MATERIALS AND METHODS
MATERIALS AND METHODS

Towards understanding the physiology of rice grown on water-logged soils and realising the objectives envisaged in the introduction, the following experiments have been conducted. All the field experiments mentioned below (Expts. I to V) were carried out on water-logged soils.

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*Kharif refers to the rainy or wet monsoon season obtained between July and December. Rabi is the dry season prevalent from January through May.*
(iii) Biochemical changes rabi, 1974
(a) Chlorophyll a, chlorophyll b and a/b ratio
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VIII. 14C photosynthesis and translocation (pot studies)

(i) Photosynthesis
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(c) Effect of nitrogen supply kharif, 1970
(ii) Translocation of 14C photosynthates kharif, 1970

A list of abbreviations used in the text is given in pages vii and viii.

I. Growth analysis and nutrient uptake pattern at different growth stages (kharif, 1970)

Thirty four day old seedlings of ten varieties viz.: MG.1231, Jagannath, Pankai, Manoharsali, Mku.32, AR.614-256-6, CR.1014, CR.70-80-2, CR.70-91-9 and T.1242
were transplanted, 2 seedlings/hill at 20 x 15 cm spacing after conventional soil preparations. (For the sake of brevity, henceforth, the varieties AR.614-256-6, CR.70-80-2 and CR.70-91-2 shall respectively be referred to as AR.614, CR.70-80 and CR.70-91. CR.70 cultures wherever so mentioned, shall again refer to the latter two varieties, viz., CR.70-80-2 and CR.70-91-2). The field was ploughed twice followed by puddling and levelling. A basal dose of 30 kg each of N and P₂O₅/ha in the form of ammonium sulphate and superphosphate respectively was incorporated into the soil. The experiment was randomised and replicated thrice in plots of 6.0 x 6.6 m.

Tiller number and height were recorded at fortnightly intervals up to flowering. Plant height was measured with the longest leaf stretched during vegetative phase and up to panicle tip during reproductive phase. For the purpose, two diagonal areas of 0.30 m² each were marked and the tiller number of the 30 plants at each site was recorded. Height was measured on the four corner plots. Every fortnight up to flowering and then at harvest, plant samples from a total of 8 hills from two diagonal sites of four adjacent hills each, were collected. After removal of roots the samples were separated into leaf, culm and panicle depending upon the stage. After measurements for leaf area they
were oven-dried at 80°C for 72 hr and then their dry weights recorded. The following observations and estimations have been computed from the plant samples.

1. total dry weight (TDW)
2. leaf area index (LAI)
3. specific leaf weight (SLW)
4. leaf length
5. relative rate of increase in height (RGR)
6. crop growth rate (CGR)
7. relative growth rate (RGR)
8. net assimilation rate (NAR)
9. leaf area ratio (LAR)
10. leaf weight ratio (LWR)
11. leaf area to leaf weight ratio (LALW)
12. nitrogen per cent in leaf blade, culm and panicle
13. phosphorus per cent in leaf blade, culm and panicle
14. potassium per cent in leaf blade, culm and panicle
15. iron per cent in leaf blade, culm and panicle (in flowering samples only)
16. manganese per cent in leaf blade, culm and panicle (in flowering samples only)
17. nitrogen per unit leaf area (N/LA)
18. nitrogen per unit ground area (N/GA)
19. total nitrogen uptake
20. total phosphorus uptake
21. total potassium uptake

While CGR, RGR, NAR, LAR and LALW were calculated up to flowering only, leaf length, LAI, SLW and LAR were recorded even after flowering at weekly intervals.

Total dry weight is the weight of dried plant expressed in g/m² land area.
Leaf area index is the area of functional leaf blade per unit area of land surface. The data was collected as follows: the length and maximum breadth of 20 representative leaves were measured and their dry weight as also that of the remaining leaves from the 8 hills recorded. The leaf area of the 20 leaves is the product of their length, breadth and the factor 0.695 (Rao et al., 1966). The leaf area of the 8 hills was computed from the total leaf blade weight and the weight per cm². The LAI was then derived by dividing the leaf area per 8 hills by the field area occupied by the 8 hills (0.24 m²).

Specific leaf weight is the dry weight of leaf blade per unit leaf area expressed in mg/dm²; RHGR was calculated following Sen and Gupta (1969) and expressed as cm/cm/day; NAR and CGR were computed following Watson (1952) and expressed respectively as g/m² leaf area/day and g/m² land area/day; LWR and RGR following Thorne (1960) and expressed as m²/g and g/g/day respectively; LWR and LALW after Cooper (1966) and expressed as g/g and m²/g respectively. The formulae are given below:

\[ RHGR = \frac{\log_{e} W_2 - \log_{e} W_1}{t_2 - t_1} \]
\[ CGR = \frac{W_2 - W_1}{t_2 - t_1} \]
\[ RGR = \frac{\log_{e} W_2 - \log_{e} W_1}{t_2 - t_1} \]
\[ NAR = \frac{(W_2 - W_1)(\log_{e} LAI_2 - \log_{e} LAI_1)}{(t_2 - t_1)(LAI_2 - LAI_1)} \]
\[ \text{LAR} = \frac{(\text{LAI}_2 - \text{LAI}_1)(\log_{10} \text{W}_2 - \log_{10} \text{W}_1)}{(\text{W}_2 - \text{W}_1)(\log_{10} \text{LAI}_2 - \log_{10} \text{LAI}_1)} \]

\[ \text{LWR} = \frac{(\text{W}_2 - \text{W}_1)(\log_{10} \text{W}_2 - \log_{10} \text{W}_1)}{(\text{W}_2 - \text{W}_1)(\log_{10} \text{LW}_2 - \log_{10} \text{LW}_1)} \]

\[ \text{LALW} = \frac{(\text{LAI}_2 - \text{LAI}_1)(\log_{10} \text{LW}_2 - \log_{10} \text{LW}_1)}{(\text{LW}_2 - \text{LW}_1)(\log_{10} \text{LAI}_2 - \log_{10} \text{LAI}_1)} \]

where \( H_2 \) and \( H_1 \), \( W_2 \) and \( W_1 \), \( \text{LAI}_2 \) and \( \text{LAI}_1 \), \( \text{W}_2 \) and \( \text{W}_1 \) are the heights, total dry weights, leaf area indices and leaf weights at times \( t_2 \) and \( t_1 \).

Nitrogen in 0.1 to 0.2 g of the oven-dried powdered sample was determined by the semi-micro-Kjeldahl method (Jackson, 1953). For the determination of other nutrients, 1.0 to 2.0 g of the ground material was digested with nitric, perchloric and sulphuric acids (Johnson and Ulrich, 1959). Phosphorus was determined calorimetrically by the sulphate-molybdate method described by Fiske and Subbarow (1925); potassium by the flame photometer (Jackson, 1953); iron and manganese calorimetrically by the thiocyanate and periodate methods respectively (Snell and Snell, 1949).

Nitrogen per unit leaf area expressed in mg/dm\(^2\) is the product of \( N \) per cent in leaf blade and specific leaf weight. Nitrogen per unit land area expressed in g/m\(^2\) is the product of \( \text{LAI} \) and \( W_{LA} \).

At maturity, the following observations, besides grain yield in kg/ha were made:
1. panicles/m²
2. grain number/panicle
3. spikelet number/panicle
4. sterility per cent
5. 1000 grain weight
6. E/T ratio
7. harvest index

Panicles/m² was derived by dividing the number of panicles obtained from the sample site by the area of the sample site (1.8 m²). Grains and spikelets/panicle, sterility per cent and 1000 grain weight were calculated from a representative sample of 20 panicles. The ratio of ear-bearing or productive tillers to the total maximum number of tillers formed is the E/T ratio. Harvest index is the percentage of grain weight to total plant weight (Donold, 1962; Singh and Stoskopf, 1971). All these data pertain to the sample area.

In the following experiments also the above mentioned estimations and observations, wherever recorded, were done or derived at, unless otherwise described, in the same manner as delineated above.

II. Effect of close and wide spacing on growth and yield (Kharif, 1970)

Ten varieties (same as in Expt. I) were transplanted at 30 day old stage after the conventional field preparations. A basal dose of 30 kg/ha each of N and P₂O₅ was given in the form of ammonium sulphate...
and single super phosphate respectively. The experi-
ment was laid out in the split-plot design, with spac-
ings in the main plot and varieties in the sub-plot, 
in plots of 3.5 x 4.75 m. The two spacings were 
20 x 15 cm and 60 x 45 cm.

Samples from 10 and 20 hills were collected 
respectively from the wide and close spacings at flower-
ing and harvest and the total dry weight and yield 
expressed both on hill and unit area basis. Tiller 
number on similar basis and height were also recorded 
at five stages (20, 30, 45, 60 and 72 days after plant-
ing). Leaf area at flowering was also calculated. The 
following data were recorded at harvest:

1. grain yield
2. number of panicles
3. grain number/panicle
4. 1000 grain weight
5. E/T ratio
6. harvest index

III. Effect of nitrogen levels on growth and yield 
attributes (Kharif, 1970)

Thirty five day old seedlings of ten varie-
ties (same as in Expts.I and II) were transplanted in 
a split-plot design with nitrogen in the main plot and 
varieties in the sub-plot, in rows 20 cm apart, with 
hills at intervals of 15 cm in plots of 6.15 x 3.20 m. 
Nitrogen at 40 or 80 kg was applied as basal dose
along with 20 kg $P_2O_5$ and 20 kg $K_2O$/ha. Ammonium sulphate was the source of nitrogen.

Tiller number and height at six growth stages viz., 17, 31, 45, 61 and 72 days after planting besides flowering were recorded on a sample area of 0.72 m$^2$. Height measurements were confined to the four corner plants in the sample area. Plant samples from 8 hills were collected at maximum tillering, flowering and harvest and the TDW worked out. Recording of leaf length apart, LAI and SLW were calculated at maximum tillering and flowering stages. Nitrogen in the plant parts was analysed in flowering and harvest samples and the total uptake derived. At the time of harvest, the following data were obtained from the sample area:

1. panicles/m$^2$
2. grain number and spikelet number/panicle
3. sterility per cent
4. thousand grain weight
5. E/T ratio
6. harvest index

IV. Effect of waterlogging on growth components, yield and yield attributes (Kharif, 1971)

Six varieties, viz., IR.2, Jagannath, Manoharsari, T.141, NS.1281 and Prasadbhog were transplanted on two different fields after proper preparations of each. One was a well drained field and the other was water-
logged especially during the tillering phase. A basal
dose of 40 kg N, 20 kg P<sub>2</sub>O<sub>5</sub> and 20 kg K<sub>2</sub>O/ha in the
form of ammonium sulphate, single super phosphate and
muriate of potash respectively was given to both the
fields. The six cultivars were replicated four times
and randomised in each field. The plot size consisted
of 9.60 x 2.70 m.

Planting of 30 day old seedlings was done at
a spacing of 20 x 15 cm with 20 cm between rows, and
2 to 3 seedlings/hill. A sample area of 0.72 m<sup>2</sup> was
marked in each plot in which the periodical tiller number
was recorded. Plant samples from 3 hills, 4 each from
2 diagonal sites were collected at the following crop
growth stages: maximum tillering, primordial initiation,
flowering, 10 DAF, 20 DAF and harvest. For TR, however, samples were collected only at three stages,
<em>viz.</em> MT, F and H.

The following observations were recorded from
the samples collected, which, after cleaning and removal
of roots, were separated into the different morphological
parts, dried and weighed.

1. TDW
2. LAI
3. SLN
4. leaf length
5. N per cent in leaf
6. N per cent in culm
7. N per cent in panicle
8. total N uptake
9. N<sub>DA</sub>
At the time of harvest, yield, panicles/m^2, grains/panicle, 1000 grain weight and HI were recorded.

Path coefficient analysis: Correlation coefficients of yield and six auxiliary characters which influence yield, viz., panicles/m^2, grains/panicle, TDW at flowering, LAI at flowering, N uptake at flowering and MGA at flowering were drawn in all possible combinations against three replication figures. Partial regression coefficients were estimated and standardised according to the method of Goulden (1966). The correlation coefficient was partitioned into its direct and indirect effects through other ancillary components on yield following Dewey and Lu (1959). This was calculated for both the normal and water-logged conditions separately.

Heritability: Heritability estimates in the broad sense were computed for TDW at flowering using the formula,

\[ h^2 = \frac{g^2}{p^2} \]

\( g^2 \) is the component of variance due to genetic differences among varieties, \( p^2 \) is the variance of variety mean and is equal to \( g^2 + e^2 \) where \( e^2 \) is the environmental variability (Rawlings et al., 1958).
V. Growth and yield as affected by higher nitrogen application under normal and water-logged conditions (kharif, 1972)

To determine the influence of nitrogen under normal and water-logged conditions on growth attributes like dry matter, LAI, nitrogen per cent and nitrogen uptake, etc., a field experiment was conducted in kharif, 1972. Eight varieties, 4 high yielding and 4 aman types viz., IR.8, Java, Vialava, Jasannath, Mancharasali, T.141, NC.1281 and Prasadbhog were included.

The experiment was laid out in two plots, one well-drained and the other water-logged and in each, nitrogen levels (40 and 20 kg/ha) were in the main plot and varieties in the sub-plot. The nitrogen was applied as basal dose along with 20 kg P<sub>2</sub>O<sub>5</sub> and 20 kg K<sub>2</sub>O at planting. The plot size was 6.40 x 1.65 m.

Thirty day old seedlings from nursery were transplanted at a spacing of 20 x 15 cm with 15 cm between hills and 2 to 3 seedlings/hill. A sample area of 0.60 m<sup>2</sup> consisting of 20 plants was demarcated for recording periodical tiller production and harvest data. Samples from 8 hills were made at the following growth stages: mid-tillering, maximum tillering, primordial initiation, flowering, mid-harvest and harvest. For IR.8, Java and

* late, photosensitive
Vi-1ava, however, sampling at only four growth stages, viz., mid-tillering, maximum tillering, flowering and harvest were done. After proper cleaning and dispensal of roots, the leaf, culm and panicles, as the case may be, were separated out and dried in a hot air oven at 80°C for 72 hr.

The following data were collected from the samples:

1. TDW  2. LAI
3. SLW  4. leaf length
5. N per cent in leaf, culm and panicle  6. N uptake

Yield, panicles/m², grains and spikelets/panicle, 1000 grain weight, E/T ratio and harvest index were recorded at harvest from the sample area.

VI. Effect of submergence of plant

(1) Growth and yield (rabi, 1970)

A pot experiment was conducted during Rabi, 1970 with eight varieties, viz., Padma, Java, CB.10-1128, P210, IR.2, Hamsa, Mtvs.15 and T(N)1 x E.65. The seedlings were transplanted 30 days after germination in 30 cm earthen pots holding 15 kg soil. Hundred ppm of N/pot was given at planting.

Thirty six days after planting the plants were submerged to 30 cm depth of water in cement tanks.
of the dimensions 3.0 m long, 2.5 m broad and 0.90 m deep. Control pots were maintained at saturation levels.

In each variety there were four control pots and four submerged pots for assessment of yield. This apart, there were 12 additional pots for each variety for sampling at pre-submergence, booting and flowering, 2 pots per treatment every stage. Raising the water depth to 30 cm was accomplished over a period of 4 days and the depth maintained till maturity with fortnightly replenishments to compensate for evaporation loss.

Periodical data on tiller count and height were recorded; the flowering samples were analysed for nitrogen and the nitrogen uptake worked out. At harvest the following observations were recorded:

1. grain yield
2. panicle/pot
3. grains/panicle
4. 1000 grain weight
5. panicle length
6. grain/straw ratio
7. root volume

Root volume was determined as follows: the soil around roots was removed by water-jet and the roots recovered. The root volume was ascertained by the displacement method.

(ii) Critical stage of submergence (kharif, 1971)

Six varieties, viz., IR.8, Jagannath, Manoharsali, T.141, NC.1281 and Prasadbhok were direct sown, 3 seeds/pot in 30 cm earthen pots filled with 15 kg soil. Hundred ppm N/pot was added at the time of sowing.
Adequate periodical prophylactic measures were observed. Submergence to 60 cm of water in cement tanks of the size 3.0 m long, 2.5 m broad and 0.90 m deep was given as indicated below:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Period of submergence (days)</th>
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</thead>
<tbody>
<tr>
<td>1. Seedling (35 days)</td>
<td>5</td>
</tr>
<tr>
<td>2. Maximum tillering</td>
<td>10</td>
</tr>
<tr>
<td>3. Flowering</td>
<td>10</td>
</tr>
<tr>
<td>4. Control</td>
<td>Saturation throughout</td>
</tr>
</tbody>
</table>

Grain yield/pot was recorded at the time of harvest. At harvest, nitrogen content in leaf blade and culm was analysed in the control plants and those submerged at seedling and flowering.

(iii) Biochemical changes (Rahil, 1974)  
(a) Chlorophyll a and chlorophyll b: Six varieties, viz., IR-8, Jagannath, Manoharsali, T-141, N-1281 and Prasadbhog were direct sown in porcelain pots. About 21 days after germination the seedlings were thinned and uniform number of seedlings retained in each pot. Hundred ppm N was added to each pot after thinning. There were four pots for each variety. When 35 days old, 2 pots in each variety were submerged totally in cement tanks and the other 2 pots kept under normal conditions. Samples were drawn at 0, 2, 4, 6 and 8 days after submergence from both normal and submerged pots.
and the chlorophyll a and chlorophyll b determined in the leaves following Arnon (1949). In each treatment, at every sampling, seedlings from the two pots were pooled and duplicates drawn for analysis.

Fifty mg of the leaf material was chopped and ground with acid washed sand of neutral pH in a pre-chilled mortar with 10 ml of 80% acetone. The extract was centrifuged at 1200 x g for 5 min to allow the debris to settle. In order to ensure complete extraction, the debris was washed with 80% acetone and again centrifuged. The supernatant obtained was made to 15 ml with 80% acetone. Absorbency was measured at 645 and 663 nm in a spectronic 20. The amount of chlorophyll was calculated using the nomogram of Kirk (1968) and expressed as mg/g fresh wt.

(b) Total sugars in leaf blade and leaf sheath:
Thirty five day old seedlings of 6 varieties, viz., IR.2, Jacannath, Manoharaali, T.141, MC.1281 and Prasadbhog, dry sown and reared as in experiment described above, were submerged completely in cement tanks. Normal plants were grown in the green-house. There were two pots/variety under each treatment and at every sampling, seedlings from the two pots were pooled. Total sugars in the leaf and sheath at 0, 3 and 6 days were assayed.

Preparation of extract: Tissue samples were chopped into small bits. Exactly 500 mg of the material
was plunged into 5 to 8 ml of boiling 80% ethanol, extracted for 5 min on a hot water bath, and cooled in a running tap. The material was homogenised by grinding in a porcelain mortar with pestle and filtered through cheese cloth. The residue was transferred back to minimum quantity of boiling 80% ethanol, reextracted for 5 min, cooled and filtered. Both the extracts were pooled and filtered through Whatman No. 41 filter paper and the final volume was adjusted to 15 ml with 80% ethanol (Mahadevan et al., 1965).

Estimation of total sugars: Total sugars were estimated by the phenol-sulphuric acid method (Hodge and Hofreiter, 1962). Aliquot of 0.5 ml of the extract and 0.5 ml of glass distilled water were pipetted into a boiling tube. One ml of 5% phenol solution was added and thoroughly mixed. Blanks contained 1 ml of distilled water. From a fast flowing pipette, 5 ml of 96% sulphuric acid was added to each tube so that the stream hit the liquid. The tube was agitated during the acid addition. After about 5 min, the mixture was diluted to 14 ml by the addition of 7 ml of distilled water. The tubes were reshaken and placed on water baths at 25 to 30°C for 20 min. The absorbency of the yellow-orange colour developed was measured at 490 nm in a spectronic 20 against the reagent blank. Sugars of unknown were calculated from glucose standards and expressed on per cent dry weight basis.
(c) Respiratory rate in leaves: Thirty five day old seedlings of 3 varieties, viz., IR-8, EC.1281 and Prasadhog raised as above were subjected to total submersion and the respiratory rate measured using a Warburg manometer at 0, 2, 4, 6 and 8 days after submersion. Corresponding samples from normal grown plants served as controls.

Weighed quantity of leaves (100 mg) was cut into 1-2 mm bits and suspended in 2 ml of 0.1 M phosphate buffer at pH 6.5 in a 15 ml Warburg manometric flask; 0.2 ml of 10% KOH was pipetted into the central well and a folded filter paper kept in the well. The flasks were equilibrated for 15 min by shaking at 30 C. Readings were taken at 15 min intervals for a period of 1 hr (Umbreit et al., 1972). Oxygen consumption in μl by the tissue was calculated and expressed as μl/g fresh wt/hr.

(d) Nitrate reductase activity: The nitrate reductase activity in leaves was assayed in the submerged and normal plants of six varieties, viz., IR-8, Jagansath, Manchorsali, T.141, NC.1281 and Prasadhog at 0, 3, 6, 24 and 48 hr following the in vivo method of Klepper et al. (1971).

Leaves were cut into small 1-2 mm pieces and placed in a 50 ml Erlenmeyer flask containing 2.5 ml of glass distilled water. Just before evacuation, 2.5 ml of 0.2 M KMnO₃ was added and the flasks evacuated for 30 sec and the vacuum released. The flasks were then incubated.
at 30 °C for 30 min. One ml of the solution from the flask was pipetted into a test tube. To this was added 1 ml sulphanilamide (1% W/V in 1 M HCl), followed immediately by 1 ml of 0.01% NEDH (N-1 napthyl ethylene diamine hydrochloride) solution. The pink colour developed was read at 540 nm in a spectronic 20. Nitrate reductase activity was computed using a standard curve of nitrite employing the above colour reaction. Enzyme activity was expressed as nM/g fresh wt/hr.

(e) RuDP carboxylase: Twenty one day old seedling of three varieties, IR.8, NC.1281 and Prasad-bhog were submerged for 5 days. RuDP carboxylase was assayed in the leaves at pre-submergence, post-submergence (5 days) and 2 days after return to normalcy. The carboxylase was assayed following Maruyama and Lane (1962).

Two hundred mg of leaf material was chopped and extracted with 4 ml of extraction mixture in an iced-cold mortar and pestle. The extraction mixture contained the following in a total volume of 600 ml: Tris buffer, 0.04 M; MgCl₂ 0.01 M; EDTA, 0.925 μM and cysteine 5 mM (pH 8.0). The slurry was filtered through a single layer of muslin cloth and the filtrate centrifuged at 12000 x g for 10 min at -5 °C. The supernatant was made up to 4 ml with extraction mixture and was used for the enzyme assay.
In a test tube, 0.2 ml of reaction mixture which consisted of the following in µM in a total volume of 40 ml: KHCO₃, 2.5; cysteine, 1.25; EDTA, 0.1; Tris, and MgCl₂, 2.5 (pH 8.1), 0.1 ml of R-5-P 2 µM, 0.1 ml of ATP 2 µM and 0.1 ml of Na¹⁴C0₃ to give 0.5 µc activity were added. Aliquot of 0.2 ml of the enzyme extract was added and the test tube given a vigorous shake. Exactly after 10 min of addition of enzyme extract, the reaction was terminated by adding 1 ml of saturated solution of 2, 4-dinitrophenyl hydrazine in 2 N HCl. After termination of reaction, 3 ml of ethyl acetate containing 3 µg of authentic oxaloacetato phenylhydrazone was added and the test tube given a shake immediately. An aliquot of 0.1 ml from the ethyl acetate phase was drawn and placed in a counting vial. The sample in the vial was dried at 30°C. The counts were obtained on Packard scintillation spectrometer using toluene containing PPO and POPOP as the liquid scintillator (4 g PPO and 10 mg POPOP were dissolved in one litre of toluene). Triplicates were maintained for each sample. The activity was expressed as cpm/g fresh wt/min after applying quench corrections.

VII. Effect of low light intensity on growth and yield (Sharif, 1971)

To assess the nature and extent of damage to low light intensity, a pot experiment with 6 cultivars,
Circular 20 cm porcelain pots holding 6 kg of soil were puddled to equal consistency. One hundred fifty ppm of nitrogen was added to the soil at the time of puddling. Thirty five day old seedlings raised in nursery beds were transplanted, 3 seedlings per pot at equidistance from one another. There were 8 pots per variety. Adequate pest and disease control measures were observed during the growth period.

At the time of anthesis, 4 pots in each variety were made over to shade imposed by covering the pots on all sides with bamboo-mats which allowed only 30 per cent of the sunlight to the interior. The other four pots in each variety were retained under normal sunlight. Care was taken to see that pots distributed over the two light treatments had almost identical tiller number.

At the time of harvest, the following observations and estimations were recorded.

1. yield/pot
2. panicle number/pot
3. grain number/panicle
4. sterility per cent
5. total dry weight/pot
6. harvest index
7. N per cent in leaf and culm
VIII. $^{14}C$ photosynthesis and translocation

(1) Photosynthesis

(a) Effect of low light (Kharif, 1970 and 71):

Leaves from 10 varieties, viz., NC-1281, Jagannath, Pankai, Manoharsali, Mtu-22, AR.614, CR.1014, CR.70-20, CR.70-91 and T.1242 grown in field were collected at maximum tillering stage, 40 leaves from each replication. Leaves from each replication were again divided into 2 lots of 20 each and each lot placed in 100 ml Erlenmeyer flask containing water to prevent their withering and shriveling. Thus there were a total of six flasks for each variety separated into two groups of 3 each.

The leaves were allowed to fix $^{14}C_2$ in a transparent glass photosynthetic chamber. The chamber was cleaved into two portions by the interpolation of a thin cloth; one half received full complement of the sunlight and the other only 30 per cent of it by virtue of it being covered on all sides by thin muslin cloth. One group of flasks was placed in the shaded half and the other kept in the exposed half of the chamber. The flasks in the exposed half were so arranged and the photosynthetic chamber so kept that there was no shading in that half. The $^{14}C_2$ was liberated from $Na_2^{14}CO_3$ contained in four petridishes (0.1 mc in each dish) hung inside the chamber by the addition of lactic acid. All joints of the
chamber were sealed with cellophane to prevent the escape of the liberated $^{14}CO_2$. To facilitate uniform distribution of the $^{14}CO_2$, the air inside the chamber was circulated through an air circulation pump. After about 1 hr of feeding, the excess $^{14}CO_2$ was trapped in NaOH. After removing the flasks from the chamber, the length and breadth of the leaves were measured and the leaves immediately killed in an hot air oven at 100°C for 30 min and later dried at 80°C for 72 hr, weights recorded and the leaves ground through 60 mesh. Activity in 20 mg of the ground material was measured in a Proportional Counting System. Photosynthetic efficiency was expressed as cps/dm$^2$.

In kharif, 1971 also the PE of detached leaves of 9 varieties, viz., IR-3, T(N)1 x T.65, Java, Vifava, Javannath, Manoharsali, T.141, NC.1231 and Prasadhphog was estimated similarly under normal and reduced light intensities.

(b) At seedling stage: In kharif, 1971, 35 day old seedlings of 6 varieties, viz., IR-3, Javannath, Manoharsali, T.141, NC.1231 and Prasadhphog raised in pots were fed with $^{14}CO_2$ in the photosynthetic chamber under normal light and the photosynthetic efficiency expressed as cps/g.
(c) Effect of nitrogen supply

Leaves from the field of 10 varieties grown under two nitrogen levels, viz., 40 kg N and 80 kg N/ha (Expt. III) were similarly fed with $^{14}C_2$ and the activity per unit leaf area recorded.

(ii) Translocation of $^{14}C_2$ photosynthates (Kharif, 1971)

Seeds of 10 varieties, M2.1231, Jagannath, Pankal, Manoharsali, Mtu.22, AR.614, CR.1014, CR.70-80, CR.70-91 and T.1242 were dry sown in 20 cm porcelain pots holding 6 kg dry soil. Twenty five days after germination the seedlings were thinned to 4 seedlings/pot. The pots received 100 ppm N, 50 ppm $P_2O_5$ and 50 ppm $K_2O$ in the form of ammonium sulphate, single super phosphate and muriate of potash respectively. Twenty five days after germination the seedlings were thinned to 3 seedlings/pot.

At flowering the plants were allowed to fix $^{14}C_2$ in the photosynthetic chamber in the manner described earlier. After feeding, half the tillers in each pot was removed, separated into leaf, culm and panicle and dried. The remaining tillers were removed 7 days after feeding and similarly dried after separation. After recording dry weights, the dried material was ground
through 60 mesh and the activity in 20 mg of each plant part determined in a Proportional Counting System. The translocation index was derived using the formula:

\[
\text{Translocation Index} = \frac{\text{Decrease in shoot activity from 0 to 7 days}}{\text{Increase in panicle activity from 0 to 7 days}}
\]