2. MATERIALS AND METHODS
Fig. 2.1. Map of Kerala State showing the study locations
2. Materials and Methods

2.1. Source of the material

Coconut ecotypes identified were mainly tall palms and the selection was made in the farmers holdings based on the scoring of the farmers participatory survey. The study was taken during the period 1999 to 2002. Ecotypes were selected in Thiruvampady panchayat in Kozhikode district, Chazhur panchayat in Trichur, Chemparakky and Neriyanangalam in Ernakulam district, Pathiyoor panchayat in Alleppey and Thazhava panchayat in Quilon district (Figures 2.1 & 2.4 to 2.8). In all the locations local West Coast Tall (WCT) were selected as check in order to make comparison in performance. All the ecotypes have attained yield stability and are grown in rainfed condition with average management practices. The area, production and productivity of coconut in the selected districts are depicted in Fig. 2.2 & 2.3. The samples selected under the study with abbreviations are given in table 2.1.

<table>
<thead>
<tr>
<th>Ecotype</th>
<th>Variety</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuttiyadi Tall (KT)</td>
<td>Tall</td>
<td>Thiruvampady, Kozhikode</td>
</tr>
<tr>
<td>West Coast Tall (WCT 1)</td>
<td>Tall</td>
<td>Thiruvampady, Kozhikode</td>
</tr>
<tr>
<td>King Coconut (KC)</td>
<td>Tall</td>
<td>Chazhur, Trichur</td>
</tr>
<tr>
<td>West Coast Tall (WCT 2)</td>
<td>Tall</td>
<td>Chazhur, Trichur</td>
</tr>
<tr>
<td>Elite Tall (ET)</td>
<td>Tall</td>
<td>Chemparakky, Ernakulam</td>
</tr>
<tr>
<td>Chowghat Green Dwarf (CGD)</td>
<td>Dwarf</td>
<td>Neriyanangalam, Ernakulam</td>
</tr>
<tr>
<td>West Coast Tall (WCT 3)</td>
<td>Tall</td>
<td>Neriyanangalam, Ernakulam</td>
</tr>
<tr>
<td>Jappanan (JPN)</td>
<td>Tall</td>
<td>Pathiyoor, Alleppey</td>
</tr>
<tr>
<td>West Coast Tall (WCT 4)</td>
<td>Tall</td>
<td>Pathiyoor, Alleppey</td>
</tr>
<tr>
<td>Komadan (KO)</td>
<td>Tall</td>
<td>Thazhava, Quilon</td>
</tr>
</tbody>
</table>
Area Production

Fig. 2.2. Share of area and production of coconut in selected districts in Kerala

Fig. 2.3. Productivity of coconut in selected districts of Kerala
2.1.1. Agro-Meteorological Variables

The ecotypes under study in Kerala are grown in different agro-climatic conditions. The climatic and soil conditions in the state of Kerala, in general, is congenial to coconut crop. Situated in the tropical region, the average annual rainfall in the state for the last ten years is 2900 mm. The average rainfall during the period under study was 2838 mm. The average maximum and minimum temperature in the state was 33.6°C and 22.6°C respectively.

The district of Kozhikode lies between north latitude 11° 7' 22'' and 11° 48' 32'' and east longitude 75°35'58'' and 76° 8'20''. The average rainfall was 4054 mm. In Thiruvampady the general terrain of land is sloppy from east to west and the soil type is red loam. The average rainfall was 3667 mm. The average temperature and humidity recorded was 27°C and 81% respectively.

The Chazhoor, in Trichur district was ideal for coconut. Average rainfall is over 3000 mm and the soil type is clayey loam. The temperature and humidity recorded were 28°C and 78 per cent respectively during the period.
Fig. 2.4. Thiruvampady panchayat in Kozhikode district
Fig. 2.5. Chazhur panchayat in Trichur district
Fig. 2.6. Chemparakky and Neriamangalam in Ernakulam district
Fig. 2.7. Pathiyoor panchayat in Alleppey district
Fig. 2.8. Thazhava panchayat in Quilon district
Ernakulam district is located between latitudes of 9°47' and 10°17' and longitudes 76°9' and 76°47'. The average rainfall in the district was 3499 mm. The districts consists of three natural divisions viz. lowland (below 25 feet from the mean sea level), midland (between 25 feet and 250 feet) and highland (250 feet and above). Chemparaky and Neriamangalam, the locations of the samples selected under the study are situated in the midland and hilly region respectively. The soil is laterite and average rainfall recorded during the study period was 3400 mm and the temperature and humidity were 28°C and 78 per cent respectively.

The Evoor in Pathiyoor panchayat is located in Alleppey district. The topography of the land is flat and the soil type is sandy loam. The average annual rainfall was around 3000mm during the study period. The average temperature noted was 29°C and humidity recorded is 66%.

In Thazhava in Quilon district, the terrain is flat and the elevation is 3m from the sea level. The average rainfall was 3250 mm. The soil type is sandy loam. The average temperature and humidity recorded during the period under study was 29°C and 64 % respectively. Average rainfall in the study areas and the state average for the years 1998, 1999 and 2000 is given in table 2.2.
Table 2.2. Average rainfall in mm in the study areas

<table>
<thead>
<tr>
<th>Districts</th>
<th>Year 2000</th>
<th>Year 1999</th>
<th>Year 1998</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quilon</td>
<td>2352.000</td>
<td>2885.100</td>
<td>2526.000</td>
<td>2587.700</td>
</tr>
<tr>
<td>Ernakulam</td>
<td>2657.300</td>
<td>3048.200</td>
<td>3307.200</td>
<td>3004.230</td>
</tr>
<tr>
<td>Aleppey</td>
<td>2638.300</td>
<td>3104.600</td>
<td>3100.800</td>
<td>2947.900</td>
</tr>
<tr>
<td>Trichur</td>
<td>2072.800</td>
<td>2761.300</td>
<td>3358.700</td>
<td>2730.930</td>
</tr>
<tr>
<td>Kozhikode</td>
<td>2529.100</td>
<td>2817.700</td>
<td>3382.200</td>
<td>2909.670</td>
</tr>
<tr>
<td>State average</td>
<td>2464.800</td>
<td>2869.557</td>
<td>3178.943</td>
<td>2837.770</td>
</tr>
</tbody>
</table>

2.1.2. Experimental Stages

In the initial stage a farmer participatory survey was conducted at nine centres representing the three major agro-climatic regions of the state. Areas known for genetically superior, high yielding cultivars or eco-types that are valued by farmers were identified as the study centres. In each location 20 to 25 farmers were interacted and required details collected on the identified eco-types. Samples were identified from these different regions of the state viz. Northern, Central and Southern. Morphological data were recorded based on the criteria adopted by the CPCRI in the Coconut Descriptors. Observations on growth, dry matter production, yield and yield components were carried out once in a year during the summer months. Biochemical analysis of tender nut water and percentage of oil in copra, free fatty acid (FFA) etc. was made for all samples annually on quarterly basis.
The procedure was repeated for three consecutive years 1999, 2000 and 2001 and the mean for the different stages during the years have been taken for the interpretation of the results. The schedule of sampling is given in table 2.3.

Table 2.3. Schedule of sampling

<table>
<thead>
<tr>
<th>Month</th>
<th>Year</th>
<th>Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>December</td>
<td>1999</td>
<td>12-8-99 to 25.11.99</td>
</tr>
<tr>
<td>February</td>
<td>2000</td>
<td>1-12-99 to 7.1.2000</td>
</tr>
<tr>
<td>March</td>
<td>2000</td>
<td>29.2.200 to 18.4.2000</td>
</tr>
<tr>
<td>June</td>
<td>2000</td>
<td>28.7.200 to 1.8.2000</td>
</tr>
<tr>
<td>August</td>
<td>2000</td>
<td>29.8.200 to 2.9.2000</td>
</tr>
<tr>
<td>March</td>
<td>2001</td>
<td>20.3.2001 to 4.4.2001</td>
</tr>
</tbody>
</table>
2.1.3. Sampling

The physiological and biochemical investigations were carried out in the 6th and 14th leaf from the top (Rajagopal et al., 1986). Four leaflets, two from each side of the rachis were collected and thoroughly cleaned with wet cotton before using for biochemical investigations. For biochemical studies sampling was done from three palms, each palm treated as a single replication and from each, duplicate samples were taken for the analysis and mean of the replications were used for the interpretation. For growth and dry matter production five palms per ecotype were selected for the observations.

2.2. Physiological parameters

2.2.1. Growth and dry matter production

a. Leaf: Growth parameters like total leaf length, breadth, leaflets leaf\(^1\), total number of leaves in the crown were recorded and the total leaf dry weight was computed non-destructively (Ramadasan and Jacob Mathew, 1987). Leaf area (LA) and leaf dry weight were determined from the total number of leaflets and by the destructive sampling of six leaflets from the middle portion of the leaf and drying to constant weight at 80\(^\circ\)C. Using the regression equations \(Y = -1.3274 + 0.049474 + 0.0192 X2\) and \(Y = -3.438 + 0.0197 (X1) + 0.0202 X2\) where \(X1 = \) dry wt. of six leaflets and \(X2 = \) number of leaflets, the LA and leaf dry weight of a single leaf were
obtained respectively. By multiplying this with the total number of leaves total LA and total dry weight was computed.

b. Stem: Annual increment in stem height and number of leaf scars in this portion were recorded once a year and annual stem dry weight was calculated non-destructively following the method of Ramadasan and Jacob Mathew (1987). The portion of the stem just below the crown was marked with red paint during March 2000. After 12 months the increase on the height of palms was measured and the number of leaf scars from this portion was counted. By using the regression equation $Y = -113.44 + 93.67X$ where $X$ is the height of three leaf scar segment, the annual stem dry matter (DM) production was calculated.

From the total leaf and stem dry weights Vegetative Dry Matter (VDM) production was calculated and expressed in kilograms.

2.3. Yield and Yield Attributes:

Observations on number of bunches produced, female flower production and total nuts harvested year$^{-1}$ were recorded. The total dry weight of the nut was determined for each experimental palm, once in three months year$^{-1}$ (4 nuts palm$^{-1}$). After dehusking the dry weight of husk, shell and copra were determined based on fresh weight dry weight ratios. From the dry weights of whole nut and whole copra, the dry weights of total nuts
as well as copra produced year\(^{-1}\) were computed. The bunches and spathes were also collected and dry weights determined by drying in hot air oven at 80\(^{\circ}\)C. By adding the dry weights of nuts, bunches and spathes produced year\(^{-1}\), the annual reproductive dry matter (RDM) production was calculated and is expressed in kilograms (Coombs and Hall, 1982).

2.4. Harvest index

The harvest index was calculated based on the total nut dry matter and the total copra yield, to the total dry matter produced per year. It is the ratio of the total economic yield to the total biomass production.

2.5. Bio-Chemical Parameters

Tender nut water analysis was made for the 6-7 months old nuts from all ecotypes. Four nuts per palm and six palms per ecotype were taken for study. Before making chemical analysis, quantity of water was measured in ml. Sweetness of water and soft meat were tested by organoleptic method.

2.5.1. Total Sugar

Total sugar content in tender nut water estimated by phenol-sulphuric acid method of Dubois et al (1951) and expressed in gm/100ml. 0.5 ml of the sample solution (0.1 ml nut water made upto 100 ml) is added with 0.5 ml 96% sulphuric acid. Working standards (10ml of 2mg / ml glucose
standard 0.2, 0.4, 0.6, 0.8 and 1 ml) were taken and made upto 1 ml with water and added 1 ml 5% phenol and 5 ml sulphuric acid, mixed for 20 minutes and read the absolute sugar at 490 nm.

A blank was run with 1 ml water, 1 ml 5% phenol and 5 ml sulphuric acid.

2.5.2. Reducing Sugars

Reducing sugars was estimated by Somogyi (1952) method. 0.8 ml of the sample (0.1ml nut water were made upto 100 ml) was added to 1.2 ml of water and 1 ml of alcoholic copper reagent. A series of standards 0.4, 0.8, 1.2, 1.6 and 2.0 ml were taken. Boiled in boiling water bath for 10 minutes and then cooled. 1ml of arsenomolybdate solution and 6ml of distilled water were added. Absorbance read at 620 nm.

A sample blank was run with 2ml water with 1 ml alcoholic copper reagent.

2.5.3. Potassium Content

Potassium content was estimated using a flame photometer (Jackson 1973) and expressed in ppm. 1ml of the nut water was diluted to 50 ml with distilled water. Value calculated by the formula:
\[
\frac{r \times 50}{1} \text{ (dilution factor)} \times \text{graph slope} = \frac{(\text{Total standard conc}^n)}{(\text{Total read conc}^n)}
\]

2.5.4. Total Free Amino Acids

Free amino acid content was estimated following the method developed by Yapenlee and Tahahashi (1966) and expressed in mg/100ml. 0.05ml nut water was added to 0.15ml distilled water as test solution. Working standards of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml was made upto 1 ml with distilled water. To this, 3.8 ml of assay medium was added in blank (2ml of distilled water) test and standards kept in boiling water bath for 12 minutes and then cooled. The absorbance read at 570 nm.

2.5.5. Leaf Polyphenol Content

Total leaf polyphenol was estimated in middle leaflets of 6th leaf using the Folin Ciocalteu method (Bray and Thorpe 1954). The estimation was based on the reaction between phenols and an oxidizing agent phosphomolybdate which resulted in the formation of a blue complex.

One ml of the alcoholic extract of middle leaflets of 6th leaf was pipetted out in a graduated test tube, and added 1ml of Folin ciocalteu reagent (Folin – ciocalteu reagent was prepared by mixing 100 g sodium tartarate, 25g sodium molybdate in 700 ml water in a flask, 50 ml of 85%
phosphoric acid and 100 ml conc HCl and boiled under reflex gently for 10 hours. Cooled and then added 150 g lithium sulphate dissolved in 50ml water and 4-5 drops of liquid bromine. Boiled the mixture without condenser for 15 minutes to remove excess bromine. Cooled and diluted to volume with water and filter. Reagent was golden yellow in colour and stable for a long period while storing) followed by 2 ml of 20% Na₂CO₃ solution and shook. The blue solution was diluted to 20ml with water and measured its absorbance at 650 nm.

A blank was run containing all the reagents devoid of plant extract.

2.5.6. Oil Percentage

The oil percentage in each sample was estimated from the copra made from the fresh nuts collected from the selected ecotypes using the Soxhlets apparatus (Anonymous, 1986). 10 gm of the dried kernel, free from foreign matter was placed in a porcelain pestle and mortar and ground well. This ground sample was placed in a thimble and covered with two pieces of absorbent cotton. This thimble was placed in the extractor of a Soxhlets apparatus with a capacity of 250 cc flask. Filled the flask with 150 ml petroleum ether of 60-80 Boiling Point. The apparatus was then fitted with the condenser and heated on a hot plate controlling the heat. The solvent from the condenser dropped on the center of the thimble, at the rate of 130-150 drops per minute. The process continued for 4 hrs. Cooled and
disconnected the flask by removing all the solvent of the thimble in the flask. The thimble was then removed from the extract and the apparatus refitted for recovering the solvent from the flask containing oil and solvent. The apparatus was then disconnected. The flask was gently heated and blew dry air by means of a foot-bellow and smelled till no odour of petroleum ether remained. Cooled the flask in a desiccator. After cooling weighed it accurately and oil percentage calculated using the formula,

\[
\text{Oil} \% = \frac{\text{Wt. of oil} \times 100}{\text{Wt. of the sample}}
\]

2.5.7. Acid value and Free Fatty Acid (FFA)

The acid value of coconut oil was determined by directly titrating the material in an alcoholic medium with aqueous sodium hydroxide solution (Anonymous, 1986). Free Fatty Acid was calculated as oleic, lauric, ricinolic and palmitic acids.

50 ml of cooled coconut oil was weighed in a 200 ml conical flask. To this 100 ml freshly neutralised hot ethyl alcohol and 1 ml phenolphthalein indicator solution was added. The mixture was boiled for about 5 minutes and titrated with standard aqueous alkali solution (NaOH) on vigorous shaking. Acid value was calculated using the formula:

\[
\text{Acid value} = \frac{56.1 \ VN}{W} \quad \text{where,}
\]

\[
V = \text{Volume in ml of standard sodium}
\]
hydroxide solution used,

\[ N = \text{Normality of standard sodium hydroxide solution,} \]

\[ W = \text{Weight in g of the material taken for test.} \]

Free Fatty Acid (FFA) – The acidity is frequently expressed as the percentage of free fatty acids present in the sample. The percentage of FFA was calculated in terms of palmitic acid (Anonymous, 1986) using the formula:

\[
\text{FFA} = \frac{25.6 \cdot VN}{W}
\]

where,

\[ V = \text{Volume in ml of standard sodium hydroxide solution used,} \]

\[ N = \text{Normality of standard sodium hydroxide solution,} \]

\[ W = \text{Weight in g of the material taken for test.} \]