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

A. Nursery Study

Materials

For the nursery study the materials used were

(1) Four types of seeds as stock sources.
   a. Assorted seeds, collected from the commercial seed sources at Kanyakumari region of the traditional rubber tract at South India. This was conventionally used in rubber nurseries for raising stock seedlings.
   b. Monoclonal seeds of three popular clones namely RRII 105, RRIM 600 and PB 28/59, readily available in Kanyakumari region, the seed source of South India. The seeds were collected from the central portion of large monoclonal areas.

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

(3) RRII 105, as scion clone. Green buds taken from the appropriate stage of green shoots collected from the green-shoot nursery raised in the central nursery of the Rubber Board, at Karikkattoor, were used for bud grafting.
(4) The bud grafted plants and budded stumps prepared after green budding.

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

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

**Lay out of the study**

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

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

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

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

At two to three whorl stage of growth the plants were transplanted to the field in a spacing recommended for bud-wood nursery (Punnose and Lakshmanan, 2000).
The plants were grown and maintained in the bud-wood nursery for two to three years and detailed studies on various aspects were undertaken.

**Design of the experiment**

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

**Observations**

**Seeds and Seedlings**

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

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

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

**Budding**

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

**Bud-grafted seedlings and budded stumps**

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

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

Position 1 - Two cm above the upper joint of the bud patch.
Position 2 - At the upper joint of the bud patch.
Position 3 - At the bud point of the bud patch.
Position 4 - At the lower joint of the bud patch.
Position 5 - Two cm below the lower joint of the bud patch.

The measurements of girth were taken using twine and scale.

Surface morphology of the graft area

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

Polybag Nursery

The observations taken from polybag nursery are

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

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

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

**Bud wood Nursery**

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

(1) Growth.

(2) Leaf diseases.

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

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

(5) Biochemical aspects.

(6) Molecular aspects.

(7) Test tap yield.
Growth

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

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

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

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

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

Test tapping was carried out by the incision method proposed by Annamma et al., (1989).
Bark anatomy of the graft union.

Sample collection

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

Processing of tissue and observations

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

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

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

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

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

Sample collection

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

Processing and observations

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

Yield recording

Yield recording was carried out using the method suggested by Annamma et al., (1989) for test tapping two to three year old rubber plants. The device used for test tapping have two blades, fixed parallel to each other on one side at a distance of 10 cm in between. The blades are fixed in such a way that the incisions made on the plant are at an angle of 25° to the horizontal.
By applying this device at a height position, 10 cm (same height on all plants), tapping was done. The latex oozed out from the incisions of each plant was collected separately on pre-dried and weighed blotting paper and dried in an oven. The dry weight was recorded and the dry weight of rubber was computed by reducing the paper weight from the dry weight of rubber with paper. Using the data on tree yield plot mean was calculated.

**Leaf Diseases**

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

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

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

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

Estimation of Peroxidase

Sample preparation

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

Preparation of Phosphate buffer

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

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

**Total Protein Estimation**

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

**Reagents used**

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

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

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

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

**Working Standard**

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

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

Detection of Esterase

Esterase enzyme activity was detected by Native PAGE gel.

Preparation of Gel

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

**Preparation of separating gel**

<table>
<thead>
<tr>
<th>Stock acrylamide solution</th>
<th>6.6 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris – HCL</td>
<td>4.0 ml</td>
</tr>
<tr>
<td>Water</td>
<td>9.0 ml</td>
</tr>
<tr>
<td>Ammonium persulphate solution</td>
<td>1 ml</td>
</tr>
<tr>
<td>TEDMED</td>
<td>10 micro lit.</td>
</tr>
</tbody>
</table>

After polymerization the combs were removed carefully.

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

**Sample Preparation**

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

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

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

Genetic analysis:

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

Sampling:

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

**Preparation of Genomic DNA**

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

**DNA amplification by PCR**

PCR was carried out in a 20μl reaction mixture containing 10-15 ng of template DNA, 250 nM of primer, 1.5mM mgCl₂, 100μM each of dATP, dGTP, dCTP and dTTP (Amersham-Pharmacia, UK), 0.5 unit of Taq DNA Polymerase enzyme and 1x reaction buffer. In order to avoid evaporation, the reaction mixture was overlaid with approximately 25 μl of mineral oil (Sigma, USA). Amplification was performed in 0.5 ml tubes placed in a 48-well thermal cycler (Perkin-Elmer DNA Thermal Cycler 480, USA). Tubes containing all the reaction components, except for the DNA template were included as a control for each primer used. The PCR programme included: a 4 min initial denaturation step at 94°C, 1 min denaturing at 94°C, 1.30 min at 38°C for annealing and 2.0 min at 72°C for extension. Thirty-five application cycles were performed and the last cycle was followed by 7 min at 72°C to ensure that primer extension reactions proceeded to completion. Eighty oligonucleotide random primers, each of 10 nucleotides long (Operon Technologies Inc; Alameda, CA, USA) were tested individually for the amplification of genomic DNA. Eight primers, which produced clear banding pattern after PCR amplification were selected for further RAPD analysis. In order to confirm whether the amplified products are reliable, amplification with each primer was repeated at least three times.
**Gel electrophoresis and photography**

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

**B. Observations on mature trees**

**Materials:**

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

**Observations**

**Total volume of latex:**

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

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

\[
\text{Oven-dry weight of rubber} \times 100
\]

\[
20
\]

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

Summer variations:

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

Growth characters:

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

**Anatomical characters:**

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

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

The characters recorded are: (plate-5).

1. Bark thickness
2. Thickness of the soft bast.

3. Number of latex vessel rows.

4. Density and width of latex vessels.

5. Height and width of phloem rays.

6. The numbers of tanniferous and untanniferous cells per unit area.

The percentage of tanniferous cells, out of total cells, in a unit area of 2.5mm² and the percentage of hard bast thickness out of total bark thickness were assessed. The ratio of ray height to the ray width was also computed which is an indicator of the inclination of axially running tissue.

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

The anatomical characters recorded from the stem are

1. Number of primary xylem points.

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

**Tapping panel dryness**

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

**Statistical Analysis**

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