Chapter 3
Materials and Methods
3. MATERIALS AND METHODS

In the present investigation, experiments were carried out at North Bank Plain Agroclimatic Zone (NBPAZ) of Assam, a state of north-east India. The details of materials and methods employed during the course of the investigation are described below.

3.1. Association of plant growth parameters with methane emission from monsoon / Sali rice

This experiment was conducted over two consecutive years (2005 and 2006) at NBPAZ of Assam with two (2) rice varieties during monsoon season under rainfed condition (monsoon / Sali rice agroecosystem). The detail technical programme of this experiment is given below.

3.1.1. Geographical location, climatic conditions and soil characteristics of the experimental site

In this agroclimatic zone, rice is the major crop grown both under irrigated and unirrigated condition. This experiment was conducted in a farmer’s field at Amolapam near Tezpur Central University campus (26°41’ N, 92°50’ E) during the monsoon rice-growing season (August-November of 2005 and 2006). Figure 3.1 shows the geographical location of the experimental site located at the NBPAZ, northeast India. The region is subtropical humid having moderately hot-wet summers and dry winters. The available meteorological data during the experimental periods were collected and are presented in Figure 3.2 and 3.3. The Agroclimatic Zone is characterized by light textured loamy alluvial
Fig. 3.1. Experimental site at North Bank Plain Agroclimatic Zone (NBPAZ) of Assam, northeast India.
soils. Various soil physiochemical properties of the experimental field are presented in Table 3.1. Light intensity above and below the canopy of rice plant was measured by a light meter (LI 250A, LI-COR, USA) and light transmission (%) through the canopy of rice plant is presented in Figure 3.4.

3.1.2. Selection of rice variety

Two high yielding rice varieties viz. Bahadur and Piolee were selected for the experiment.

3.1.2.2. Description of varieties

1. Bahadur: This variety was developed at Regional Agricultural Research Station (RARS), Titabor of Assam Agricultural University, Jorhat, India, by cross combination between ‘Pankaj’ and ‘Mahsuri’. It is a blast tolerant, non-lodging variety recommended for shallow water (0-30 cm) agroecosystem for monsoon (Salī) season. Duration and average yield in ideal field condition is 150-155 days and 5.0-5.5 t ha⁻¹, respectively.

2. Piolee: It was developed at Regional Agricultural Research Station (RARS), Titabor of Assam Agricultural University, Jorhat, India, by cross combination between ‘Pankaj’ and ‘Mahsuri’. It is a blast tolerant variety, having very good grain quality. It is recommended for shallow water (0-30 cm) agroecosystem for monsoon (Salī) season. Duration and average yield in ideal field condition is 150-155 days and 5.0-5.5 t ha⁻¹, respectively.
Fig. 3.2. Meteorological parameters during the experimental period of 2005 (Sali season).

Fig. 3.3. Meteorological parameters during the experimental period of 2006 (Boro, Ahu and Sali season).
Fig. 3.4. Light transmission (%) through the canopy of varieties Bahadur and Piolee. Data presented are means ± SE (vertical bars).

Fig. 3.5. Meteorological parameters during the experimental period of 2007 (Boro season).
3.1.3. Field preparation

The experimental plot was ploughed, puddled thoroughly to 15-cm depth and levelled. Fertilizer was applied at the rate of 40:20:20 kg N-P-K ha\(^{-1}\) in the form of urea, single super phosphate (SSP) and murate of potash (MOP). One third (1/3\(^{rd}\)) of full dose of urea along with full dose of SSP and MOP was applied at the time of final land preparation. Remaining part of urea was applied at the time of tillering (1/2 of the remaining part) and panicle initiation stage (another 1/2 of the remaining part), as recommended in the package of practice of Assam Agricultural University. Rice seedlings (25-day old) of two varieties, viz. Bahadur and Piolee were transplanted (spacing: 20 cm x 20 cm; 2 seedling per hill) in four replicated plots (5 x 5m = 25 m\(^2\)).

3.1.4. Gas sampling and estimation of methane emission

Methane flux from rice field was recorded at seven (7) different growth stages viz. early tillering (42 DAT: Days after transplanting), late tillering (56 DAT), panicle initiation (70 DAT), flowering (84 DAT), grain development (98 DAT), ripening (112 DAT) and maturation (119 DAT). Two additional methane samplings were done at 7 and 14 days after harvest. Methane flux was recorded by using a static chamber technique (Plate 3.1) described by Parashar et al. (1996). Briefly, chambers 50 cm long, 30 cm wide and 70 cm tall made of 6-mm-thick clear acrylic sheet were used for gas sampling. Rectangular U shaped aluminium channel (50 cm X 30 cm) supported on an aluminium frame (50 cm X 30 cm X 15 cm) was used to support the chambers. The aluminium channels were inserted into the soil to a depth of 15 cm 7 days before transplanting. During gas sampling, the aluminium channels were filled with water to a depth of 2.5 cm. This acted as an air seal for the flux boxes. A battery-operated fan inside each box thoroughly mixed the air in the chamber before sampling. Gas samples were drawn from the chambers using a 50 ml airtight syringe fitted with a three-way stop-cock and a fine needle that was inserted through a self-sealing rubber septum. Gas
samples were collected at intervals of 15 min at 09-00h for four times and again at 14-00 h. During each sampling, temperature and water level inside the chamber were measured. Atmospheric pressure was also recorded at the time of gas sampling. This permitted calculation of air volumes at standard temperature and pressure (STP). Soil temperature was measured with the help of soil thermometer inserted in to the soil near the chamber (at 5cm distance) at the time of sampling. The average of morning and evening values (°C) were considered as the soil temperature value for the day.

Methane in gas samples was determined (Plate 3.2) using a gas chromatograph (Varian, Model 3800, The Netherlands) fitted with flame ionization detector (FID) and Chromopack capillary column (50 cm long, 0.1µm inside diameter). Column, injector and detector temperature were maintained at 50, 90 and 150°C respectively. Gas chromatograph was calibrated with a methane standard (5.5ppm) obtained from National Physical Laboratory, New Delhi. Nitrogen, hydrogen and zero air was used as carrier gas, fuel gas and supporting gas respectively. Methane flux was calculated from the temporal increase in the CH₄ concentration inside the box using the equation of Parashar et al. (1996):

\[
Flux = \frac{BV_{STP} \times C'_{CH₄} \times 16 \times 1000 \times 60}{10^6 \times 22400 \times A \times t} \text{mg m}^{-2} \text{h}^{-1}
\]

Where, \(BV_{STP}\) is the box air volume in C.C. at STP. It was calculated by:

\[
BV_{STP} = \frac{BV \times BP \times 273}{(273 + T) \times 760}.
\]

\(BV\) (Box air volume) was calculated by:

\[
BV = [(H - h) LW - \text{biomass volume inside box}]
\]

Where,

- \(H\) = Box height (cm)
- \(h\) = Water level above the channel (cm)
\[ L = \text{Box length (cm)} \]
\[ W = \text{Box width (cm)} \]
\[ \text{BP} = \text{Barometric pressure (mm Hg)} \]
\[ T = \text{Box air temperature at the time of sampling (°C)} \]
\[ \text{C}_{\text{CH}_4} = \text{Change in CH}_4 \text{ concentration in ppmv from 0 to t min. and} \]
\[ A = \text{Paddy area covered by the box (m}^2) \]

The average of morning and evening fluxes were considered as the flux value for the day.

3.1.5. Morpho-physiological parameters of plant

3.1.5.1. Plant height

Plant height was measured at weekly interval in cm from the base of the plant to the end of the top leaf with the help of a scale. The average plant height of ten samples from each variety was taken and expressed as plant height (cm plant\(^{-1}\)).

3.1.5.2. Leaf number per hill

Number of leaves per hill was counted at weekly interval. The average leaf number of ten hills from each variety were taken and expressed as leaf number hill\(^{-1}\).
3.1.5.3. *Tiller number per hill*

Total number of tillers including the main shoot was counted at weekly interval. The average tiller number of ten hills from each variety were taken and expressed as tiller number hill$^{-1}$.

3.1.5.4. *Leaf area per hill*

Leaf area per hill was measured at weekly interval with a portable laser leaf area meter (Plate 3.3) (CID, Model CI-203). The average leaf area of ten hills from each variety were taken and expressed as leaf area (cm$^2$ hill$^{-1}$).

3.1.5.5. *Leaf area index*

Leaf area index was measured at weekly interval using the following formula:

$$\text{Leaf area index (LAI) = } \frac{\text{Leaf area (cm}^2\text{)}}{\text{Ground area (cm}^2\text{)}}$$

3.1.5.6. *Root length and volume*

Total root length per hill was measured at weekly interval by a portable laser leaf area meter (CID, Model CI-203) with root measurement attachment. The average root length of ten hills from each variety were taken and expressed as root length (cm hill$^{-1}$). Root volume
was determined by standard water displacement method. The average root volume of ten hills from each variety were taken and expressed as root volume (ml hill⁻¹).

3.1.5.7. Culm, leaf blade, leaf sheath and root dry weight

Root and shoot portion from ten hills were collected separately from each variety at weekly interval. The shoot portion was carefully separated into culm, leaf blade and leaf sheath. The root portion was washed thoroughly to remove the soil and sand particles under running water over a sieve. Appropriate care was taken so that the minute parts of roots can be collected. Both root and shoot parts were oven dried at 75°C till the weight become constant. The average culm, leaf blade, leaf sheath and root dry weight of ten hills from each variety were taken and expressed as dry weight (g hill⁻¹).

3.1.5.8. Leaf photosynthetic rate

Leaf photosynthesis was measured at weekly interval (from 7 days after transplanting till harvest) by an infra-red gas analyzer (LI-6400 portable photosynthesis system, LICOR, USA), under ambient environmental conditions (Plate 3.4). The photosynthetic rate (μ mol CO₂ m⁻² s⁻¹) of intake leaf was measured following the method of Baig et al. (1998). The middle portion of a fully expanded, healthy-green 2nd leaf from the top was used for measurement up to the pre-flowering stage, and the flag leaf was used for photosynthesis measurement from the panicle initiation stage of the crop. Leaves were held in the chamber until values of photosynthesis were observed to be as constant as possible (steady state). Leaves were kept at steady state for 1 min before measurements were taken.
3.1.6. Yield and yield attributing parameters

3.1.6.1. Thousand-grain weight

At harvest, the average thousand grain weight of ten samples from each variety were determined and expressed as thousand grain weight (g).

3.1.6.2. Panicle length

Panicle length was measured from the nodal base of the panicle to the tallest part of the main rachis. Average length of panicles from ten plants of each variety was taken and expressed as panicle length (cm).

3.1.6.3. Dry weight of developing panicle

After panicle initiation, the developing panicles were collected separately from each variety at weekly interval and were oven dried at 75°C till the weight became constant. The average dry weight of developing panicle from ten hills were taken and expressed as dry weight (g hill⁻¹).
3.1.6.4. *Filled grain*

Filled grains were separated from the unfilled grains by soaking them in water. The percentage was worked out to get the filled grain %. The average filled grain of ten samples from each variety were taken and expressed as filled grain (%)..

3.1.6.5. *Yield*

The mature plants were harvested from one square meter area from each plot avoiding the border rows. After threshing and cleaning, the grain yield was recorded. Values presented here are average of four replications and expressed as tone hectare\(^{-1}\).

3.1.7. *Soil physico-chemical properties*

Soil samples were collected from the root zone of rice plants from 15cm depth from the four replicate plots with the help of a soil-sampling core and composite samples were prepared from the collected soils. Root fragments were removed carefully from the soil and various physico-chemical properties were analyzed.

3.1.7.1. *Soil pH*

On each methane flux measurement day, soil pH was measured at 1:1.25 soil to water ratio (soil water slurry) using a digital pH meter.
3.1.7.2. Cation exchange capacity

Soil samples were collected from the experimental field before the start of the experiment. The cation exchange capacity (CEC) of the soil samples were determined by 1N ammonium acetate (pH 7.0) method (Jackson, 1973).

3.1.7.3. Determination of clay, sand and silt content

Soil samples collected from the experimental field before the start of the experiment were analyzed for clay, silt and sand fraction by employing Buyoucous hydrometer method (Black, 1965).

3.1.7.4. Soil organic carbon

Organic carbon content of the soils was determined at weekly interval on each methane flux measurement day by standard wet oxidation method. One gram of soil sample was treated with ten (10) ml of 1 N potassium dichromate and 20ml of concentrated H$_2$SO$_4$ was added in the solution. After 30 minutes, 200ml of water, 10 ml of 1N phosphoric acid and 1ml of diphenylamine indicator was added. The solution turned bluish purple in colour. This was titrated against 0.5N standard ferrous ammonium sulphate solution till the contents become grayish green in colour. Simultaneously, a blank determination was also conducted and the value was recorded. The organic carbon was calculated by the method of Walky and Black (Jackson, 1973).
3.1.7.5. Soil nutrient content

Estimation of soil nitrogen content was carried out by Micro Kjeldahal method (Jackson, 1973). Potassium and phosphorus content of soils were estimated by Flame photometric method and Colorimetric method (Jackson, 1973), respectively. Estimation of total Fe, Cu, Mn and Zn, were done in an atomic absorption spectrophotometer (Model AA200, Perkin Elmer, USA).

3.1.8. Statistical analysis

Measurements of different parameters for all the growth stages were replicated for four times. The significance or non-significance of a given variance was determined by calculating the respective ‘t’ and SE ± values (Gomez and Gomez, 1984), considering the variety as source of variation. Correlation of methane flux with other parameters (means of all different growth stages) was done by Pearson correlation method.

3.2. Association of plant growth parameters with methane emission from irrigated / Boro rice

This experiment was conducted at irrigated rice agroecosystem of NBPAZ of Assam with two (2) rice cultivars during spring season (Boro rice) under irrigated condition. The detail technical programmes of this experiment are given below.
Table 3.1. Soil characteristics of the experimental fields of different agroecosystems.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Year / Cropping season</th>
<th>Sali</th>
<th>Ahu</th>
<th>Boro</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2005</td>
<td>2006</td>
<td>2006</td>
</tr>
<tr>
<td>pH</td>
<td>5.43 ± 0.02</td>
<td>5.36 ± 0.01</td>
<td>5.40 ± 0.06</td>
<td>5.45 ± 0.01</td>
</tr>
<tr>
<td>EC (mmhos/100g)</td>
<td>0.47 ± 0.02</td>
<td>0.45 ± 0.01</td>
<td>0.43 ± 0.02</td>
<td>0.47 ± 0.01</td>
</tr>
<tr>
<td>CEC (m eq. 100g⁻¹)</td>
<td>9.54 ± 0.23</td>
<td>9.31 ± 0.43</td>
<td>10.20 ± 0.55</td>
<td>10.87 ± 0.15</td>
</tr>
<tr>
<td>Bulk density (g cc⁻¹)</td>
<td>0.66 ± 0.01</td>
<td>0.66 ± 0.02</td>
<td>0.85 ± 0.02</td>
<td>0.87 ± 0.01</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>28.60 ± 0.23</td>
<td>28.53 ± 0.56</td>
<td>30.10 ± 0.49</td>
<td>31.57 ± 0.12</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>41.15 ± 0.28</td>
<td>41.38 ± 0.53</td>
<td>41.40 ± 0.90</td>
<td>39.50 ± 0.21</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>30.25 ± 0.43</td>
<td>30.10 ± 0.55</td>
<td>28.50 ± 0.44</td>
<td>28.93 ± 0.09</td>
</tr>
<tr>
<td>Organic carbon (%)</td>
<td>0.93 ± 0.01</td>
<td>0.97 ± 0.01</td>
<td>0.94 ± 0.01</td>
<td>0.95 ± 0.01</td>
</tr>
<tr>
<td>Available Nitrogen (kg ha⁻¹)</td>
<td>373.13 ± 1.03</td>
<td>375.38 ± 2.07</td>
<td>375.40 ± 1.73</td>
<td>378.67 ± 1.62</td>
</tr>
<tr>
<td>Available Phosphorus (kg ha⁻¹)</td>
<td>35.50 ± 0.79</td>
<td>37.00 ± 0.79</td>
<td>36.20 ± 0.83</td>
<td>39.43 ± 0.64</td>
</tr>
<tr>
<td>Available Potassium (kg ha⁻¹)</td>
<td>234.63 ± 1.16</td>
<td>238.13 ± 1.21</td>
<td>237.70 ± 2.03</td>
<td>240.90 ± 0.81</td>
</tr>
<tr>
<td>Total Iron (ppm)</td>
<td>448.00 ± 1.78</td>
<td>454.00 ± 2.08</td>
<td>445.00 ± 2.08</td>
<td>431.00 ± 2.65</td>
</tr>
<tr>
<td>Total Manganese (ppm)</td>
<td>20.75 ± 0.85</td>
<td>22.25 ± 0.85</td>
<td>20.00 ± 1.00</td>
<td>19.67 ± 0.88</td>
</tr>
<tr>
<td>Total Copper (ppm)</td>
<td>17.75 ± 0.85</td>
<td>19.75 ± 0.85</td>
<td>17.00 ± 1.53</td>
<td>17.67 ± 1.45</td>
</tr>
<tr>
<td>Total Zinc (ppm)</td>
<td>25.50 ± 1.26</td>
<td>26.00 ± 0.82</td>
<td>23.00 ± 1.53</td>
<td>24.00 ± 1.53</td>
</tr>
</tbody>
</table>

a = Standard error
3.2.1. Geographical location, climatic conditions and soil characteristics of the experimental site

The geographical location and climatic conditions of the experimental area are described in 3.1.1. The experiment was conducted over two consecutive years (2006 and 2007) in a farmer’s field at Amolapam near Tezpur Central University campus (26°41’ N, 92°50’ E) during the spring rice-growing season (February–June). The available meteorological data of the experimental periods were collected and are presented in Figure 3.3 and 3.5. The experimental site comprises light textured loamy alluvial soils. Soil samples were collected from the experimental field before the start of each experiment and analyzed. Various soil physiochemical properties of the experimental field are presented in Table 3.1. Light transmission (%) through the canopy of rice plant is presented in Figure 3.6.

3.2.2. Selection of rice variety

Two rice varieties viz. Ranjit and Agni were selected for this experiment.

3.2.2.2. Description of varieties

1. Ranjit: This variety was developed at Regional Agricultural Research Station (RARS), Titabor of Assam Agricultural University, India, by cross combination between ‘Pankaj’ and ‘Mahsuri’. This semi-dwarf variety is recommended for shallow water (0-30 cm) agroecosystem. It is also grown under irrigated condition. Duration and average yield in ideal control field condition is 150-155 days and 5.0-5.5 t ha⁻¹ respectively.

2. Agni: It is an indigenous traditional rice cultivar, generally grown under irrigated condition during Boro season.
3.2.3. Field preparation

The experimental plot was ploughed, puddled thoroughly to 15-cm depth and levelled. Fertilizer was applied at the rate of 60: 30: 30 kg N-P-K ha$^{-1}$ in the form of urea, single super phosphate (SSP) and murate of potash (MOP). One third (1/3$^{rd}$) of full dose of urea along with full dose of SSP and MOP was applied at the time of final land preparation. Remaining part of urea was applied at the time of tillering (1/2 of the remaining part) and panicle initiation stage (another 1/2 of the remaining part), as recommended in the package of practice of Assam Agricultural University. Thirty-five days old rice seedlings (5-6 leaf stage) of cultivars Agni and Ranjit were transplanted (spacing: 20cm $\times$ 20 cm; 2 seedling per hill) in four replicated plots (5 x 5m $=$ 25 m$^2$). Fields were submerged by applying irrigation from transplanting to panicle initiation (70 DAT) stage of the crop.

3.2.4. Gas sampling and estimation of methane emission

Methane flux was recorded from the first day of transplanting (0 DAT) and thereafter 7-day intervals till harvest. Two additional methane samplings were done at 7 and 14 days after harvest. Details of materials and methods employed are described in 3.1.4. Cumulative methane emissions from rice varieties for the entire growth period were computed by the method of Naser et al. (2007) by using the following formula:

$$\text{Cumulative gas emission} = \frac{n^{-1} \sum_{i=1}^{n} (R_i \times D_i)}{D_i},$$

Where, $R_i$ is the mean gas emission (mg m$^{-2}$ day$^{-1}$) of the two sampling times, $D_i$ is the number of days in the sampling interval, and $n$ is the number of sampling times. Cumulative methane emissions are expressed as seasonal integrated flux ($E_{int}$) in g m$^{-2}$.
3.2.5. *Morpho-physiological parameters of plant*

Details of the methodology employed for the determination of morpho-physiological parameters of plants are described in 3.1.5.

3.2.6. *Yield and yield attributing parameters*

Details of the methodology employed for the determination of yield and yield attributing parameters are described in 3.1.6.

3.2.7. *Soil physico-chemical properties*

Details of the methodology employed for the determination of soil physico-chemical properties are described in 3.1.7.

3.2.8. *Statistical analysis*

Details of the method of statistical analysis are described in 3.1.8.

3.3. *Association of plant growth parameters with methane emission from rainfed upland / Ahu rice*

This experiment was conducted at rainfed rice agroecosystem of North Bank Plain Zone of Assam with two (2) rice varieties during summer season (*Ahu rice*). The detail technical programme of this experiment is given below.
Fig. 3.6. Light transmission (%) through the canopy of cultivars Agni and Ranjit. Data presented are means ± SEd (vertical bars).

Fig. 3.7. Light transmission (%) through the canopy of varieties Disang and Luit. Data presented are means ± SEd (vertical bars).
3.3.1. Geographical location, climatic conditions and soil characteristics of the experimental site

The geographical location and climatic conditions of the experimental area are described in 3.1.1. Methane emission from paddy fields was estimated during the rainfed summer rice (locally known as Ahu) growing season (April - July) of 2006. The available meteorological data during the experimental periods were collected and presented in Figure 3.3. Various soil physiochemical properties of the experimental field are presented in Table 3.1. Light transmission (%) through the canopy of rice plant is presented in Figure 3.7.

3.3.2. Selection of rice variety

Two rice varieties viz. Disang and Luit were selected for this experiment.

3.3.2.2. Description of varieties

1. Disang: This variety was developed at Regional Agricultural Research Station (RARS), Titabor of Assam Agricultural University, Jorhat, India, by cross combination between ‘Heera’ and ‘Annada’. This semi-dwarf variety is recommended for flood-prone areas before the onset of flood in Ahu season. Duration and average yield in field condition is 95-100 days and 3.5-4.0 t ha\(^{-1}\) respectively.

2. Luit: This variety was developed at Regional Agricultural Research Station (RARS), Titabor of Assam Agricultural University, Jorhat, India, by cross combination between ‘Heera’ and ‘Annada’. It is recommended for flood-prone areas in Ahu season (April-July). Duration and average yield in ideal field condition is 95-100 days and 3.5-4.0 t ha\(^{-1}\) respectively.
3.3.3. **Field preparation**

The methodology of field preparation is described in 3.1.3.

3.3.4. **Gas sampling and estimation of methane emission**

Methane flux was recorded from the day of transplanting at 7-days intervals. Two additional methane samplings were done at 7 and 14 days after harvest. Details of materials and methods employed are described in 3.1.4. Cumulative methane emissions from two rice varieties for the entire growth period were computed as described in 3.2.4.

3.3.5. **Morpho-physiological parameters of plant**

Details of the methodology employed for the determination of morpho-physiological parameters of plant are described in 3.1.5.

3.3.6. **Yield and yield attributing parameters**

Details of the methodology employed for the determination of yield and yield attributing parameters are described in 3.1.6.

3.3.7. **Soil physico-chemical properties**

Details of the methodology employed for the determination of soil physico-chemical properties are described in 3.1.7.
3.3.8. **Statistical analysis**

Details of the methods of statistical analysis are described in 3.1.8.

3.4. **Analysis of intervarietal difference in methane flux from rice plants grown during monsoon season as biological mitigation option**

This experiment was conducted at shallow water rice agroecosystem of North Bank Plain Zone of Assam with ten (10) rice cultivars during monsoon season (*Sali* rice). The detail technical programme of this experiment is given below.

3.4.1. **Geographical location, climatic conditions and soil characteristics of the experimental site**

The geographical location and climatic conditions of the experimental area are described in 3.1.1. Methane emission from paddy fields was estimated during the rainfed monsoon rice (locally known as *Sali*) growing season (August - November) of 2006. The available meteorological data during the experimental periods were collected and presented in Figure 3.3. Various soil physicochemical properties of the experimental field are presented in Table 3.1. Light transmission (%) through the canopy of rice plant is presented in Figure 3.8.
Fig. 3.8. Light transmission (%) through the canopy of ten rice cultivars. Data presented are means ± SEd (vertical bars).
3.4.2. Selection of rice variety

This experiment was conducted with ten (10) rice cultivars. Among the cultivars, five (5) were traditional rice genotypes popularly grown in this agro-climatic zone, viz. Basmuthi (V₁), Bogajoha (V₂), Choimora (V₃), Rashmisali (V₄) and Lalkalomdani (V₅); and the other five (5) were high yielding varieties viz. Mahsuri (V₆), Moniram (V₇), Kushal (V₈), Prafulla (V₉) and Gitesh (V₁₀).

3.4.2.2. Description of varieties

1. Basmuthi (V₁): It is an indigenous tall traditional rice cultivar. It is grown under rainfed low-land condition during monsoon (Sali) season.

2. Bogajoha (V₂): It is an indigenous traditional rice cultivar. It is generally grown under rainfed low-land condition during monsoon (Sali) season. This tall cultivar has good cooking quality.

3. Choimora (V₃): It is also an indigenous traditional rice cultivar. It is grown under rainfed low-land condition during monsoon (Sali) season.

4. Rashmisali (V₄): It is an indigenous traditional rice cultivar. It is generally grown under rainfed low-land condition during monsoon (Sali) season.

5. Lalkalomdani (V₅): This is also a local collection. It is a popular traditional cultivar of the agroclimatic zone. This cultivar has good cooking quality and is grown during monsoon season.

6. Mahsuri (V₆): It is a derivative of Indica and Japonica hybridization programme. The cross combination was between Taichung 65 and Mayong ebos 80/2. It has medium slender grain type with very good cooking quality. Duration and average yield in ideal field condition is 140-145 days and 3.5-4.0 t ha⁻¹ respectively.

7. Moniram (V₇): This variety was developed at Regional Agricultural Research Station (RARS), Titabor of Assam Agricultural University, Jorhat, India, by cross combination
between 'Pankaj' and 'Mahsuri'. This semi-dwarf variety is recommended for shallow water (0-30cm) submergence in flood prone areas. It is a blast tolerant non-lodging variety. Duration and average yield in ideal control field condition is 150-155 days and 4.5-5.0 t ha\(^{-1}\) respectively.

8. Kushal (V\(8\)): This variety was developed at Regional Agricultural Research Station (RARS), Titabor of Assam Agricultural University, Jorhat, India, by cross combination between 'Pankaj' and 'Mahsuri'. This semi-dwarf variety is recommended for shallow water (0-30cm) submergence in flood prone areas. It is a non-lodging variety. Duration and average yield in ideal control field condition is 150-155 days and 4.5-5.0 t ha\(^{-1}\) respectively.

9. Prafulla (V\(9\)): This variety was developed at Regional Agricultural Research Station (RARS), Titabor of Assam Agricultural University, Jorhat, India, by cross combination between 'Akisali' and 'Kushal'. This semi-dwarf variety is recommended for shallow land flood-plane and flood prone areas. Duration and average yield in ideal control field condition is 150-155 days and 5.0-5.5 t ha\(^{-1}\) respectively.

10. Gitesh (V\(10\)): This variety was developed at Regional Agricultural Research Station (RARS), Titabor of Assam Agricultural University, Jorhat, India, by cross combination between 'Akisali' and 'Kushal'. This semi-dwarf variety is recommended for shallow land flood-plane and flood prone areas. Duration and average yield in ideal control field condition is 150-155 days and 5.0-5.5 t ha\(^{-1}\) respectively.

3.4.3. Field preparation

The experimental plot was ploughed, puddled thoroughly to 15-cm depth and levelled. Fertilizer was applied at the rate of 40: 20: 20 kg N-P-K ha\(^{-1}\) in the form of urea, single super phosphate (SSP) and murate of potash (MOP). One third (1/3\(^{rd}\)) of full dose of urea along with full dose of SSP and MOP was applied at the time of final land preparation. Remaining part of urea was applied at the time of tillering (1/2 of the remaining part) and panicle initiation stage (another 1/2 of the remaining part), as recommended in the package.
of practice of Assam Agricultural University. Rice seedlings (25-day-old) of ten rice cultivars were transplanted (spacing: 20 cm x 20 cm; 2 seedling per hill) in four replicated plots (5 x 5 m = 25 m²).

3.4.4. Gas sampling and estimation of methane emission

Methane flux was recorded from the first day of transplanting (0 DAT) and thereafter at 7-days intervals till harvest. Two additional methane samplings were done at 7 and 14 days after harvest. Details of materials and methods employed are described in 3.1.4. Cumulative methane emissions from two rice varieties for the entire growth period were computed as described in 3.2.4.

3.4.5. Morpho-physiological parameters of plant

Transpirational rates (mmol H₂O m⁻² sec⁻¹) of intake leaf were measured at weekly interval (from 7 DAT till harvest) by an infra-red gas analyzer (LI-6400 portable photosynthesis system, LICOR, USA), under ambient environmental conditions. The middle portion of a fully expanded, healthy-green 2nd leaf from the top was used for measurement up to the pre-flowering stage, and the flag leaf was used for transpiration measurement from the panicle initiation stage of the crop. Leaves were held in the chamber until values of transpiration were observed to be as constant as possible (steady state). Leaves were kept at steady state for 1 min before measurements were taken.

Details of the methodology employed for the determination of other morpho-physiological parameters of plant are described in 3.1.5.
3.4.6. Leaf and stem anatomy

For scanning electron microscopic (SEM) analysis, leaf sections were prepared from the middle portion of the flag leaf at panicle initiation stage. Nodal sections of the stems were taken from about 15 cm above the ground surface. Fresh leaf and stem samples of different rice cultivars were fixed and dehydrated following the method of Neinhuis and Edelmann (1996). After fixation and dehydration samples were fixed to metal stubs with carbon adhesive tape, coated with platinum by Auto Fine Coater (JEOL, JFC-1600) and examined in a Scanning Electron Microscope (JEOL, JSM-6390LV, Japan). Observations and photographs with the scanning electron microscope were made at 15KV. Stomatal frequency was measured from four random fields of each sample (from adaxial surface) and mean values are expressed as number of stomata mm\(^{-2}\). For the measurement of the diameter of medullary cavity, stem sections were taken from the rice plants from about 15cm above the ground surface. Leaf sheaths were carefully removed and fine sections of the culm were examined under Stereo Microscope (Stemi 2000-C, ZEISS, Germany) at 40 X. Observations were recorded from four microscopic fields of each sample and diameters of medullary cavity (mm) were computed by using Axiovision LE Documentation Software (Germany).

3.4.7. Yield and yield attributing parameters

Details of the methodology employed for the determination of yield and yield attributing parameters are described in 3.1.6.
3.4.8. Soil physico-chemical properties

Details of the methodology employed for the determination of soil physico-chemical properties are described in 3.1.7.

3.4.9. Statistical analysis

Measurements of different parameters at all the growth stages were replicated for four times. The significance of the difference was assessed by ANOVA and subsequently by Duncan’s multiple range test (DMRT), and differences are reported at probability (P) <0.05, considering cultivars as source of variation. Correlations of physiological and anatomical parameters (mean values of different growth stages) with mean CH$_4$ flux values from the cultivars were done by using SPSS package programme (Version 10.0).
Plate 3.1. Gas sampling in rice field by static chambers

Plate 3.2. Gas-sample analysis in Gas Chromatograph

Plate 3.3. Portable laser leaf area meter

Plate 3.4. Portable photosynthesis system