3. MATERIALS AND METHODS

3.1. General

The present investigation entitled “Performance of different Casuarina species and its half sib population in Northern dry Zone of Karnataka” was conducted at Regional Agricultural Research Station, Gangavati during March 2004 to September 2009. Ten competitive candidate trees were selected from each *Casuarina* species. The details of plant material in the present investigation are furnished in Table 2.

3.1.1. Climate

Regional Agricultural Research Station (RARS), Gangavati, is situated in the North East Dry Zone of Karnataka at 16° 15’ N latitude and 77° 21’ E longitude with an altitude of 389 meters above the mean sea level and an average rainfall of 660 mm. The monthly rainfall data for the year 2008 was obtained from the meteorological observatory of Regional Agricultural Research Station, Gangavati in Raichur district (Table 1).

3.1.2. Experimental material

The experiment material for present study consisted of known provenance of five different *Casuarina* species viz., *Casuarina equisetifolia*, *Casuarina obesa*, *Casuarina glauca*, *Casuarina cunninghamiana* and *Casuarina cristata* planted during the year 1998-99 at Regional Agricultural Research Station, Gangavati. The plantation is in Randomized Block Design, with four replications in black cotton soil, which comprises ten trees in each the five species of *Casuarina*. Properties of black cotton soil as follows viz., bulk density (1.38), soil pH (8.31), soil EC (1.17 dS/ml), nitrogen (134.14 kg ha$^{-1}$), phosphorous (15.33 kg ha$^{-1}$) and potassium (401.75 kg ha$^{-1}$). Trees were planted at spacing of 2.5 m. X 2.5 m. (line X rows) in square planting design (Fig. 1). The description of the species is presented in Table 2.
Table 1. Weather data for *Casuarina* plantation site at Gangavati, Northern dry zone of Karnataka

<table>
<thead>
<tr>
<th>Month</th>
<th>Rain fall (mm)</th>
<th>Temperature (°C)</th>
<th>Relative Humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum</td>
<td>Minimum</td>
<td>8 am.</td>
</tr>
<tr>
<td>Apr.07</td>
<td>14.3</td>
<td>3.5</td>
<td>38.85</td>
</tr>
<tr>
<td>May</td>
<td>29.30</td>
<td>97.7</td>
<td>38.79</td>
</tr>
<tr>
<td>June</td>
<td>66.71</td>
<td>164.0</td>
<td>35.70</td>
</tr>
<tr>
<td>July</td>
<td>64.87</td>
<td>19.5</td>
<td>32.69</td>
</tr>
<tr>
<td>August</td>
<td>87.58</td>
<td>74.75</td>
<td>29.67</td>
</tr>
<tr>
<td>September</td>
<td>122.25</td>
<td>405.75</td>
<td>30.77</td>
</tr>
<tr>
<td>October</td>
<td>120.21</td>
<td>56.25</td>
<td>29.65</td>
</tr>
<tr>
<td>November</td>
<td>26.71</td>
<td>--</td>
<td>30.27</td>
</tr>
<tr>
<td>December</td>
<td>11.63</td>
<td>--</td>
<td>29.84</td>
</tr>
<tr>
<td>January-08</td>
<td>0.84</td>
<td>--</td>
<td>30.58</td>
</tr>
<tr>
<td>February</td>
<td>0.79</td>
<td>5.0</td>
<td>32.66</td>
</tr>
<tr>
<td>March</td>
<td>0.66</td>
<td>190.2</td>
<td>34.94</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>546.91</strong></td>
<td><strong>1016.65</strong></td>
<td></td>
</tr>
<tr>
<td>RI</td>
<td>RII</td>
<td>RIII</td>
<td>RIV</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>------</td>
<td>-----</td>
</tr>
<tr>
<td>φ φ</td>
<td>Ω Ω</td>
<td>Ψ Ψ</td>
<td>Α Α</td>
</tr>
<tr>
<td>Ζ Ζ</td>
<td>φ φ</td>
<td>Α Α</td>
<td>Ψ Ψ</td>
</tr>
<tr>
<td>Ψ Ψ</td>
<td>Α Α</td>
<td>Ζ Ζ</td>
<td>Ω Ω</td>
</tr>
<tr>
<td>Ω Ω</td>
<td>Ψ Ψ</td>
<td>φ φ</td>
<td>Ζ Ζ</td>
</tr>
<tr>
<td>Α Α</td>
<td>Ζ Ζ</td>
<td>Ω Ω</td>
<td>φ φ</td>
</tr>
</tbody>
</table>

Fig. 1 Plan layout of the experiment of five *Casuarina* species
Table 2. Description of characters of different *Casuarina* species

<table>
<thead>
<tr>
<th>Casuarina species</th>
<th>Number of leaves (teeth)</th>
<th>Type of tree</th>
<th>Male flower</th>
<th>Female cones</th>
<th>Seeds</th>
<th>Tree habits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A) <em>C. equisetifolia L.</em> (Wilson and Johnson)</td>
<td>17-50, occasionally 6, erect 0.3-0.8 cm diameter, needle 17-28 cm long.</td>
<td>Dioecious</td>
<td>7.0-40 mm long &amp; 7-11.5 whorls cm⁻¹</td>
<td>Peduncle 3-13 mm long, cone 10-24 mm long, 9-13 mm dia &amp; ridges 16-17.</td>
<td>5-10 mm long, 0.4-0.8 dia, 3-4 mm width</td>
<td>Stem furrows usually densely pubescent. Height 12.75 – 17.5 m.</td>
</tr>
<tr>
<td>1B) <em>C. equisetifolia L.</em> (Woodall and Geary)</td>
<td>18-48, mostly 7, occasionally brown tipped, but becoming whiter towards the tip and edges, needle 13-25 cm long</td>
<td>Monoecious</td>
<td>6.0-25.5 mm long &amp; 7-9.5 whorls cm⁻¹</td>
<td>10-20 mm dia, mostly 16, slightly longer than wide &amp; cone 18-23 mm long.</td>
<td>5-9 mm long, 0.3-0.7 dia, 3-4 mm width</td>
<td>Longitudinal rib sharp, prominent stomata trench usually filled with hairs &amp; Height 12.25 – 15.0 m.</td>
</tr>
<tr>
<td>2) <em>C. obesa</em> (Miq.)</td>
<td>Small, erect, leaf teeth are Slender, and occur in whorls of 22-29 and needle 8.5-21 cm long</td>
<td>Dioecious</td>
<td>--</td>
<td>Cone 11-20 mm long, cones 9-15.25 dia &amp; ridges 14-15.</td>
<td>5-9 mm long, 0.3-0.7 dia, 2-3 mm width</td>
<td>Erect tree, 1.5-10 m tall with a width of 4 m. There are separate male and female trees.</td>
</tr>
<tr>
<td>3) <em>C. glauca</em> (Wilson and Johnson)</td>
<td>18-37, erect 0.6-0.9 mm long, usually marc scent and needle 6.5-18.5 cm long.</td>
<td>Dioecious</td>
<td>--</td>
<td>Peduncle 3-12mm long, cone 9-18 mm long, 9-14.5 mm dia &amp; ridges 15.</td>
<td>4-6.5 mm long, 0.3-0.5 dia, 2.5-3.0 mm width</td>
<td>Branchlets long to 38mm. Branch let ridges broad, flat or slightly rounded. Brown band at base of teeth. Tree height 11.50 – 13.25 m.</td>
</tr>
<tr>
<td>4) <em>C. cunninghamiana</em> Miq.</td>
<td>17-36, sharply pointed, mostly apprised, central portion brown with gray tip and needle 16.5–23.5 cm long.</td>
<td>Dioecious</td>
<td>8-40 mm long &amp; 8-10.5 whorls cm⁻¹</td>
<td>Cone 6-10 mm dia, 18-23.5 mm length &amp; ridges 15 – 16.</td>
<td>6-12 mm long, 0.4-0.7 dia, 3-4 mm width</td>
<td>Branch et ridges narrow and prominently angular. Teeth tips browned. Tree height 11.00 – 13.75 m.</td>
</tr>
<tr>
<td>5) <em>C. cristata</em> (Miq.)</td>
<td>16-29, sharply pointed, mostly apprised and needle 9.5-18.5 cm long.</td>
<td>Dioecious</td>
<td>--</td>
<td>Cones 11-18 mm dia &amp; cones 15 mm 21 mm long.</td>
<td>5-8 mm long, 0.3-0.6 dia, 3-4 mm width</td>
<td>Erect tree 1.5-10 m tall with a width of 4 m. Tree height 10.75 – 13.0 m.</td>
</tr>
</tbody>
</table>
3.2. Field observations on quantitative characters
Following observations were recorded on the quantitative and qualitative characters of 10 candidate trees of each Casuarina species randomly taken from four replications and the average values were computed as species means for each replication.

3.2.1. Total tree height (m)
Tree height was measured from ground level to tip of the tree by using ravi- altimeter based as per Chaturvedi and Khanna (1982) and expressed in meter.

3.2.2. Clear bole height (m)
Clear bole height of tree from ground level up to the first green branch of the trunk was measured using ravi-altimeter as per Chaturvedi and Khanna (1982) and expressed in meters.

3.2.3. Diameter at breast height (cm)
Diameter was measured by using the tree calliper at breast height of the bole as per Chaturvedi and Khanna (1982) and expressed in centimeters.

3.2.4. Number of branches tree\(^{-1}\)
Total numbers of live branches arising from the main stem were counted.

3.2.5. Crown area (m\(^2\))
Crown area was computed by measuring the spread of crown identified by the drip line on the ground, along two axes at right angles to each other from the base of the trunk. The average of the two components of both the axes were used to compute the correct width by simple multiplication as in the case of a square or rectangle (length X breadth) and expressed in square meters as per Schmidt (1993).

3.2.6. Bark thickness (mm)
Bark thickness was measured by using a vernier callipers correct to first decimal place.

3.2.7. Needle length (cm)
Average length of 50 randomly selected needles from middle part of the tree was measured and expressed in centimeters.

3.2.8. Number of inter nodes needle\(^{-1}\)
The number of inter nodes per needle were counted.

3.2.9. Volume of tree (m\(^3\))
Volume of tree was estimated by using formula as per Chaturvedi and Khanna (1982).
Volume (m³ tree⁻¹) = Height X Basel area X Form factor
where,

\[
\text{Basel area (m}^2) = \frac{g^2}{4 \pi}
\]

3.2.10. Volume index

Volume index tree⁻¹ = (Diameter (cm))² x Height (cm)

(Hatchet, 1985 and Manavalan, 1990)

3.2.11. Tree biomass

Biomass yield tree⁻¹ was estimated by using formula as per Anonymous (2004)

Biomass (kg tree⁻¹) = Density of wood X volume of tree

3.2.1. Field observation on qualitative characters

3.2.1.1. Branching habits (Denpb)

The density and appearance of the branches were estimated on a 1 – 4 scale as per Pinyopusarerk et al., (2004).

1 = very high- regular branching with internodes length around 15 cm
2 = high- irregular branching with internodes length mainly around 15 cm
3 = low - irregular branching with internodes length mainly around 30 cm
4 = very low - irregular branching with internodes length generally > 30 cm

3.2.1.2. Stem straightness (Strut)

Stem form corresponded to the extent of straightness of the stem depending on the number of bends observed. Stem form was estimated on 1 – 6 scale as described by Pinyopusarerk et al., (2004).

1 = Not vertical with more than two bends
2 = roughly vertical with more than two bends
3 = not vertical with one to two bends
4 = roughly vertical with one to two bends
5 = roughly vertical and straight
6 = completely vertical and straight
3.2.1.3. Angle of branching
The angle made by the primary, secondary and tertiary branches correspondingly with the main trunk, primary and secondary branches respectively was assessed on a 1 – 2 scale as per Pinyopusarerk et al., (2004).

Scale 1 = < 60 degree, 2 = > 60 degree

3.2.1.4. Length of branches
Lengths of the branches were estimated on a 1 – 4 scale as per Pinyopusarerk et al., (2004).

1 = long - generally > one - quarter of total height
2 = short - generally < one - quarter of total height

3.2.1.5. Health
Stem and foliage were assessed as healthy or unhealthy, while damage normally caused either by insect or disease was recorded as per Pinyopusarerk et al., (2004)

3.2.1.6. Stem health
Stem assessed as healthy or unhealthy based on a 1-2 scale.
Stem classes damage 1= yes (healthy), 2 = No (unhealthy)

3.2.1.7. Foliage health
Foliage assessed as healthy or unhealthy based on a 1-2 scale.
Foliage classes damage scale, 1= yes, 2 = No

3.2.1.8. Flowering
Male and female trees flower blooming was observed based on scale as per Pinyopusarerk et al., (2004).

Flowering scale, 0: No flowering, 1: Flowering

3.2.1.9. Fruiting
Commencement of fruiting was estimated on a 1–2 scale as per Pinyopusarerk et al., (2004).

Fruiting scale- 0: No fruiting, 1: Fruiting

3.2.1.10. Survival percentage
The actual number of trees survived after planting in field were counted and expressed as survival per cent from the total number of seedlings planted.

3.2.2. Observation on physical properties of wood
3.2.2.1. Specific gravity
Rectangular wood specimens of size 2 X 2 cm in cross-section and 6 cm length was made. These were weighed and volume was calculated by multiplying all three dimensions as per IS (1986).

\[
\text{Specific gravity} = \frac{\text{Weight in g of test specimen}}{\text{Volume in cm}^3 \text{ of test specimen}}
\]

3.2.2.2. Green wood density (g/cm³)
Wood density of test specimens were determined as per IS (1986)

\[
\text{Wood density (g/cm}^3\) = \frac{\text{Mass}}{\text{Volume}}
\]

Where, Mass = Weight in g. of fresh test specimen
Volume = Volume in cm³ of test specimen

3.2.2.3. Dry wood density (g/cm³)
Test specimen of oven dried wood was used for wood density determination as per IS (1986)

\[
\text{Wood density (g/cm}^3\) = \frac{\text{Mass}}{\text{Volume}}
\]

3.2.2.4. Calorific value (kcal kg⁻¹)
An isothermal oxygen bomb calorimeter conforming to the requirements of Indian Standard Institution (IS:1359-1959), British Standards (BS 1016 : part 5:1967) and the Indian Institute of Petroleum (IP 12/63T) was used to determine the test calorific value of different Casuarina species.

Procedure: Initially, the cleaned and dried crucible was weighed and one g of wood sample was added. A piece of fuse wire made of platinum was tied across the two electrodes such that the tip of the wire was just above the surface of the sample. A cotton thread of length 15 cm was rounded over the fuse wire. Two milliliter of distilled water was poured in the bomb. The lid of the bomb was lightened by hand and bomb was charged by oxygen without replacing the inside air until pressure gauge indicated 25 kg/sq cm. The charged bomb was checked for leakage and placed inside the calorimeter vessel, which was then placed in the calorimeter bucket. A known amount of water was poured in the vessel until the bomb was completely
submerged except its terminals. Bomb terminals were connected to the ignition circuit through firing unit of the calorimeter and over plate was placed along with the thermometer and stirrer which was rotated at 600 rpm. The vibrator and magnifier units of the calorimeter were set and apparatus as kept idle for 10 minutes. The charge filled in the bomb as fired by closing the circuit and temperature was recorded for every one minute interval. The temperature was recorded until the rate of change of temperature becomes a constant. Thereafter, the decreasing temperature was recorded for five minutes at the interval of one minute each.

The temperature values obtained were recorded in three periods’ viz., preliminary period, chief period and after period. The wood test procedure was initiated for three times for each test sample and the arrange values were tabulated.

**The calorific values of wood test samples were calculated as follows**

Calorific value = Heat liberated - Nitrogen correction - sulphur correction - correction for heat of ignition (cal)

Heat liberated = Effective mean heat capacity of calorimeter x corrected temperature rise

$$\text{Heat liberated} = \text{EMPHC} \times \text{CTR} \text{ (cal)}$$

Thereafter, the decreasing temperature was recorded for five minutes at the interval of one minute each.

The temperature values obtained were recorded in three periods’ viz., preliminary period, chief period and after period. The wood test procedure was initiated for three times for each test sample and the arrange values were tabulated.

**The calorific values of wood test samples were calculated as follows**

Calorific value = Heat liberated - Nitrogen correction - sulphur correction - correction for heat of ignition (cal)

Heat liberated = Effective mean heat capacity of calorimeter x corrected temperature rise

$$\text{Heat liberated} = \text{EMPHC} \times \text{CTR} \text{ (cal)}$$

Nitrogen and sulphur corrections were neglected because of their minor effect on calorific value.

Correction for heat of ignition (CHI) was calculated considering the length of platinum wire equal to the distance between the poles of bomb and cotton thread rounded on it and their calorific values were as below.

Calorific value of platinum wire = 100 cal/g

Calorific value of cotton thread = 4180 cal/g

CHI= Weight of platinum wire X 100 + weight of cotton thread x 4180, cal

Effective mean heat capacity of calorimeter was taken directly from the test manual of the calorimeter = 2871cal / °C

Corrected temperature rise (CTR) = UCTR-CR

Where, UCTR = Uncorrected temperature rise, °C

$$\text{CR} = \text{Cooling correction, °C}$$

[The thermometer correction was neglected]

Cooling correction (CR) was calculated using the Renault Pfaundler formula as given below
\[ \frac{V2 - V1}{t2 - t1} \]

\[ CR = nV_1 + \frac{X(t)+1/2 (to+tn)-nt1}{cal} \ldots (3.4) \]

Where,

- \( n \) = Number of minutes in the chief period
- \( V_1 \) = Rate of fall of temperature in the preliminary period, °C/min
- \( V_2 \) = Rate of fall of temperature in the after period °C/min
- \( t_1 \) = Average temperature during preliminary period, °C
- \( t_2 \) = Average temperature during after period, °C
- \( to \) = Firing temperature, °C
- \((t)\) = Sum of the temperatures \((t_1,t_2 \ldots \ldots ,t_{n-1})\) recorded during chief period.
- \( t_1, t_2, t_3 \ldots \ldots , t_n \) were successive temperatures recorded during the chief period.

The final temperature \((t_n)\) was the temperature after which the rate of change of temperature remained constant.

Uncorrected temperature rise (UCTR) = \( t_n - to \)

### 3.2.3. Effect of species on physico-chemical parameters of soil

Nutrient enrichment by growing *Casuarina* species in the black cotton soil areas was analyzed by assessing the soil for bulk density, soil pH, EC (do/ml), N (kg ha\(^{-1}\)), P (kg ha\(^{-1}\)) and K (kg ha\(^{-1}\)). Samples were drawn randomly in four replications from soil profiles dug at 0.3 X 0.3 X 0.3 m dimensions in the 10 years old plantations. Representative samples from four pits were pooled and analyzed in duplicate. The samples were drawn from 0 - 23 cm profile range from underneath of ten trees of each species from four replications.

#### 3.2.3.1. Soil bulk density (g/cc)

Soil bulk density was determined by using Keen Raczkowski brass cup method as per Piper (1966).

#### 3.2.3.2. Soil pH

Soil pH was determined in 1:2.5 soil: water ratio suspensions by using a pH meter, as per Piper (1966).

#### 3.2.3.3. Soil EC (ds ml\(^{-1}\))
Electrical conductivity of 1:2.5 soil: water ratio suspension was determined with a conductometer bridge as per Piper (1966).

3.2.3.4. Available Nitrogen
Available soil nitrogen was determined by alkaline potassium permanganate method as per Subbaiah and Asija (1956).

3.2.3.5. Available Phosphorus
Available Phosphorus in the soil was extracted with Olsen’s extract (0.5 N NaHCO₃) and the extracted phosphorus was determined by molybdenum blue method using ascorbic acid as a reducing agent as per Olsen et al., (1954).

3.2.3.6. Available Potassium
Available potassium was extracted with neutral normal ammonium acetate and the potassium in the extract was determined with flame photometer as per Hanway and Heidal (1975).

3. 3. Observation for monoecy and dioecy
Number of trees with only male or female flowers as well as both male and female flowers were counted and classified as dioecious and monoecious respectively in plantations.

3.3.1. Phenology of Casuarina species
Following observations were recorded for the phenology and floral morphological characters of 10 candidate trees of each Casuarina species randomly taken from four replications and the average values were computed as species means under each replication.

3.3.2. Observation on male floral morphological traits of Casuarina species
3.3.2.1. Male flower length (cm)
Fifty male flowers were randomly selected during peak flowering period from each tree and length measured in centimeter.

3.3.2.2. Male flower width (mm)
Fifty flowers were randomly selected during peak blooming period from the each tree and width recorded in millimeter.

3.3.2.3. Number of floral whors male inflorescence⁻¹
Fifty flowers were randomly selected during peak blooming period from each tree and total number of floral whors centimeter⁻¹ inflorescence⁻¹ was recorded.
3.3.2.4. Stamen length (mm)
Fifty stamens were randomly taken from inflorescence during peak blooming period from each tree and length recorded in millimeters.

3.3.2.5. Stamen diameter (mm)
Fifty stamens were randomly taken from inflorescence during peak blooming period from each tree and diameter recorded in millimeter.

3.3.2.6. Pollen formation time
Pollen appearing time was observed in every 2 hours of interval over 24 hours and right time of pollen formation was recorded.

3.3.2.7. Pollen dehisce time
Pollen dehiscing time was recorded an interval of every one hour after right time of pollen formation.

3.3.2.8. Pollen dehiscing temperature
Pollen dehiscing or pollen releasing time and temperature was recorded an interval of every one hour after right time of pollen formation.

3.3.3. Observation on female floral morphological parameters in Casuarina species

3.3.3.1. Female flower length (mm)
Fifty flowers were randomly selected during peak blooming period from each tree and flower length was measured in millimeter.

3.3.3.2. Flower width (mm)
Fifty flowers were randomly selected during peak blooming period from the each tree and flower diameter (width) was recorded in millimeter.

3.3.3.3. Stigma length (mm)
Fifty flowers were randomly selected during peak blooming period from the each of tree and stigma length was recorded in millimeter.

3.3.3.4. Width of stigma (mm)
Fifty flowers were randomly selected during peak blooming period from the each tree and diameter of stigma was recorded in millimeter.

3.3.3.5. Total ovules ovary \(^{-1}\) (number)
Fifty flowers were randomly selected during peak blooming period from the each tree and number of ovules ovary \(^{-1}\) was recorded by using electronic microscope.
3.3.3.6. Total floral buds inflorescence$^{-1}$ (number)
Fifty inflorescences were randomly selected from each tree and total number of floral buds borne inflorescence$^{-1}$ was recorded.

3.3.3.7. Shape of flower
Fifty flowers were randomly selected from each tree and shape was recorded as either disk or oval.

3.3.4. Reproductive biology of different *Casuarina* species

3.3.4.1. Fruit set in open pollination of monoecy and dioecy
Fifty inflorescences were randomly selected from each of tree, labeled and number of floral buds per inflorescence counted. Total number of matured fruits recorded after 18 to 20 weeks in monoecious and dioecious trees.

3.3.4.2. Hand pollination
Fifty inflorescences were randomly selected and labeled from each tree in both monoecious and dioecious species and covered with brown paper bag before opening the flower. Pollens were collected from male tree and used for pollinating the female flower and after 18 to 20 weeks number of matured fruits were recorded.

3.3.4.3. Total fruit set inflorescence$^{-1}$ (number)
Fifty inflorescences were randomly selected from each tree and after 18 to 20 week duration, total number of matured fruits inflorescence$^{-1}$ was recorded.

3.3.4.4. Fruit set inflorescence$^{-1}$ (%)

\[
\text{Fruit set} \, (\%) = \frac{\text{Total number of fruits matured per inflorescence}}{\text{Total number of floral buds per inflorescence}} \times 100
\]

3.3.4.5. Seeds packed cone$^{-1}$ (%)
Hundred cone samples collected randomly from each selected tree were labeled and packed in butter cover. Number of ridges cone$^{-1}$, number of seeds ridge$^{-1}$ and total number of seeds cone$^{-1}$ was counted after opening cone.

\[
\text{Seeds packed} \, (\%) = \frac{\text{Actual number of seeds cone}^{-1}}{\text{Number of seeds ridge}^{-1} \times \text{Number of ridges cone}^{-1}} \times 100
\]
3.3.4.6. Days to fruit maturity (weeks)
The number of weeks from the first week of fruit initiation to the last week when fruit matured and turned brown recorded in weeks.

3.4.1. Morphological quantitative parameters of cone
Quantitative morphological characters of cone from 10 candidate competitive trees in each species were randomly chosen from four replications. The average values were computed as species means from each replication for the following parameters

3.4.1.1. Cone fresh weight (g)
Hundred fresh cones were collected randomly from each selected tree and weighed in g using weighing balance at 0.001 mg accuracy.

3.4.1.2. Cone dry weight (g)
Hundred dry cones were collected at random from each selected tree and weighed in g using weighing balance at 0.001 mg accuracy.

3.4.1.3. Cone length (cm)
Hundred cones were collected at random from each selected tree and its length was measured using vernier caliper in cm.

3.4.1.4. Cone width (mm)
Hundred cones were collected at random from each selected tree and its width was measured using vernier caliper in mm.

3.4.1.5. Ridges cone\(^{-1}\) (number)
Hundred cones were collected randomly from each selected tree and total number of ridges on each cone counted.

3.4.1.6. Seeds ridge\(^{-1}\) (number)
Hundred cones were collected randomly from each selected tree and total number of seeds in each ridge per cone recorded.

3.4.1.7. Seeds cone\(^{-1}\) (number)
Hundred cones were collected randomly from each selected tree and total number of filled seeds from each cone was counted.

3.4.1.8. Cone dry matter (%)
Per cent of cone dry matter was calculated by using formula

\[
\text{Dry matter (\%) = Cone dry weight ÷ Cone fresh weight x 100}
\]
3.4.1.9. Cone shape
Hundred fresh cones were collected at random from each selected tree and shape of cone (either cylindrical or ellipsoid) was recorded by visual observation.

3.4.1.10. Cone color
Hundred matured cones were collected at random from each selected tree and color, either brown or green was recorded by visual observation.

3.4.2. Morphological qualitative parameters of seed
Qualitative morphological characters of seeds from ten candidate competitive trees of each study species randomly chosen from four replications were recorded. The average values were computed as species means from each replication for the following parameters

3.4.2.1. Seed length (mm)
Hundred seeds were collected randomly from 100 processed hundred cones of each selected tree and length was measured using vernier caliper.

3.4.2.2. Seed width (mm)
Hundred seeds were collected at random from 100 processed cones and width was measured by vernier caliper.

3.4.2.3. Seed thickness (µm)
Hundred seeds were collected at random from 100 processed cones and their thickness was measured by screw gauge.

3.4.2.4. 1000 seeds weight (g)
Thousand seeds were collected at random from matured cones and weighed by using weighing balance at 0.001 mg accuracy.

3.4.2.5. Number of seeds g⁻¹
One gram of seeds was weighed by using weighing balance and number of seed g⁻¹ recorded

3.4.2.6. Seed coat
Thousand seeds were collected at random from 100 processed cones and seed coat was categorized either smooth or hard.

3.4.2.7. Colour of seed membrane
Hundred seeds were collected at random from 100 processed cones and color of seed membrane; either grayish or golden cream was recorded.
3.4.2. Seed layer
Hundred seeds were collected at random from processed 100 cones and presence/absence of seed layer gelatinous was recorded.

3.4.3. Morphological traits for seed grade
Matwed cones (2.0 kg) were collected at random from each species and spread out on a canvas in room condition to open the bracteole. The seeds were then separated by gentle shaking and rotation.

3.4.3.1. Seed grade
The unprocessed seeds were graded by sieving through a series of screens with allotted perforation of sizes as follows:

1. Sieve size 1.98 mm
2. IFGTB Sieve size 2.25 mm.
3. Sieve size 2.78 mm
4. Control

A part of the unprocessed (ungraded) seed fraction was kept as control. Standard germination test was conducted as per the procedure laid down by ISTA (1993). The experiment was laid out in a completely randomized design consisting of four replications of four treatments; each treatment was randomly allotted in each of the five Casuarina species in sand tray method. Seed germination was recorded after 6 – 18 days.

3.5. Observation on seed germination

3.5.1. Speed of seed germination
Germination count was taken on the 6th day, 12th day and on 18th day of incubation period. The numbers of seedlings that emerged in each roll were counted and germination percentage worked out. The seeds were considered germinated when radical was visible and from 6th day onwards number of seedlings emerged recorded.

3.5.2. Seed vigor index
Seedlings were selected at random from the normal germination test from each replication on the sixth day of incubation. Root length and shoot length were measured and expressed in mm. Vigor index was computed by multiplying mean length of seedlings with percentage of normal germination (Abdulbaki and Anderson, 1973)
3.5.3. Effect of different substratas on seed germination
The pooled seeds of each of the *Casuarina* species were used for germination.

3.5.3.1. Design and layout
The experiment was laid out in a completely randomized block design comprising of four replications of three substratas viz., between paper, sand seed tray and raised nursery bed and each treatment was randomly allotted to species of *Casuarina*.

3.5.3.2. Between papers
A standard non toxic germination paper was used for the between paper method prescribed by the ISTA (Anon, 1984). Hundred seeds from each species of *Casuarina* in four replications were placed on paper towels and then towels were rolled and placed in the germination chamber. The temperature maintained in the germination chamber was 29 ± °C with relative humidity of 95 per cent. Seed germination was recorded after 6 to 18 days and expressed in percentage.

3.5.3.3. Seed tray
The sand was sieved, moistened and filled in seed germination trays of size 40 × 30 cm and 5 cm deep. Hundred seeds from each species of *Casuarina* were sown in four replications and trays were kept under ambient condition in the lab. Seed germination was recorded after 6 to 18 days and expressed in percentage.

3.5.3.4. Raised seed bed
The raised seed beds of dimension 10.0 x1.0 x 0.2 m were prepared and soil medium consisted of sand, red earth and farm yard manure in the ratio of 1:1:1. Seedbeds were compacted to ensure good contact between the top layer in which the seeds were sown and the deeper layers. The soil and sand was sufficiently compacted then 100 seeds from each species of *Casuarina* in four replications were sown in raised bed. Seed germination observation was recorded after 6 – 18 days and expressed in percentage.

3.5.3.5. Tetrazolium chloride
Rapid viability tests such as tetrazolium chloride (TZ) was carried out using fresh seeds and seeds stored up to 21 months. For TZ test, seeds were de-winged completely and soaked in tap water for 24 hours. The soaked seeds were removed, cut longitudinally into two equal halves to expose the embryo and placed in petri dishes in four replications of 100 seeds from each *Casuarina* species for rapid determination of viability of seeds. Tetrazolium solution
per cent was prepared by dissolving 2,3,5 triphenyl tetrazolium chloride salt in double distilled water of 6.5 to 7.0 pH. The seeds were soaked in distilled water for 24 hours and then placed in 1 % of TZ solution and incubated in dark at 30°C for 24 hours. Finally, the TZ solution was decanted and the seeds were rinsed in running tap water. The staining pattern of the embryo was subsequently examined. Seeds that were stained deep red were recorded viable while those that showed uneven or no staining were interpreted non-viable. Viability was expressed in percentage (ISTA, 1993).

3.5.4. Influence of pre-sowing treatments on seed germination
In order to break seed-coat dormancy of Casuarina species with soft coated seeds and to promote rapid and uniform germination, it was necessary to subject seeds for pre-sowing treatments. Hundred seeds from each species of Casuarina were sown in four replications in sand tray and were kept under ambient condition in the lab as per ISTA, 1993.

3.5.4.1. Potassium nitrate (KNO₃) treatment
KNO₃ (2.0 %) solution was prepared by dissolving 2 g of KNO₃ in 1.0 liter of water and seeds from each species were soaked in solution for 10-12 hours.

3.5.4.2. Hot water or lukewarm water
Seeds were soaked in hot water at 54 ºC for 10-12 hours.

3.5.4.3. Pre chilling
Temperature in refrigerator was maintained at 0ºC and seeds were stored in chilled temperature over one week.

3.5.4.4. Gibberellic acid (GA₃)
Seeds were soaked in 500 ppm GA₃ (concentrations of 50 mg/liter) for 10-12 hours.

3.5.4.5. Cold water
Seeds were soaked in cold water treatment at 24ºC for 10-12 hours.

3.5.4.6. Control or untreated seeds
Seeds were not presoaked.

3.6.1. Performance of seedlings in half sib population
Twenty five seedlings were taken from each Casuarina species from 4 replications. The following observations were made at 6 month after sowing (MAS) followed by 9 MAS and 12 MAS for seedling growth performance.
3.6.1. Seedling survival (%) 
The number of seedlings survived at 3 month after sowing (MAS) followed by 6 MAS and 12 MAS were counted.

3.6.1.2. Seedling height (cm) 
The seedling height was measured by scale from collar region to the tip of the shoot.

3.6.1.3. Collar diameter (mm) 
Collar diameter was measured by vernier caliper at the base of the stem at the root collar region.

3.6.1.4. Number of branches seedling\(^{-1}\) (number) 
All the primary branches arising on main stem were counted.

3.6.1.5. Number of needles seedling\(^{-1}\) 
All live primary and secondary needles were counted.

3.6.1.6. Needle length (cm) 
Length of 25 randomly selected needles from middle part of the seedling was measured by scale and average noted.

3.6.1.7. Number of inter nodes needle\(^{-1}\) 
All the inter-nodes within each needle were counted.

3.6.1.8. Number of roots seedling\(^{-1}\) 
Twenty five seedlings were selected from each species at random and were uprooted from container, washed with running water and roots were counted.

3.6.1.9. Root length (cm) 
The length from collar region to the tip of root was recorded by scale.

3.6.1.10. Number of root nodules seedling\(^{-1}\) 
Twenty five seedlings were selected from each species at random and were removed from container. The roots were carefully washed with running water to remove the adhered soil and then the number of nodules on roots were counted and expressed in numbers.

3.6.2. Total seedling biomass productivity 
Twenty five competitive seedlings were taken from each *Casuarina* species from 4 replications. The following observations for total seedling biomass production were made on 12 months after sowing.
3.6.2.1. Fresh stem weight (g)
Green stem fresh weight was recorded using weighing balance (0.001 mg accuracy) separating it from the needles and roots.

3.6.2.2. Fresh needle weight (g)
Needle fresh weight was recorded using weighing balance after separating it from the stem.

3.6.2.4. Fresh root weight (g)
Root fresh weight was recorded using weighing balance after separating it from the collar stem region.

3.6.2.5. Total seedling fresh weight (g)
Total seedling fresh weight was recorded using weighing balance.

3.6.2.6. Dry stem weight (g)
Stem was dried in oven at 103 ± °C over night and the dry weight of stem was recorded.

3.6.2.7. Dry needle weight (g)
Needles were dried in oven at 103 °C overnight and the dry weight was recorded.

3.6.2.8. Root dry weight (g)
Root was dried in oven at 103 ± °C over night and dry weight of root was recorded.

3.6.2.9. Total seedling dry weight (g)
Entire seedling was dried in oven at 103 ± °C over night and the dry weight was recorded.

3.6.2.10. Seedling dry biomass (%)
Seedling dry biomass was calculated by using following formula

\[
\text{Total seedling dry biomass (\%)} = \frac{\text{Total seedling dry weight (g)}}{\text{Total seedling fresh weight (g)}} \times 100
\]

3.6.2.11. Suitability index
It is the summation of height, diameter and survival of each seedling expressed as percentage of the respective maxima (Ghosh et al., 1981).

3.6.2.12. Volume index
Volume index = (collar diameter in cm) \(^2\) x seedling height (cm)

(Hatchell, 1985 and Manavalan, 1990)
3.6.3. Estimation of biochemical parameters in half sib population

3.6.3.1. Nutrient content

The free proline, chlorophyll and nitrogen contents of *Casuarina* species were chemically analyzed.

3.6.4.2. Total nitrogen content (%)

Ten seedlings from each species were collected in four replications, oven dried (103 ± °C) and ground to a fine powder and samples were taken for nitrogen analysis. Nitrogen content was estimated by Micro Kjeldhal Distillation method (Diacid extraction with H2SO4: HClO4 in the ratio of 5:2) following Jackson (1973).

\[
\text{Nitrogen (\%) = } \frac{\text{TV} \times \text{N} \times \text{acid} \times 0.014 \times \text{V1}}{\text{Weight of sample} \times \text{V2}} \times 100
\]

Where,

- TV = Titre value,
- N = Normality of each acid (HCl)
- V1 = Volume of digested sample
- V2 = Volume taken for distillation.

3.6.4.3. Stem nitrogen content (%)

Stem nitrogen was estimated by Micro Kjeldhal Distillation method (Jackson, 1973).

3.6.4.4. Needles nitrogen content (%)

Needle nitrogen was estimated by Micro Kjeldhal Distillation method (Jackson, 1973).

3.6.4.5. Root Nitrogen content (%)

Root nitrogen was estimated by Micro Kjeldhal Distillation method (Jackson, 1973).

3.6.4.6. Estimation of chlorophyll content

The chlorophyll a, b and “total” chlorophyll were estimated using the method of Yoshida et al., (1971) and expressed in mg/ fresh weight of needle. A sample of 0.5 g. of needle tissues from each *Casuarina* species in 4 replications at 12 MAS were taken and washed thoroughly with distilled water. Then the needle tissues were macerated with 10 ml of 80 per cent acetone using pestle and mortal until it got thoroughly homogenized. The homogenized material was centrifuged at 5000 rpm for 10 minutes and the supematant was transferred to 25 ml volumetric flask. Then the final volume was made up with 80 per cent acetone. The absorbance of the solution was measured in the (UV spectrophotometer at 645 and 663 nm using the solvent (80 per cent acetone) as blank. The chlorophyll present in the extract was calculated using the following formulas.
3.6.4.6.1. Chlorophyll ‘a’ = 12.7 (A 663) - 2.69 (A 645) x V / 1000 x W
3.6.4.6.2. Chlorophyll ‘b’ = 22.9 (A 645) - 4.68 (A 663) x V / 1000 x W
3.6.4.6.3. Total chlorophyll = 20.2 (A 645) + 8.02 (A 663) x V / 1000 x W

Where, V = Final volume made up
W = Weight of plant tissues taken

3.6.4.7. Free proline content (µg/g fresh weight)

Free proline content in the leaves of different species of Casuarinas was determined calorimetrically as per the method of Bates et al., (1973). A known weight (0.5 g) of fresh needle sample was ground well in a mortar using fine sand and was extracted with 10 ml of 3 % sulfosalicylic cyclic acid. The extract was filtered and 2 ml of filtrate was used for proline estimation. To the 2 ml of filtrate, 2 ml of acid ninhydrin reagent (2.5 g ninhydrin was dissolved in 40 ml of 6 M orthophosphoric acid and 60 ml of glacial acetic acid) and 2 ml of glacial acetic acid were added and placed in a boiling water bath for one hour.

Proline content (µg/g fresh weight) = (36.2311 x OD x V x d) / (2 x f)

Where, OD - Optical Density at 520 nm
V - Total volume of extract in ml
d - Fresh weight / dry weight ratio
f - Fresh weight taken (mg)
2 - Volume of the extract taken
3.7. Statistical analysis

3.7.1. Analysis of variance (ANOVA)

Estimates of mean, variance and standard error were worked out after Panse and Sukhatme (1961). The analysis of variance was carried out for different characters in order to assess the variability among the five species of *Casuarina*.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degree of freedom</th>
<th>Sum of squares</th>
<th>Mean sum of squares</th>
<th>F-ratio Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications</td>
<td>(r-1)</td>
<td>RSS</td>
<td>( \frac{RSS}{r-1} = \frac{M_r}{M_e} )</td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>(s-1)</td>
<td>VSS</td>
<td>( \frac{VSS}{s-1} = \frac{M_v}{M_e} )</td>
<td>( \frac{M_r}{M_e} )</td>
</tr>
<tr>
<td>Error</td>
<td>(s-1)(r-1)</td>
<td>ESS</td>
<td>( \frac{ESS}{(s-1)(r-1)} = Me )</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>sr-1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Where, \( r \) = Number of replications and \( s \) = Number of species.

3.7.2. Estimation of genetic variability

Both genotypic and phenotypic coefficients of variability for all the characters were computed as per the method given by Burton and De Vane (1953).

3.7.2.1. Genotypic Coefficient of Variability (GCV)

\[
GCV = \frac{\sigma_g}{\overline{X}} \times 100
\]

Where,

\( \sigma_g \) = Genotypic standard deviation
\( \overline{X} \) = General mean of the character
3.7.2. Phenotypic Co-efficient of Variability (PCV)

\[
\text{PCV} = \frac{\sigma_p}{\overline{X}} \times 100
\]

where:
\(\sigma_p\) = Phenotypic standard deviation
\(\overline{X}\) = General mean of the character

GCV and PCV were classified as follows (Robinson *et al.*, 1949).
- Low = 0 to 10%
- Moderate = 10 to 20%
- High = > 20%

3.7.3. Estimation of heritability

Broad sense heritability for all the characters was worked out using the formula (Hanson *et al.*, 1953).

\[
h^2 \text{ (\%)} = \frac{\sigma^2_g}{\sigma^2_p} \times 100
\]

where;
\(\sigma^2_g\) - genotypic variance.
\(\sigma^2_p\) - phenotypic (total) variance.

Heritability was classified into
- Low = 0 to 30%
- Moderate = 30-60%
- High = > 60% (Robinson *et al.*, 1949)

3.7.4. Genetic advance (GA)

Genetic advance as per cent of mean for each character was worked out by adopting the formula given by Johnson *et al.*, (1955).

Genetic advance (GA) = \(h^2 \cdot \sigma_p \cdot K\)

where; \(h^2\) - Heritability in broad sense
\(K\) - Selection differential, \(K = 2.06\) at 5 per cent intensity of selection
\(\sigma_p\) - Phenotypic standard deviation (Lush, 1949)
Further, genetic advance as per cent of mean (GAM) was worked out by using the formula given below:

\[
\text{GAM} = \frac{\text{GA}}{\overline{X}} \times 100
\]

where;
- \(\text{GAM}\) - Genetic advance as per cent mean
- \(\text{GA}\) - Genetic advance
- \(\overline{X}\) - General mean of the character

Genetic advance as per cent mean was classified as follows (Johanson et al., 1955).
- Low \(= 0\) to 10\%
- Moderate \(= 10\) to 20\%
- High \(> 20\%\)

### 3.7.5. Association of morphological traits

#### 3.7.5.1. Correlation studies

Phenotypic and Genotypic correlation coefficients were calculated according to the method suggested by Goulden (1952).

\[
\text{r}(X_1X_2) = \frac{\text{COV}(X_1X_2)}{\sqrt{\text{V}(X_1)\text{V}(X_2)}} \times 100
\]

Where, \(\text{r}(X_1X_2)\) - is the correlation between \(X_1\) & \(X_2\)

- \(\text{COV}(X_1X_2)\) - is the covariance between \(X_1\) and \(X_2\)
- \(\text{V}(X_1)\) - is the variance of \(X_1\)
- \(\text{V}(X_2)\) - is the variance of \(X_2\)

#### 3.7.5.2. Phenotypic correlation

Phenotypic covariance between 1 and 2

\[
\text{rp} \ 1.2 = \frac{\text{Phenotypic covariance between 1 and 2}}{(\text{Phenotypic variance of 1 x Phenotypic variance of 2})^{\frac{1}{2}}}
\]
3.7. 5.3. Genotypic correlation

Genotypic covariance between 1 and 2
\[ rg_{1.2} = \frac{\text{Genotypic variance of 1} \times \text{Genotypic variance of 3}}{\left(\frac{1}{2}\right)} \]

3.7. 6. Path coefficient analysis

Path coefficient analysis was carried out by using the phenotypic correlation coefficients to know the direct and indirect effects of the yield components on tree biomass as suggested by Wright (1921) and as illustrated by Dewey and Lu (1959).

Standard path coefficients which are the standardized partial regression coefficients were obtained by solving the following set of \( P \) simultaneous equations through the use of "DOO LITTLE TECHNIQUE" as described by Goulden (1959),

\[
P_{01} + P_{02}r_{12} + \ldots + P_{0n}r_{1n} = r_{01} \\
P_{01}r_{12} + P_{12}r_{02} + \ldots + P_{0n}r_{2n} = r_{02} \\
P_{01}r_{1n} + P_{02}r_{2n} + \ldots + P_{0n}r_{0n} = r_{0n}
\]

Where, \( P_{01}, P_{02} \ldots \ldots P_{0n} \) are the direct path coefficients of variables 1, 2, \ldots, \( n \) on the dependent variable 0, \( r_{12}, r_{13} \ldots \ldots r_{1p} \ldots \ldots rp \) (p-1) are the possible correlation coefficients between various independent variables and \( r_{01}, r_{02}, \ldots \ldots r_{0p} \) are the correlations between dependent variable and independent variable.

\[
P^2_{0x} = 1 - (P^2_{01} + 2P_{01} P_{02}r_{12} + 2P_{01} P_{03}r_{13} + \ldots \ldots P^2_{02} + 2P_{02} P_{03} r_{13} + \ldots \ldots + P^2_{0p})
\]

\[
\text{Residual effect} = \sqrt{P^2_{0x}}
\]

3.7. 7. Estimation of genetic diversity

3.7. 7.1. Mahalanobis \( D^2 \) analysis

The formula given by Mahalanobis (1936) was used to compute the distances between different populations. The square of the Mahalanobis generalized distance between any two populations is given by the formula,

\[
\delta^2 = \sum \delta_i \delta_j r_{ij}
\]
Where; $\delta^2 = \text{Square of generalized distances}$

$r_{ij} = \text{Reciprocal of the common dispersion matrix}$

$\delta_i = (U_{i1} - U_{i2})$

$\delta_j = (U_{j1} - U_{j2})$

$U = \text{Vector of mean values for all the characters}$

3.7.7.2. Clustering of $D^2$ values

All the $[n (n-1)/2]$ $D^2$ values were clustered using Tocher's method as described by Rao (1952).

3.7.7.3. Intra-cluster distance

The intra-cluster distances were calculated following Singh and Chaudhary (1977).

Square of intra-cluster distance = $\sum D^2_i / n$

Where; $\sum D^2_i = \text{Sum of distances between all possible combination of the entries included in a cluster and } n = \text{Number of all possible combinations}$

3.7.8.4. Inter-cluster distance

The inter-cluster distances were calculated following Singh and Chaudhary (1977).

Square of inter-cluster distance = $\sum D^2_i / n_{ij}$

Where;

$\sum D^2_i = \text{Sum of distances between all possible combination (n}_{ij}) \text{ of the entries included in the cluster study (i and j)}$

$n_i = \text{Number of entries in cluster i}$,

$n_j = \text{Number of entries in cluster j}$