seguin, M., Legrun, P. and Lanaud, C. (1993). Ribosomal DNA variations in wild and cultivated rubber tree (*Hevea brasiliensis*). *Theoretical and Applied Genetics,* **36**: 1049 - 1057.

Bobilioff, W. (1920). Correlation between yield and number of latex vessel rows of *Hevea brasiliensis. Archief Voor De Rubber Cultuur Deel.* **4**: 391.

Bobilioff, W. (1923). *Anatomy and Physiology of Hevea brasiliensis*. Part I. Zurich, Art. Inst. Orell fussli, 150 p.

Brown, A.D.H., Clegg, M. T., Kahler, A. L. and Weir, B. S. (1990). *Plant Population Genetics, Breeding and Genetic Resources.* Sinauer Assoc. Sunderland, Massachusetts.

Brozozowska - Hanower, J., Cretin, H., Hanower, P. and Michel, P. (1979). Variations du pH entre compartiments vacuoline et cytoplasmique au sein du latex d' *Hevea brasiliensis*: Influence saisonniere et action due traitment par l' Ethrel generateur d' ethylene, Repercussion sur La production et l' apparition d' encoches seches. *Physiologie Vegetale,* **17**: 851.

Bryce, G. and Campbell, L.E. (1917). On the mode of occurrence of latex vessels in *Hevea brasiliensis. Department Agriculture Ceylon Bull*etin, **30:** 1 - 22.

Burfield, D. R., Chew, L. C. and Gan, S. N. (1976). Distribution of abnormal groups in natural rubber. *Polymer*, **17**: 713 - 714.

Burton, G.W. (1952). Quantitative inheritance in grasses. *Proceedings of 8th International Grassland Congress,* **1:** 277 - 283.

Burton, G.W. and Devane, E.H. (1953). Estimating heritability in tall fescue from replicated clonal material. *Agronomy Journal*, **45:** 478 - 581.

Buttery, B.R. and Boatman, S.G. (1976). Water deficit and flow of latex. In: *Water Deficit and Plant Growth.* V 4. (Ed. T.T. Kozlowsky). Academic Press, New York, pp. 233 - 288.

Campbell, D.S. and Fuller, K.N.G. (1984). Factors influencing the mechanical behaviour of row unfilled natural rubber. *Rubber Chemistry Technology,* **57**: 104 - 117.

Chandrasekar, T.R. (1994). Correlation and path analysis of yield and its components, some factors of water relations and soil moisture in *Hevea brasiliensis. Indian Journal of Natural Rubber Research*, **7**(2): 89 - 94.

Chandrasekar, T.R., Gawai, P.P. and Saraswathyamma, C.K. (2002). Yield performance of trees grown from polycross seeds of rubber (*Hevea brasiliensis*) in a dry subhumid climate in India. *Indian Journal of Natural Rubber Research,* **15**(1): 19 - 27.

Chandrasekar, T.R., Marattukalam, J.G. and Nazeer, M.A. (1996). Growth reaction of *Hevea brasiliensis* heat and drought stress under dry subhumid climatic conditions. *Indian Journal of Natural Rubber Research*, **9**(1): 1 - 5.

Chandrasekar, T.R., Nazeer, M.A., Marattukalam, J.G., Gawai, P., Annamalainathan, K. and Joseph, T. (1998). An analysis of growth and drought tolerance in rubber during the immature phase in a dry subhumid climate. *Experimental Agriculture,* **34:** 1 - 14.

Chandrasekar, T.R., Nazeer, M.A., Marattukalam, J.G. and Premakumari, D. (1995). Studies on the nature of variation and covariation of some quantitative traits in *Hevea brasiliensis*. *Indian Journal of Natural Rubber Research*, **8**(1): 54 - 56.

Chapman, C.G.D. (1989). Collection strategies for the wild relatives of field crops. In: *The Use of Plant Genetic Resources*. (Eds. A. D. H. Brown, D. R. Marshall, O.H. Frankel and J.T. Williams). Cambridge University Press, Cambridge, pp. 263 - 279.

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

Chevallier, M.H., Soleille, B. and Nicolas, D. (1985). Polymorphisme Enzymatique et Variabilite Ge'ne'tique de I' *He'vea. Caoutchoucs et Plastiques*, **625**: 83.

Chrestin, H. and Gidrol. X. (1985). Contribution of lutoidic tonoplast in regulation of cytosotic pH of latex from *Hevea brasiliensis*: Effect of ethephon. *Proceedings of the International Rubber Conference*, 1985, Kuala Lumpur, Malaysia, V **3**, pp. 66 - 87.

Cole, O.D. (1958). Natural rubber. In: *The Vanderbilt Rubber Handbook.* (Ed. G.G. Winspear). R.T. Vandderbilt Company, New York, pp. 13 - 39.

Coupe, L. and Lambert, C. (1977). Absorption of citrate by the lutiods from the latex vessels and rubber production by *Hevea. Phytochemistry,* **16**: 455.

Cousineau, C.J. and Donnelly, D. J. (1992). Use of isozyme analysis to characterise raspberry cultivars and detect cultivar mislabelling. *Hort Science*, **27**(9): 1023 - 1025.

Crawford, D.J. (1983). Phylogenetic and systematic inferences from electrophoretic studies. In: *Isozymes in Plant Genetics and Breeding*. Part A. (Eds. S. D. Tanksley and T. J. Orton). Elsevier, Amsterdam, pp. 257 - 287.

Cretin, H., Jacob, J.L., Prevot, J.C. and D'Auzac, J. (1980). Le pH du latex: son influence sur la production et les elements due sa regulation. *Reveue Generale des Caoutchoucs et Plastiques,* **603**: 111.

Das, S., Rajagopal, J., Bhatia, S., Srivastava, P. S. and Lakshmikumaran, M. (1999). Assessment of genetic variation within *Brassica campestris* cultivars using amplified fragment length polymorphism and random amplification of polymorphic DNA markers. *Journal of Biosciences*, **24**: 433 - 440.

Demange, A.C., Legnaté, H., Seguin, M.P.C., Guen, V.L., Chapuset, T. and Nicolas, D. (2001). Rubber tree. In: *Tropical Plant Breeding* (Eds. André Charrier, Michel Jacquot, Serge Hamon and Dominique Nicolas). Oxford and IBH Publishing Co. New Delhi, pp. 455 - 480.

Desalphine (2002). Indian Natural Rubber Industry to Face the Challenge. *Souvenir, 100 years of commercial Rubber Cultivation in India.* 1902 - 2002. p. 3.

Dijkman, M.J. (1951). *Hevea: Thirty years of research in the Far East.* University of Miami Press, Coral Gabler, Florida, 329 p.

Dijkman, M.J. and Ostendorf, F.W. (1929). Seedling test garden. Mede de clingen van HAPD AVROS, 453 p.

Doyle, J.J. and Doyle, J.L. (1990). Isolation of DNA from small amounts of plant tissues. *Focus,* 12: 13 - 15.

Ebi, G.C. and Kolawole, E.G. (1992). Some aspects of stabilization and degradation of Nigerian natural rubber. (11): The environmental degradation of DBHBT - Modified unprocessed, unvulcanized Nigerian natural rubber. *Journal of Applied Polymer Science,* **45**(10-12): 2239 - 2244.

Edgar, A.T. (1987). *Manual of Rubber Planting*. The Incorporated Society of Planters, Kuala Lumpur, 705 p.

Esah, Y. (1990). Clonal characterization of latex and rubber properties. *Journal of Natural Rubber Research,* **5**(1): 52 - 80.

Eschbach, J.M., Roussel, D., Van De Sype, H. and Jacob, J.L. (1984). Relationship between yield and clonal physiological characteristics of latex from *Hevea brasiliensis. Physiologie Vegetale,* **22**: 295.

Evans, D.A. and Sharp, W.R. (1986). Somaclonal and gametoclonal variation. In: *Handbook of Plant Cell Culture.* (Eds. D. A. Evans, W. R. Sharp and P. V. Ammirato). Macmillan, New York, V **4,** pp. 97 - 132.

Falconer, D.S. (1981). *Introduction to Quantitative Genetics*. Longman, New York, pp. 340.

Falconer, D.S. (1989). *Introduction to Quantitative Genetics*. Ed. 3. Longman, Burnt Mill.

Filho, K.A.N., Filho, M.J.B., Cardoso, M. and Ortalani, A.A. (1982b). Phenotypic correlations and climate influence on the yield of rubber clones. *Silviculture en Sao Paulo,* **16**(A): 1004 - 1007.

Fisher, R.A. and Yates, F. (1963). *Statistical Tables for Biological, Agricultural and Medical Research.* Longman, England, 146 p.

Friedman, H.P. and Rubin, J. (1967). On some invariant criteria for grouping data. *Journal of the American Statistics Association,* **62**: 1159 - 1178.

Fuller, K.N.G. (1988). In: *Natural Rubber Science and Technology*. (Ed. A. D. Roberts). Oxford University Press, Oxford, pp. 159 - 165.

George, P.J. and Jacob, C.K. (2000). *Natural Rubber: Agromanagement and Crop Processing*. Rubber Research Institute of India, Kottayam, 648 p.

George, P.J., Nair, V.K.B. and Panikkar, A.O.N. (1980). Yield and secondary characters of the clone RRII 105 in trial plantings. *International Rubber Conference*, 1980, Kottayam, India.

George, P.J., Panikkar, A.O.N. and Nair, V.K.B. (1967). Observations on the floral biology and fruit set in *Hevea brasiliensis* Muell. Arg. *Rubber Board Bulletin*, **9**(2): 18 - 27.

George, S.P., Gopimony, R. and Gangadharan, P. (1984). Seedling progeny analysis of Cashew types. *Agricultural Research Journal of Kerala*, **22**(2): 124 - 128.

Gilbert, N.E., Dodds, K.S. and Subramaniam, S. (1973). Progress of breeding investigations with *Hevea brasiliensis*. V. Analysis of data for earlier crosses. *Journal of the Rubber Research Institute of Malaysia*, **32**: 365 - 380.

Gomez, J.B. (1982). *Anatomy of Hevea and its Influence on Latex Production.* Malaysian Rubber Research and Development Board, Kuala Lumpur, 75 p.

Gottilieb, L.D. (1981). Electrophoretic evidence and plant populations. In: *Progress in Phytochemistry*. (Ed. L. Finhold). Pergamon Press, New York, V **7**, pp. 1 - 46.

Granger, A.R., Clarke, G.R. and Jackson, J.F. (1992). Sweet cherry cultivar identification by leaf isozyme polymorphism. *Theoretical and Applied Genetics*, **86**: 458 - 464.

Grantham, J. (1925). Periodical tapping of *Hevea*. *Malaysian Agricultural Journal*, **8**: 342.

Hamaz, S.B. and Gomez, J.B. (1982). Some structural factors affecting the productivity of *Hevea brasiliensis*. III. *Journal of the Rubber Research Institute of Malaysia*, **30**: 148 - 160.

Hamrick, J.L. and Godt, M.J. (1990). Allozyme diversity in plant species. In : *Plant Population Genetics, Breeding and Genetic Resources.* (Ed. A. D. H. Brown). pp. 43 - 63.

Harland, S.G. (1957). The improvement of coconut palm by breeding and selection. *Bulletin No. 15. Coconut Research Institute,* Ceylon, pp. 14 - 21.

Heusser, C. and Holder, H.J.V.S. (1931). Quoted by Dijkman, M. J., 1951.

Ho, C.Y. (1976). Clonal characters determining the yield of *Hevea brasiliensis. Proceedings of the International Rubber Conference*, 1975, Kuala Lumpur, Malaysia, V **2**, pp. 27 - 44.

Ho, C.Y., Subramaniam, A. and Yong, W.M. (1976). Lipids associated with the particles in *Hevea* latex. *Proceedings of the International Rubber Conference*, 1975, Kuala Lumpur, Malaysia, V **2**, pp. 441 - 456.

Ho, C.Y., Narayanan, R. and Chen, K.T. (1973). Clonal nursery studies in *Hevea*.1. Nursery yields and associated structural characteristics and their variations. *Journal of the Rubber Research Institute of Malaya*, **23**: 305 - 316.

International Rubber Research and Development Board IRRDB (2001). *Annual Report* 2000. IRRDB, Kuala Lumpur, Malaysia.

Jacob, C.K. (1997). Diseases of potential threat to rubber in India. *Planters' Chronicle*, **92**(10): 451 - 461.

Jacob, J. (2002). Ecofriendly credentials of natural rubber. *Rubber Planters Conference*, India. 2002: Global Competitiveness of Indian Rubber Plantation Industry, 21 - 22 November 2002, Rubber Board, Kottayam, pp. 47 - 49.

Jain, J.P. (1982). *Statistical Techniques in Quantitative Genetics.* Tata McGraw Hill, New Delhi, pp. 281.

John A., Varghese, Y.A., Mydin, K.K., Sebastian, T., Thomas, V. and Saraswathyamma, C.K. (2002). Performance of certain introduced *Hevea* clones in India. *Indian Journal of Natural Rubber Research* (in press).

Johnson, H.W., Robinson, H.F. and Constock, R.E. (1955). Estimates of genetic environmental variability in Soybean. *Agronomy Journal*, **47:** 314 - 318.

Kahler, A.L. and Price, S.C. (1987). Isozymes in population genetics and evolution. In: *Grass Systematics and Evolution*. T. R. Sodestrom, K. W. Hilu.

Kamalam, P., Licy, J. and Hrishi, N. (1978). Association of characters in some selfed progenies of Cassava, *Manihot esculenta* (CRANTZ). *Journal of Root Crops*, **4:** 27 - 29.

Kang, L.C. and Hashim, I. (1982). Factors influencing the dry rubber content of *Hevea* latex. *Planters' Bull*etin, **172:** 89 - 98.

Kempthrone, O. (1977). *An Introduction to Genetic Statistics.* Iowa State University Press, U.S.A., 545 p.

Krishnakumar, A.K. (2003). India needs advance planning to maetch growing NR consumption: *Rubber Asia.* March - April, 57 - 72.

La Rue, C.D. (1921). Structure and yield in *Hevea brasiliensis. Archief Voor De Rubber Cultuur Deel,* **5:** 574.

Leconate, A., Lebrun, P., Nicolas, D. and Seguin, M. (1994). Electrophoresis application a l'identification Clonale de l' *Hevea. Plantations, Recherche, Development*, **1:** 28.

Lee, C.K. and Tan, H. (1979). Daily variation in yield and dry rubber content in four *Hevea* clones. *Journal of the Rubber Research Institute of Malaysia,* **27**(3): 117 - 126.

Lespinasse, D., Rodier - Goud, M., Grivet, L., Leconte, A., Legnate, H. and Seguin, M. (2000). A saturated genetic linkage map of rubber tree (*Hevea spp.)* based on RFLP, AFLP, microsatellite and isozyme markers. *Theoretical and Applied Genetics,* **100:** 121 - 138.

Liang, M., Yuntong, W., Dong Quiong, H., Dehi, Z., Li Desum, Seng Xian, W., Zhnocai, C. and Jaialin, F. (1980). Preliminary analysis of genetic parameters of some characters in *Hevea* seedling. *Chinese Journal of Tropical Crops*, **1:** 50 - 52.

Licy, J. (1997). *Variability, correlations and heterosis for yield and yield components in certain hybrid clones of the para rubber tree, Hevea brasiliensis* (Willd.ex Adr.de Juss) Muell. Arg. Ph.D. Thesis, University of Kerala, Trivandrum, 158 p.

Licy, J., Panikkar, A.O.N., Premakumari, D., Varghese, Y.A. and Nazeer, M.A. (1992). Genetic parameters and heterosis in *Hevea brasiliensis*. I. Hybrid clones of RRII 105 × RRIC 100. *Indian Journal of Natural Rubber Research*, **5** (1 & 2): 51 - 56.

Licy, J., Panikkar, A.O.N., Premakumari, D., Saraswathyamma, C.K., Nazeer, M.A. and Sethuraj, M.R. (1996). Genetic parameters and hetrosis in rubber (*Hevea brasiliensis*) Muell. Arg. IV. Early versus mature performance of hybrid clones. *Placrosym XII*, 1996, Kottayam, India. *Developments in Plantation Crops Research*, pp. 9 - 15.

Licy, J., Saraswathyamma, C.K., Premakumari, D., Meenakumari, T., Rajeswari Meenattoor, J. and Nazeer, M.A. (2002). Genetic parameters and heterosis in rubber *Hevea brasiliensis* Muell Arg: V. Hybrid vigour for yield and yield component, among the RRII 400 series clones in small scale trial. *Indian Journal of Natural Rubber Research*, (In press).

Link, W., Dixkens, C., Singh, M., Schwall, M. and Melchinger, A.E. (1995). Genetic diversity in European and Mediterranean faba bean germplasm revealed by RAPD markers. *Theoretical and Applied Genetics*, **90**: 27 - 32.

Liu, N. (1980). A review of quantitative genetic studies on *Hevea* by RRIM. *Chinese Journal of Tropical Crops*, **1**: 42 - 46.

Liyanage, A.D.S., Jayasinghe, C.K. and Liyanage, N.I.S. (1989). Losses due to Corynespora leaf fall disease and its eradication. *Proceedings of the RRIM Rubber Growers Conference*, 1989, Malacca, Malaysia, pp. 401 - 410.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randell, R.J. (1951). Protein measurement with the Folin's phenol reagent. *Journal of Biological Chemistry*, **193**: 265 - 275.

Luo, H., Van Coppenolle, B.V., Seguin, M. and Boutry, M. (1995). Mitochondrial DNA polymorphism and phylogenetic relationships in *Hevea brasiliensis*. *Molecular Breeding*, 1: 51 - 63.

Mahalanobis, P.C. (1928). A statistical study at Chinese head measurement. *Journal of the Asiatic Society of Bangladesh*, **25:** 301 - 377.

Mahalanobis, P.C. (1936). On the generalized distance in statistics. *Proceedings of the National Academy of Sciences, India*, **2:** 49 - 55.

Marattukalam, J.G. and Mercykutty, V.C. (2000). Propagation techniques. In: *Natural Rubber: Agromanagement and Crop Processing*. (Eds. P.J. George and C. Kuruvilla Jacob). Rubber Research Institute of India, Kottayam, pp. 75 - 96.

Markose, V.C. (1984). *Biometric analysis of yield and certain yield attributes in the Para rubber tree, Hevea brasiliensis,* Muell. Arg. Ph.D. Thesis, Kerala Agricultural University, Trichur, pp. 113 - 114.

Markose, V.C. and George, P.J. (1980). Broad sense heritability value of certain characters and possible genetic gain in clonal selections of *Hevea brasiliensis*. *International Rubber Conference India,* 1980, Kottayam.

Martin, G. (1961). Natural rubber. In: *The Applied Science of Rubber*. (Ed. W. J. S. Naunton) Edward Arnold, London, Chapter II, Part two, pp. 157 - 164.

Mass, J.G.J.A. and Bokma, F.T. (1950). In: *De Landbouw in den Indischen Archipal.* 111, pp. 237 - 426.

Mathew, N.M. (2002). Natural Rubber Research in India: Yesterday, Today and Tomorrow. *Souvenir, 100 years of Commercial Rubber Cultivation in India,* 1902 - 2002. p. 33 - 41.

Mercykutty, V.C., Marattukalam, J.G., Saraswathyamma, C.K. and Meenakumari, T. (2002). *Identification of Hevea clones: A manual.* Rubber Research Institute of India, Kottayam, Kerala, India, 103 p.

Milford, G.F.J., Paardekooper, E.C. and Ho Chai Yee (1969). Latex vessel plugging, its importance to yield and clonal behaviour. *Journal of the Rubber Research Institute of Malaya*, **21**: 274.

Morris, J.E. and Sekhar, B.C. (1959) Recent developments in the production and processing of natural rubber in Malaya. *Proceeding of the International Rubber Conference,* 1959, pp. 277.

Mydin, K.K. (1990). Polyclonal seed gardens: Their role in rubber improvement and production. *Rubber Board Bulletin*, **26**(1): 3 - 4.

Mydin, K.K. (1992). *Genetic divergence, prepotency and inbreeding depression in Para rubber (Hevea brasiliensis Muell. Arg.).* Ph.D. Thesis, Kerala Agricultural University, Trichur, India, 321 p.

Mydin, K.K., Nair, V.G., Panikkar, A.O.N., Saraswathy, P. and Sethuraj, M.R. (1990). Prepotency in rubber. 1. Early estimation through juvenile traits. *Proceedings of the National Symposium on New Trends in Crop Improvement of Perennial Species*, 1990, Rubber Research Institute of India, Kottayam, pp. 114 - 123.

Mydin, K.K., Nair, V.G., Sethuraj, M.R., Panikkar, A.O.N., Nazeer, M.A. and Saraswathy, P. (1996). Prepotency in rubber. 2. Seedling progeny analysis for yield and certain yield attributes. *Indian Journal of Natural Rubber Research,* 9(1): 63 - 66.

Mydin, K.K., Nair, V.G., Sethuraj, M.R., Saraswathy, P. andd Panikkar, A.O.N. (1992). Genetic divergence in *Hevea* brasiliensis. *Indian Journal of Natural Rubber Research,* **5** (1 & 2): 120 - 126.

Mydin, K.K., Sankariammal, L., Thomas V. and Saraswathyamma, C.K. (2002). Prepotency in rubber: Half-sib progeny evaluation. *Placrosym* XV, 10-13 December 2002, Mysore, Karnataka, pp. 25 - 26.

Nair, V.K.B. and George, P.J. (1969). The Indian clones RR11 100 series. *Rubber Board Bulletin,* **10** (3): 115 - 139.

Nair, V.K.B., George, P.J. and Saraswathyamma, C.K. (1976). Breeding improved *Hevea* clones in India. *Proceedings of the International Rubber Conference*, 1975, Kuala Lumpur, Malaysia, V 2, pp. 45 - 54.

Nair, V.K.B and Panikkar, A.O.N. (1966). Progress of investigations on the improvement of rubber planting materials in India. *Rubber Board Bulletin*, **8:** 201 - 210.

Narayanan, R., Gomaz, J.B. and Chen, K.T. (1973). Some structural factors affecting the productivity of *Hevea brasiliensis*. II. Correlation studies between structural factors and yield. *Journal of the Rubber Research Institute of Malaya,* **23:** 285.

Narayanan, R. and Ho, C.Y. (1973). Clonal nursery studies in *Hevea.* II. Relationship between yield and girth. *Journal of the Rubber Research Institute of Malaya*, **23** (5): 332 - 338.

Narayanan, R., Ho, C.Y. and Chen, K.T. (1974). Clonal nursery studies in *Hevea.* III. Correlation between yield, structural characters, latex constituents and plugging index. *Journal of the Rubber Research Institute of Malaysia,* **24**(1): 1 - 6.

Nazeer, M.A., Markose, V.C., George, P.J. and Panikkar, A.O.N. (1986). Performance of a new *Hevea* clones from RRII 100 series in large scale trial. *Journal of Plantation Crops,* **14**(2): 99 - 104.

Ng, N. P., Abdullah, Sepien, C.B., Ooi and Leong. W. (1979). Report on various aspects of yield, growth and economics of a density trial. *Proceedings of the Rubber Research Institute of Malaysia Planter's Conference,* 1979, Kuala Lumpur, pp. 303 - 331.

Nga, B.H. and Subramaniam, S. (1974). Variations in *Hevea brasiliensis*. *Journal of the Rubber Research Institute of Malaysia*, **24** (2): 69 - 74.

Nielsen, G. (1984). The use of isozymes as probes to identify and label plant varieties and cultivars. In : *Isozymes : Current Topics in Biological and Medical Research.* (Eds. M. C. Rattazzi, J. G. Scandalios and G. S. Whitt). Alan R. Liss, New York, pp. 1-32.

Ninane, F. (1967). Evapotranspiration velle et eroissance de genues *Heveas* Sourns a differenter humiditaden Sol. *Revu Plastiques*, **44** (2): 207.

Ninane, F. (1970). *Les aspects Ecophysiologiques de la Productive ches Hevea brasiliensis* Au Cambodge. Thesis, University Catholique de Louvain, France, 1970.

Ong, S.H. (1981). Correlation between yield, girth and bark thickness of RRIM clone trials. *Journal of the Rubber Research Institute of Malaysia*, **29**(1): 1 - 13.

Ong, S.H. and Tan, H. (1987). Utilisation of *Hevea* genetic resources in the RRIM. *Malaysian Applied Biology*, **16**: 145 - 165.

Oraon, P., Prakash, R. and Alagne, M.E. (1977). Correlation studies in Chickpea (*Cicer arietinum L.*). *Tropical Grain Leg. Bull.*, **7**: 18 - 19.

Ostendorf, F.W. (1932) Resultaten der tootsing van *Hevea* Cloonern in den proeftuin Tjiomas. I. *Archief Voor de Rubber Cultuur inn Nederlandsch Indie*, **16**: 215 - 235.

Panikkar, A.O.N. (1974). *Anatomical studies in Hevea brasiliensis*. Ph.D Thesis, Birla Institute of Technology and Science, Pilani, India.

Panse, V.G. (1957). Genetics of quantitative characters in relation to plant breeding. *Indian Journal of Genetics*, **17**: 318 - 328.

Panse, V.G. and Sukhatme, P.V. (1985). *Statistical Methods for Agricultural Workers*. Indian Council of Agricultural Research, New Delhi, pp. 145 - 149.

Pardekooper, E.C. and Somosorn, S. (1969). Clonal variation in latex flow pattern. *Journal of the Rubber Research Institute of Malaya*, **21**: 264.

Pardekooper, E.C. and Sookmook, S. (1969). Diurnal variations in latex yield and dry rubber content and relation to saturation and deficit of air. *Journal of the Rubber Research Institute of Malaya*, **21**: 341 - 347.

Pavia, J.R., de., Filho, J.B.M., Siqueria, E.R. and Valois, A.C.C. (1982). Prediction of genetic gain for some rubber characters utilising three different schemes of selection. *Pesquisa Agropecuria Brasileira*, **17**(1): 1647 - 1654.

Pearce, S.C. (1953). Field experimentation with fruit trees and other perennial plants. Technical communication No: 23. Commonwealth Agricultural Bureaux, England.

Peries O.S. (1970). *A Handbook of Rubber Culture and Processing*. Rubber Research Institute of Ceylon, Agalawatta, 188 p.

Pillay, P.N.R. (1980). *Handbook of Natural Rubber Production in India.* Rubber Research Institute of India, Kottayam, 668 p.

Polhamus, L.G. (1962). *Rubber: Botany, Production and Utilisation.* Inter-science Publishers Inc., New York, 449 p.

Pollinere, J.P. (1966). Introduction to the study of genetical selection of *Hevea brasiliensis*. *Tropical Abstracts,* **21**: 6.

Premakumari, D. (1992). *Variability, correlation and path coefficient analysis for yield in relation to anatomical characters in Hevea brasiliensis* (Willd.ex Adr.de Juss.). Ph.D. Thesis, University of Kerala, Trivandrum, 260 p.

Premakumari, D., George, P.J. and Panikkar, A.O.N. (1989). An attempt to improve test tapping in *Hevea* seedlings. *Placrosym* VII, Coonoor, India, 1986. *Journal of Plantation Crops*, 16 (Supplement): 383 - 387.

Premakumari, D. and Panikkar, A.O.N. (1984). Organographic Variability of Stomatal characters in *Hevea brasiliensis* Muell. Arg. and its possible significance in clonal susceptibility to leaf fall disease. *Proceedings of the International Rubber Conference,* 1984, Colombo, Sri Lanka, pp. 317 - 324.

Premakumari, D., Panikkar, A.O.N., Annamma, Y. and Leelamma, K.P. (1981). Studies on the cambial activity in *Hevea brasiliensis*, Muell. Arg. *Proceedings of PLACROSYM IV,* 1981, Mysore, pp. 425 - 430.

Premakumari, D., Marattukalam, J.G. and Panikkar, A.O.N. (1988). Influence of the orientation of laticifiers and quantity of laticiferous tissue on yield in *Hevea brasiliensis* Muell. Arg. *Journal of Plantation Crops*, **16**(1): 12 - 18.

Premakumari, D., Panikkar, A.O.N. and Marattukalam, J.G. (1992). Genetically controlled traits for disease escape in rubber. *Gregor Johann Mendel Birthday Lecture series and International Symposium,* 1992, Department of Botany, University of Calicut, India.

Prevot, J.C., Jacob, J.L. and Vidal, A. (1984). The redox potential of latex criterion of the physiological state of the laticiferous system. *Compte - Rendu du Colloque Exploitation physiologie et Amerlioration de I' Hevea,* 1984, Montpellier, France, pp. 227.

Ramanujam, S. and Thirumalachar, D.K. (1967). Genetic variability of certain characters in red pepper (*Capsicum annum L.*). *Mysore Journal of Agricultural Sciences*, **1**(1): 30 - 36.

Rao, A.N. (1975). Periodic changes in the cambial activity of *Hevea brasiliensis*. *Journal of the Indian Botanical Society*, **51:** 13 - 17.

Rebailler, D. (1972). *Quelques aspects du role des lutoides dans la physiologie et l'ecoulement du latex d' Hevea brasiliensis.* Thesis submitted to University of Abidjan.

Rubber Board (2003). *Indian Rubber Statistics.* V 26. Rubber Board, Kottayam, 93 p.

Rubber Research Institute of Malaysia (1971). Wintering. *Planters' Bulletin*, **117**: 295.

Rubber Research Institute of Malaysia (1992). SMR Bulletin. No. 7.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual.* Ed. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Sanderson, A.R. and Sutcliffe, H. (1921). *Brown Bast.* Rubber Grower's Association, London.

Sanderson, A.R. and Sutcliffe, H. (1929). Vegetative characters and yield of *Hevea. Journal of the Rubber Research Institute of Malaya,* **1**: 75 - 90.

Saraswathyamma, C.K. (1990). *Morphological, cytological and genetic investigation on induced and spontaneous male sterile clones of para rubber tree (Hevea brasiliensis Willd. ex Adr. de Juss.) Muell. Arg.* Ph.D. Thesis, University of Kerala, India, 176 p.

Saraswathyamma, C.K. (2002). Advances in crop improvement in *Hevea* in the traditional rubber growing tract of India. *Rubber Planters' Conference*, India 2002. Global competitiveness of Indian Rubber Plantation Industry, 21-22 November 2002, Rubber Board, Kottayam, pp. 47 - 49.

Saraswathyamma, C.K., Licy, J. and Marattukalam, J.G. (2000). Planting materials. In: *Natural Rubber: Agromanagement and Crop Processing.* (Eds. P.J. George and C. Kuruvilla Jacob). Rubber Research Institute of India, Kottayam, pp. 58 - 74.

Saraswathyamma, C.K. and Marattukalam, J.G. (1996). *Rubber Planting Materials: Development and Propagation.* Rubber Board, Kottayam, 114 p. (Malayalam).

Saraswathyamma, C.K., Marattukalam, J.G. and George, P.J. (1992). Introduction and performance of exotic clones in India. *Rubber Reporter*, **17**: 5 - 10.

Saraswathyamma, C.K., Markose, V.C., Licy, J., Annamma, Y., Panikkar, A.O.N. and Nair, V.K.B. (1980). Triploidy in *Hevea brasiliensis* Muell. Arg. *International Rubber Conference,* 1980, Kottayam, India.

Saraswathyamma, C.K.; Markose, V.C., Panikkar, A.O.N. (1981). Studies on fruit characteristics of *Hevea PLACROSYM IV,* Mysore, 1981.

Saraswathyamma, C.K. and Sethuraj, M.R. (1975). Clonal variation in latex flow characteristics and yield in the rubber. *Journal of Plantation Crops,* **3**: 14 - 15.

Schultez, R.E. (1970). The history of taxonomic studies in *Hevea*. *The Botanical Review,* **36**(3): 197 - 276.

Schultez, R.E. (1977). Wild *Hevea*: An untapped source of germplasm. *Journal of the Rubber Research Institute of Sri Lanka*, **54:** 227 - 257.

Schultez, R.E. (1987). Studies in the genus *Hevea*. 8. Notes on intraspecific variants of *Hevea brasiliensis* (Euphorbiaceae). *Economic Botany*, **41**(2): 125 - 147.

Schweizer, J. (1936). Over de physiologische bet cekins van latex van *Hevea brasiliensis*. 16th Annual meeting. V V Proefst. Pers. Djember.

Schweizer, J. (1941). *Hevea* plant material in Besoeki. *De Bergcultures,* **15**: 598 - 610.

Sebastian, T., Saraswathyamma, C.K. and Thomas, V. (2002). Morphological studies on seed coat of certain clones of *Hevea brasiliensis* Muell. Arg. using SEM. *Indian Journal of Natural Rubber Research,* **15**(1): 40 - 43.

Sethuraj, M.R. (1968). Studies on physiological aspects of rubber production. 1. Theoretical consideration and preliminary observations. *Rubber Board Bulletin*, **9**: 47 - 62.

Sethuraj, M.R. (1977). *Studies on the physiological factors influencing yield in Hevea brasiliensis.* Ph.D. Thesis, Banaras Hindu University, Banaras.

Sethuraj, M.R. (1981). Yield components in *Hevea brasiliensis:* Theoretical considerations. *Plant Cell and Environment*, **4**: 81 - 83.

Sethuraj, M.R. (1985). Physiology of growth and yield in *Hevea brasiliensis*. *Proceedings of the International Rubber Conference,* 1985, Kuala Lumpur, Malaysia, pp. 3 - 19.

Sethuraj, M.R. (1992). Yield Components in *Hevea brasiliensis*. In: *Natural Rubber: Biology, Cultivation and Technology.* (Eds. M.R. Sethuraj and N.M. Mathew). Elsevier, Amsterdam, pp. 137 - 163.

Sethuraj, M.R., Gururaja Rao, G. and Raghavendra, A.S. (1984). The pattern of latex flow from rubber tree *(Hevea brasiliensis)* in relation to water stress. Paper presented at the *Symposium on Cellular and Molecular Biology of Plant Stress,* Keystone, Colorado, 1984.

Sethuraj, M.R., Marattukalam, J.G., George, P.J. and Markose, V.C. (1974). Certain physiological parameters to determine the high yielding characteristics in rubber. Paper presented at the *IRRDB Scientific Symposium*, Part 1, Cochin, 1974.

Sethuraj, M.R. and Mathew, N.M. (1992). *Natural Rubber: Biology, Cultivation and Technology.* Elsevier, Amsterdam, 610 p.

Sethuraj, M.R. and Raghavendra, A.S. (1987). Rubber. In: *Tree Crop Physiology.* (Eds. M.R. Sethuraj and A.S Raghavendra). Elsevier, Amsterdam, pp. 193 - 223.

Sethuraj, M.R., Sulochanamma, S. and George, M.J. (1974). Influence of initial rate of latex flow rows of latex vessels and plugging index on the yield of the progenies of *Hevea brasiliensis*. Muell. Arg. derived from crosses involving Tjir 1 as the female parent. *Indian Journal of Agricultural Sciences*, **44**: 354 - 356.

Sethuraj, M.R., Usha Nair, N., George, M.J. and Sulochanamma, S. (1974b). Studies on the physiological aspects of yield stimulation in *Hevea brasiliensis.* Paper presented at the *IRRDB Scientific Symposium,* Part 1, Cochin, 1974.

Shoucai, C., Hansuang, S., Dong Qiong. H., Sheng, L. and Xueqin, Z. (1994). Identification of mildew resistance gene from *Hevea* tree by RAPD technique. *Chinese Journal of Tropical Crops*, **15:** 26.

Shylaraj, K.S. and Gopakumar, K. (1987). Testing for prepotency in coconut palm. *Agricultural Research Journal of Kerala,* **25**(2): 259 - 262.

Simmonds, N.W. (1969). Genetical basis of plant breeding. *Journal of the Rubber Research Institute of Malaya,* **21**: 1 - 10.

Simmonds, N.W. (1986a). The strategy of rubber tree breeding. *Proceedings of the International Rubber Conference,* 1985, Kuala Lumpur, Malayasia, pp. 115 - 126.

Simmonds, N.W. (1986b). Theoretical aspects of synthetic/polycross populations of rubber seedlings. *Journal of Natural Rubber Research,* **1**(1): 1 - 15.

Simmonds, N.W. (1989). Rubber breeding. In: *Rubber.* (Eds. C.C. Webster and W.J. Baulkwill). Longman Scientific and Technical, England, pp. 85 - 124.

Simpson, M.J.A. and Withers, L.A. (1986). Characterisation of plant genetic resources using isozyme electrophoresis: *A Guide to the Literature, International Board for Plant Genetic Resources*, Rome.

Singh, R.K. and Chaudhary, B.D. (1985). *Biometrical Methods in Quantitative Genetic analysis*. Kalyani Publishers, New Delhi, 318 p.

Sreelatha, S., Saraswathyamma, 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.

Stagracznski, R. and Kunst, K.G. (1993). Natural Rubber and Specialities. *Kautschuk Gummi Kunststoffe*, **46:** 1-2, 795 - 798

Subramaniam, A. (1975). Molecular weight and other properties of natural rubber: A study of clonal variations. *Proceedings of the International Rubber Conference,* 1975, Kuala Lumpur, Malaysia, **V 4,** pp. 3 - 27.

Sunderasan, E., Wickneswari, R., Ma. Zain Abd. Aziz and Yeang, H.Y. (1994). Incidence of self and cross pollination in two *Hevea brasiliensis* clones. *Journal of Natural Rubber Research,* **9** (4): 253.

Suresh, K.M. and Unnithan, V.K.G. (1996). A computer orientented interactive algorithm for clustering. *Indian Journal of Genetics and Plant Breeding,* **56:** 412 - 424.

Swaminathan, M.S. (1977). Recent trends in plant breeding with special reference to the improvement of the yield potential of rubber. *Journal of Rubber Research Institute of Sri Lanka,* **54:** 11 - 16.

Tanaka, Y. (1985). Structural characterization of Cis-polyisoprenes from sunflowed, *Hevea* and guayule. *Proceedings of the International Rubber Conference,* 1985, Kuala Lumpur, V **2**, pp. 73.

Tan, H. (1975). Estimate of parental combining abilities in rubber based on young seedling progeny Euphytica, **27**: 817 - 823.

Tan, H. (1987). Strategies in rubber tree breeding. In: *Improving Vegetatively Propagated Crops.* (Eds. Abbot and Atkin). Academic Press, London, pp. 27 - 62.

Tan, H. (1998). A study on nursery selection in *Hevea* breeding. *IRRDB Symposium on Natural Rubber (Hevea brasiliensis).* Vol. 1. *General, Soils and Fertilization, Breeding and Selection.* International Rubber Research and Development Board, Brickendonbury, U.K., pp. 114 - 120.

Tan, H., Mukerjee, T.K. and Subramaniam, S. (1975). Estimates of genetic parameters of certain characters in *Hevea brasiliensis. Theoretical and Applied Genetics*, **46:** 181 - 190.

Tan, H. and Subramaniam, S. (1976). A five-parent diallel cross analysis for certain characters of young *Hevea* seedlings. *Proceedings of the International Rubber Conference*, 1975, Kuala Lumpur, Malaysia, V **2**, pp. 13 - 26.

Tanksley, S.D. (1983). Gene mapping. In: *Isozymes in Plant Genetics and Breeding.* Part A. (Eds. S.D. Tanksley and T.J. Orton). Elsevier, Amsterdam, pp. 109 - 138.

Tanksley, S.D. and Orton, T. J. (1983). *Isozymes in Plant Genetics and Breeding.* Part A. Elsevier, Amsterdam, 516 p.

Tanksley, S.D. and Orton, T. J. (1983). *Isozymes in Plant Genetics and Breeding.* Part B. Elsevier, Amsterdam, 472 p.

Tanksley, S.D. and Orton, T.J. (1983). Molecular markers in plant breeding. *Plant Molecular Biology, Reporter,* **1**(1): 3 - 8.

Tata, S.J. (1980). Distribution of proteins between fractions of *Hevea* latex separated by ultracentrifugation. *Journal of the Rubber Research Institute of Malaysia*, **28**(2): 77.

Taylor, R.A. (1926). The interrelationship of yield and the various vegetative characters in *Hevea brasiliensis. Rubber Research Scheme Ceylon Bulletin*, **43**.

Templeton, J.K. (1968). Growth studies in *Hevea brasiliensis* I. Growth analysis upto seven years after budgrafting *Journal of the Rubber Research Institute of Malaya*, **20**(3): 136 - 146.

Templeton, J.K. (1969). Partition of assimilates. *Journal of the Rubber Research Institute of Malaya*, **21**(3): 259 - 263.

Thomas, V., Sailajadevi, T., Nair, R.B., Shankar, S. and Saraswathyamma, C.K. (2002). Seasonal activity of cambium and changes in bark structure of *Hevea brasiliensis. Indian Journal of Natural Rubber Research*, **15**(1): 55 - 65.

Tuy, L.M., Tran Thi Thuy Hoa, Lai Van Lam, Pham Hai Duong and Le Gia Trung Phuc (1997). The adaptation of promising rubber clones in the Central Highlands of Vietnam. *IRRDB Symposium on Natural Rubber (Hevea brasiliensis).* V **1.** General, Soils, Fertilization, Breeding and Selection session. Hochi Minh City, Vietnam, 14 - 15 October, 1997. pp. 155 - 163.

Van De Peer, Y. and De Wachter, R. (1994). TREECON for Windows: A software package for the construction and drawing of evolutionary trees for Microsoft Windows. *Computer Application in Biosciences*, **10**: 570 - 596.

Varghese, Y.A. (1992). Germplasm resources and genetic improvement. In: *Natural Rubber: Biology, Cultivation and Technology.* (Eds. M. R. Sethuraj and N. M. Mathew). Elsevier, Amsterdam, pp. 85 - 115.

Varghese, Y.A., John, A., Premakumari, D., Panikkar, A.O.N. and Sethuraj, M.R. (1993). Early evaluation in *Hevea*: Growth and yield at the juvenile phase. *Indian Journal of Natural Rubber Research*, **6**(1&2): 19 - 23.

Varghese, Y.A., Knaak, C., Sethuraj, M.R. and Ecke, W. (1997). Evaluation of random amplified polymorphic DNA (RAPD) markers in *Hevea brasilinesis*. *Plant Breeding*, **116**: 47 - 52.

Varghese, Y.A., Marattukalam J.G., Premakumari, D., Saraswathyamma, C.K., Licy, J. and Panikkar, A.O.N. (1990). Promising rubber planting materials with special reference to Indian clones. *Planters' Conference*, 1990, Kottayam, India, pp. 62 - 70.

Vellejos, 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.

Venkatachalam, P., Thomas, S., Priya, P., Thanseem, I., Gireesh, T., Saraswathyamma, C.K. and Thulaseedharan, A. (2002). Identification of DNA polymorphism among clones of *Hevea brasiliensis* (Muell.) Arg. using RAPD analysis. *Indian Journal of Natural Rubber Research*, **15**(2): 173 - 181.

Vischer, W. (1921). Over de resultaten verkregen met het oculeeren van *Hevea brasiliensis*. Op de onderneming pasir waringen (results obtained with budded trees of *Hevea brasiliensis* on pasir waringen estate). *Archief Voor De Rubber Cultuur Deel*, **5**: 17.

Vischer, W. (1922). Resultaten bereiki met oculaties van *Hevea brasiliensis* op de onderneming pasir waringen, een bijdrage tot de vraag. In Hoeverre aantal latex - vaten en productiviteit raskenmerken zijn (results obtained with buddings on the pasir warigen estate). *Archief Voor De Rubber Cultuur Deel*, **6**: 426.

Vollema, J.S. (1941). Over de invloed van het tappen op de diktegroel van *Hevea brasiliensis*. *Archief Voor de Rubber Cultuur inn Nederlandsch Indie,* **25**: 417 - 423.

Vollema, J.S. and Dijkman, M.J. (1939). Resultaten der toetsing van *Hevea* cloonen in den; proeftuin Tjiomas II. *Archief Voor de Rubber Cultuur inn Nederlandsch Indie,* **23:** 47 - 130.

Webster, C.C. and Baulkwill, W.J. (1989). *Rubber.* Longman Scientific and Technical, England, 614 p.

Webster, C.C. and Paardekooper, E.C. (1989). The botany of the rubber tree. In: *Rubber.* (Eds. C.C. Webster and W.J. Baulkwill). Longman Scientific and technical, England, pp. 57 - 84.

Whitby, G.S. (1919). Variation in *Hevea brasiliensis. Annals of Botany,* **31:** 313 - 332.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalsk, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research,* **18:** 6531 - 6535.

Wimalaratna, S.D. and Pathiratna, L.S.S. (1974). Observation on wintering in *Hevea. Rubber Research Institute of Sri Lanka Bulletin,* **9:** 5 - 9.

Wittshire, J.L. (1934). Variations in the composition of latex from clone and seedling rubber. *Journal of the Rubber Research Institute of Malaya,* **5:** 1.

Wright, H. (1998). *Para Rubber or Hevea brasiliensis: Its botany, cultivation, chemistry and diseases.* Biotech books, Delhi, 179 p.

Wycherley, P.R. (1968). Introduction of *Hevea* to the Orient. *Planters' Bulletin,* **44**: 127 - 137.

Wycherley, P.R. (1969). Breeding of *Hevea. Journal of the Rubber Research Institute of Malaya,* **21**: 38 - 55.

Wycherley, P.R. (1992). The genus *Hevea:* Botanical aspects. In: *Natural Rubber: Biology, Cultivation and Technology.* (Eds. M.R. Sethuraj and N.M. Mathew). Elsevier, Amsterdam, pp. 50 - 66.

Yeang, H.Y. (1988). Destabilization of *Hevea* latex by bark sat: Involvement of high density rubber particles in latex. *Journal of Natural Rubber Research.* V 3(2): 115 - 126 p.

Yeang, H.Y. and Paranjothy, K. (1982). Initial physiological changes in *Hevea* latex and latex flow characteristics associated with intensive tapping. *Journal of the Rubber Research Institute of Malaysia,* **30**: 31.

Yeang, H.Y., Sunderasan, E., Wickneswari, R., Napi, D. and Zamri, A.S.M. (1998). Genetic relatedness and identities of cultivated *Hevea* clones determined by isozymes. *Journal of Rubber Research,* **1**(1): 35 - 47.

Yeang, H.Y., Wickneswari, R., Sunderasan, E., Leong, S.K., Ahmad Asri Hussain, Mohamed Napi David, Zamri, A.S.M. and Mohamed Noor Abdul Ghani (1995). Isozymes in *Hevea* crop improvement. *IRRDB Symposium on Physiological and Molecular Aspects of the Breeding of Hevea bresiliensis,* 6 - 7 November 1995, pp. 52 - 57.

Yeet, Y.H., Ghandimathi, H. and Paranjothi, K. (1977). Protein and enzyme variation in some *Hevea* cultivars. *Journal of the Rubber Research Institute of Malaysia,* 25(1): 9 - 18.