CHAPTER II

MATERIALS AND METHODS

MATERIALS

The seeds of nine rice varieties namely AU1, CO36, CO43, CSC1, CSC2, GR3, IR20, TKM4 and TKM9 were procured from Annamali University, Annamalai nagar, Tamil Nadu Agricultural University, Coimbatore and Gujarat Rice Research Institute, Navagam, India.

METHODS

I. Germination of the grains to study early seedling growth

Selected seeds of uniform size were surface-sterilized with 0.1 per cent mercuric chloride for three minutes, subsequently washed thoroughly with distilled water and blotted dry. These seeds were transferred to sterilized petri dishes containing Whatman No.1 filter paper moistened with test solution of sodium chloride. The seeds were allowed to germinate at electrical conductivity (EC) of 5, 10 and 15 mmhos/cm concentrations of sodium chloride. Each petri dish contained 20 seeds and each treatment was replicated five times. The seeds were set for germination at 30 $\pm$ 1°C under dark conditions. Dry weight of shoot system was recorded using

CHAPTER II

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10 day old seedlings. From this vegetative growth studies, the optimum concentration of EC 10 mmhos/cm sodium chloride was selected for use in further studies.

II. Early growth studies in hydroponics

Among nine rice varieties, two salt tolerant varieties CO 43, and CSC1 and three salt sensitive varieties CSC2. TKM4 and TKM9 were selected for early growth studies. The seeds were germinated on moistened filter paper for six days. The seven day old seedlings were transferred to 150 ml of half strength of unaerated Hoagland's solution (Hoagland and Arnon, 1950) containing 100 mM NaCl having an EC value near to 10 mmhos/cm. The NaCl was omitted in control nutrient solution. The nutrient solution was renewed twice a week. All plants were grown under 10 h of light at 11,000-16,000 lux with incandescent lamps and 14 h dark at temperature of 30-32°C. Shoots and roots were harvested at 7, 14, 23 and 30 days after initial salinization for further studies. Growth, inorganic compounds such as Na^+, K^+ and Cl^- and organic compounds such as free amino acids, protein and total nitrogen were assayed after harvesting as described below. Growth studies were means of five replicates, each consisting of two plants and analysed statistically using analysis of variance (Freund, 1977). Other assays were repeated twice.

1. Inorganic constituents

a. Determination of inorganic elements

Sodium and potassium content were estimated by the method of storey and Wyn Jones (1977). Plant sample was ashed and dissolved in 1N HCl. Sodium and potassium were estimated using flame emission spectrophotometry. Chloride was determined by titration method using silver nitrate (Yoshida et al., 1976).

2. Organic constituents

a. Extraction of free amino acids

The method employed for the extraction of free amino acids is essentially that of Singh et al.(1973). 200 mg of tissues were homogenized with 2 ml of methanol-chloroform-water (MCW) (12:5:1/V) at room temperature. The homogenate was briefly centrifuged to collect the clear supernatant. The residue was shaken with a further 2 ml MCW for 5 min and centrifuged. The supernatants were combined and separated into a lower chlorophyll-containing chloroform layer and an upper methanol-water phase by adding water (1.5 ml) and chloroform (1 ml) to break the stable emulsion formed during extraction. The upper phase was diluted with 10 ml water and used for estimation of free amino acids and free proline.

b. Free amino acid estimation

The method employed for the estimation of free amino acids is essentially that of Rosen (1957) by ninhydrin method using glycine as standard.

c. Free proline estimation

The proline estimation was followed by Singh et al. (1973) method. 10 ml of above extract was shaken for 10 min with permutit resin (375 mg Folin Decalso F). The solution was decanted off the resin into a boiling tube, and 5 ml glacial acetic acid and 5 ml acidic ninhydrin reagent (125 mg ninhydrin: 3 ml glacial acetic acid: 2 ml 6 M Othophosphoric acid) were added. The mixture was then boiled for 45 min, cooled at room temperature, and shaken with a known amount of benzene (5-15 ml depending upon proline concentration). The optical density of the ninhydrin product dissolved in the benzene was measured at 515 nm and the proline concentration estimated from a standard curve.

d. Extraction and estimation of protein-bound-proline

Protein-bound-proline was estimated by Singh et al. (1973) method. Free proline in the tissue sample was removed by extraction with MCW three times; no free proline was detected in the third extract. A water-soluble protein

fraction which could be precipitated by trichloroacetic acid was obtained from the residue and hydrolysed with 6 N HCl at 110°C for 20 hr. Residual HCl was removed from the hydrolysates by repeated redrying before they were dissolved in MCW. Proline was estimated by the ninhydrin method.

e. Extraction of protein

Protein was extracted as described by Prisco and Vieira (1976). 200 mg of tissue was ground in 0.01 M Na-K-phosphate buffer, pH 7.6, containing 0.1 M NaCl. The ratio of tissue to the grinding medium was 1:10 (w/v). The homogenate was centrifuged at 3000 r.p.m. for 10 min., and the supernatant was assayed for total protein. The pellet was resuspended in a volume of 0.1 N NaOH equal to the initial supernatant, and the insoluble protein was assayed. Soluble protein was determined as the difference between total and insoluble protein.

f. Determination of protein

The protein was determined by the modified Lowry et al. method (Hartree, 1972).

g. Determination of total nitrogen

Total nitrogen was estimated as described by Umbriet et al. (1959). 50 mg of dry powder was digested with 2 ml of 2N H_2SO_4 containing copper selenate (20 mg/100 ml of 2N H_2SO_4)

and made up to volume 25 ml. An aliquot of the sample was taken and the nitrogen content was estimated using double-iodide reagent.

III. Later growth studies

All nine rice varieties namely AU1, CO36, CO43, CSC1, CSC2, GR3, IR20, TKM4 and TKM9 were used for this study. The pot culture experiments were carried out in earthen pots with 7 kg of soil under the net-house conditions. Salinization was imposed on three week-old-seedlings by the addition of sodium chloride solution of electrical conductivity (EC) 10 mmhos/cm once in a week with a combination of normal watering on other days for 7 weeks. Controls received only water. Soil samples were collected just before the salt treatments for analysis of pH, EC, sodium, chloride, potassium, calcium, magnesium, carbonate, bicarbonate, sulphate, sodium absorption ratio (SAR), potassium absorption ratio (PAR), exchangable sodium percentage (ESP), exchangable potassium percentage (EPP), total cations and total anions. Shoot system was harvested at 2, 4 and 6 weeks after initial salinization for growth measurements and biochemical estimations.

Growth, inorganic compounds such as Na^+, Cl^-, K^+, Ca^{2+} and Mg^{2+}, organic compounds such as free amino acids, RNA, DNA, protein, total nitrogen, reducing sugars, non-reducing

sugars, total soluble sugars, starch, total carhobydrate, ascorbic acid, titrable acid number, chlorophyll and activity of different enzymes were determined from the shoot portion as mentioned below. Growth studies were means of five replicates and analysed statistically as explained before. Other assays were averages of two determinations.

1. Soil determinations

a. Preparation of saturation extract

Saturation extract was prepared according to the method of Ghopra and Kanwar (1980) and used for determination of pH, EC and inorganic ions.

b. Analysis of inorganic ions

The method employed for the determination of inorganic ions present in the soil extract, was essentially that of Jackson (1967). Sodium and potassium were estimated using flame emission spectrophotometry and chloride was estimated by titration method using silver nitrate. Calcium, magnesium and sulphate were determined by titration method using EDTA. Carbonate and bicarbonate were estimated by titration method using H_2SO_4. Exchangable sodium percentage, exchangable potassium percentage, sodium absorption ratio and potassium absorption ratio were calculated using the formula described by Richards (1953). All determinations were repeated twice.

2. Growth studies

Height, fresh weight and dry weight of shoot system were studied at 2, 4 and 6 weeks after initial salinization. Total leaf area, number of tiller per plant, fresh weight and dry weight of tiller and percentage of survival were studied at the sixth week after initial salinization and were replicated five times.

3. Inorganic constituents of shoot system

Shoot system was harvested at 2, 4 and 6 weeks after initial salinization and analysed for the inorganic ions. Sodium and potassium were estimated by Storey and Wyn Jones (1977) method as described above in the early growth studies (1.a) using flame emission spectrophotometry. Chloride was estimated by titration method (Yoshida et al., 1976) as described above in the early growth studies (1.a). Calcium and Magnesium were estimated by titration method using EDTA as described by Derderian (1961). All the estimations were repeated twice.

4. Studies of enzyme activities

Shoot system was harvested at six weeks after initial salinization. The fully developed third leaf from the top was selected for all the enzymatic studies. All the assays were repeated twice.

buffer (pH 5.7), according to the method of Dawson and Magee (1955). The homogenate was centrifuged for 15 minutes at 5,000 x g and the supernatant was used for the enzyme assay. The entire operation was carried out at 4°C.

The reaction mixture consisted of 1 ml of the enzyme, 2 ml of 0.15 M citrate phosphate buffer (pH 5.7), 1 ml of substrate (one mg/ml) and 1 ml of distilled water. The ascorbicacid solution was added last and immediately 1 ml aliquot was withdrawn from the reaction mixture and placed in a test tube containing 1 ml of 3% metaphosphoric acid. Other samples were removed in the same manner at 30 and 60 minutes. The ascorbic acid in the aliquots was determined as described by Jayaraman (1981) using 2, 4-dinitrophenylhydrazine reagent. The assay was conducted at 30°C and the result was expressed as μ moles of ascorbic acid oxidised per hour per gram fresh weight.

c. Polyphenol oxidase (EC 1.10.3.1)

500 mg of leaf material was homogenized in phosphate buffer (0.05 M, pH 6.7) in a chilled mortar and pestle. The homogenate was centrifuged at 10,000 r.p.m. for 15 minutes. The supernatant served as crude extract for the assay of polyphenolase activity. All operations was carried out at 0-4°C.

The method of enzyme assay is essentially that of Taneja and Sachar (1974). The assay system contained 4 ml

This was incubated for 10 minutes in boiling waterbath, cooled and diluted with 10 ml of water. Colour developed was read at 540 nm and compared with a standard curve of maltose. The enzyme activity was expressed as µ moles of maltose released per 10 minutes per mg protein.

e. Invertase (EC 3.2.1.26)

The crude amylase extract was used for invertase assay.

The method of assay of enzyme was that of Malik and Singh (1980). The assay system contained 0.4 ml of 0.1 M acetate buffer (pH 4.6), 0.25 ml of 0.4 M sucrose and 0.35 ml of enzyme extract to give a total volume of one ml. In the control tubes sucrose solution was added only when enzyme was inactivated by boiling for about 5 minutes. After incubation at 30°C for 1 hour, 1 ml of 3,5-dinitrosalicyclic acid reagent was added. The tubes were placed in boiling water bath for 10 minutes. Then the contents were diluted to 10 ml and the O.D. was recorded at 560 nm. Glucose was used as a standard to calculate the reducing sugar released. The activity was expressed as µ moles of reducing sugar produced per hour per mg protein.

f. Phosphorylase (EC 2.4.1.1)

One gram of leaf material was ground for 10 minutes with equal amount of glass powder in a chilled mortar and

the enzyme was extracted with 10 ml of cold distilled water. The homogenate was centrifuged at 6,000 r.p.m. for 15 minutes at 0 to 2°C. The supernatant was used as the source of the enzyme.

Phosphorylase activity was assayed using the modified method of Ozbun et al. (1973). The reaction mixture contained in a total volume of 2.5 ml. 0.2 ml of 5% starch solution, 0.5 ml of 0.5 M citrate buffer (pH 6.5), 1.0 ml enzyme and water. The enzyme-buffer mixture was equilibrated at 35°C for 5 minutes and 1.0 ml of 0.1 M glucose-1-phosphate was added. The mixture was then incubated at 35°C for 10 minutes before terminating the reaction by the addition of 5 ml of 5% TCA. The reaction mixture was centrifuged to separate the supernatant from the pellet and 1.0 ml of aliquot was used in the determination of inorganic phosphate by the method of Fiske and Subbarow (1925). The activity was expressed as μ moles of phosphate released per 10 minutes per mg protein.

g. Acid phosphatase (EC 3.1.3.2) and ATPase (EC 3.6.1.3)

The method employed for the extraction and assays of acid phsophatase and ATPase is essentially that of Hasson-Porath and Poljakoff-Mayber (1971). One gram of leaf material was homogenized with 7 ml of cold Tris-Maleate

buffer (0.1 M, pH 7.0), containing 0.1 M sucrose and 0.003 M magnesium sulphate. The homogenate was centrifuged at 25,000 r.p.m. for 20 minutes in a refrigerated centrifuge at 0-2°C. The supernatant was collected and the residue was washed once with 3 ml of the same buffer. The combined supernatant was used for the assay of acid phosphatase. The residue was dissolved with 5 ml of the same buffer and used for the assay of membrane-bound ATPase.

The assay system of phosphatase contained 2.5 ml of Tris-Maleat buffer (0.1 M, pH 5.0), 1 ml of p-nitrophenyl phosphate (8 μ moles/ml) and 0.5 ml of crude enzyme extract. The reaction was started by the addition of enzyme. The reaction mixture was incubated at 30°C for 30 minutes before terminating the reaction by the addition of 3 ml of 0.2 N NaOH. The samples were allowed to stand for 1 hour and colour development was read colorimetrically at 410 nm. p-nitrophenol released during the enzyme activity was calculated from the standard curve. The activity was expressed as μ moles of p-nitrophenol released per 30 minutes per mg protein.

The assay system of membrane bound ATPase contained 2.5 ml of 0.1 M Tris-Maleatebuffer (pH 7.5), 1 ml of ATP (Na salt) (2.5 μ moles/ml) and 0.5 ml of membrane fraction. The reaction was initiated by the addition of enzyme. The

reaction mixture was incubated at 30°C for 30 minutes before terminating the reaction by the addition of 2.5 ml of 10% TCA. An aliquot of the reaction mixture was used for the determination of inorganic phosphorus by the method of Fiske and Subbarow (1925). The enzyme activity was expressed as μmoles of phosphate released per 30 minutes per mg protein.

h. Nitrate reductase (EC 1.6.6.1)

One gram of leaf material was ground to a fine paste with a pinch of neutral glass powder in 6 ml of the extraction medium (1m M EDTA, 10 mM cysteine, 25 mM potassium phosphate adjusted to a final pH of 8.5 with KOH) using chilled mortar and pestle at 0-3°C. The macetrate was passed through a 4 layers of cheese cloth and the filtrate was centrifuged at 10,000 r.p.m. for 15 min at 0°C. The supernatant was used as the crude enzyme preparation.

The nitrate reductase activity was assayed essentially according to the procedure described by Hageman and Flesher (1960). 3 ml reaction mixture contained 1 ml of 0.1 M potassium phosphate buffer, pH 7.5, 0.2 ml of 0.1 M KNO_3, 0.5 ml of 2 mM NADH, 0.2 ml of enzyme extract and distilled water. The reaction was carried out at 30°C for 60 minutes and terminated by the addition of 1% (w/v) sulphanilamide in 3 N HCl and the

nitrite formed was estimated according to Snell and Snell (1949). The activity of the enzyme was expressed as μ moles of NO_2^- formed per hour per gram fresh weight.

i. Glutamic oxaloacetic transaminase (GOT) (EC 2.6.1.1) and glutamic pyruvic transaminase (GPT) (EC 2.6.1.2)

The method followed for GOT and GPT is essentially that of Harper and Paulsen (1969). 500 mg of leaf material was homogenized with 5 ml of cold grinding medium containing 3.3 mM Tris-HCl buffer (pH 7.2), 3.3 mM cysteine and 0.1 mM sodium salt of EDTA. The resultant suspension was centrifuged in refrigerated centrifuge at 10,000 r.p.m. for 15 minutes at 2°C. Supernatant served as enzyme source.

For GOT, 1 ml of crude enzyme was incubated with 1 ml of reaction mixture containing 0.02 M aspartate and 0.02 M α-Ketoglutarate in 0.2 M K-phosphate buffer (pH 7.5). After 0 minute and 60 minutes the reaction was stopped with 1 ml of colour reagent (2, 4-di-nitrophenylhydrazine 1 mg/ml of 1.35 N HCl). After 30 minutes 5 ml of 0.4 N NaOH was added followed by 5 ml distilled water. The absorbance of oxaloacetate-phenylhydrazine was read at 504 nm against reagent blank. Oxaloacetate formed during the reaction was calculated from the standard curve of oxaloacetate. The enzyme activity is expressed as n moles of oxaloacetate formed per 30 minutes per mg protein.

j. Protease (EC 3.4.2.2)

Extraction and assay of protease activity was followed by Prisco et al. (1975). 250 mg of leaf material was ground in a mortar with 5 ml of cold 1% NaCl in phosphate buffer (pH 7.6). The homogenate was centrifuged at 10,000 r.p.m. for 15 minutes. The supernatant served as the crude enzyme extract. All operations were carried out at 0-4°C.

The assay system contained 1 ml enzyme extraction and 5 ml of 1% casein solution in sodium phosphate buffer (pH 7.6) and incubated at 50°C. The reaction was stopped after 60 minutes with 1 ml of 40% TCA. The free amino acids liberated during the reaction was estimated by ninhydrin method using glycine as standard (Rosen, 1957). The activity was expressed as μ moles of glycine released per hour per mg protein.

k. α-Ketoglutaric dehydrogenase (EC 1.2.4.2), pyruvic dehydrogenase (EC 1.2.4.1), and Succinic dehydrogenase (EC 1.3.99.1)

250 mg of leaf material was ground with 7 ml of of 0.1 M potassium phosphate buffer at pH 7.2. The homogenate was centrifuged at 6,000 r.p.m. for 15 minutes at 0 to 2°C and the supernatant served as enzyme source.

The assays of enzymes were carried out by the method of Kusunose et al.(1956). The system contains in 6 ml: (in μ moles); potassium phosphate buffer, pH 7.4, 200; Mg^{2+}, 20; KCN, 20; substract, 150 (sodium salt of succinic acid or pyruvic acid or α-ketoglutaric acid); in addition 0.1 ml of dye (DCPIP, 0.0025% for α-ketoglutaric dehydrogenase; potassium ferricyanide, 0.03% for succinic and pyruvic dehydrogenase) and 1 ml of enzyme extract. The reduction of DCPIP was measured at 600 nm and potassium ferricyaride at 420 nm. The dye reduction was calculated from the standard curve prepared by different concentration of DCPIP and potassium ferricyanide. The activity was expressed as μ moles of DCPIP or potassium ferriyanide reduced per minute per mg protein.

5. Organic constituents

Shoot system was harvested at 2,4 and 6 weeks after initial salinization. The third leaf from the top was selected for the estimations of free amino acids, free proline, protein, ascorbic acid, titrable acid number and chlorophyll. The whole shoot system was dried and used for the determinations of total nitrogen, RNA, DNA and carbohydrates.

a. Extraction and estimation of free amino acids

Extraction and estimation of free amino acids wasedone as given above in the early growth studies (2.a and b).

b. Extraction and estimation of free proline

Free proline was estimated according to the method of Bates et al.(1973). 500 mg of leaf material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate filtered through Whatman No.2 filter paper. Two ml of filtrate was allowed to react with 2 ml acid ninhydrin (prepared by mixing 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid and warming with agitation, until dissolved) and 2 ml of glacial acetic acid in a test tube for 1 hour at 100°C, and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously for 15 to 20 seconds. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene as a blank.

c. Extraction and estimation of nucleic acids

Extraction and estimation of RNA and DNA were followed by Jayaraman (1981) method. 200 mg of dry powder of shoot system was dissolved with 10 ml of cold 10% TCA and centrifuged at 3,000 r.p.m. for 10 minutes. The supernatant was discarded. The precipitate was dissolved with 5 ml of cold 10% TCA and centrifuged. The supernatant was discarded. The precipitate was suspended in 5 ml of ethanol-ether mixture (1:1) and centrifuged. The supernatant was discarded. The precipitate was dissolved with 5 ml of 0.5 N NaOH and

kept at 37°C for 18 hours. The sample was again centrifuged. The supernatant was precipitated with equal volume of 10% TCA and centrifuged. The precipitate was discarded and the supernatant was taken for the estimation of ribose sugars.

The precipitate obtained from centrifugation after the addition of 0.5 N NaOH, was suspended with 1 ml of perchloric acid. The sample was heated in a boiling water bath for 1 hour, cooled and centrifuged. The precipitate was discarded and supernatant was taken for estimation of deoxyribose sugars. The ribose and deoxyribose sugars were assayed using orcinol and diphenyl amine reagent respectively.

d. Protein determination

Extraction and estimation of soluble, insoluble and total protein have been carried out as given above in the early growth studies (2.e and f).

e. Determination of total nitrogen

50 mg of dry powder of shoot system was used for estimation as given above in the early growth studies (2.g).

f. Extraction and estimation of soluble and reducing sugars

An aliquots of 100 mg dry powder of shoot system was taken and few drops of cold 80 per cent ethanol and

0.5 ml distilled water were added to prevent clumping. 5 ml of warmed 80 per cent ethanol was added to each tube and stirred well to extract soluble and reducing sugars (McCready et al., 1950). The samples were spinned at 10,000 r.p.m. for 15 minutes. The supernatant was collected and the residue was repeatedly extracted four times with hot ethanol. All the supernatants were pooled together and evaporated to dryness at 40°C. The residue was dissolved in a small quantity of warm water and the final volume was made upto 5 ml. The contants of total soluble and reducing sugars were determined using Anthrone method (Yemm and Willis, 1954) and Folin and Malmrose method (Umbreit et al., 1959) respectively.

g. Extraction and estimation of starch

The residue obtained after the ethanolic extraction of sugar was used further for the extraction of starch with 52 per cent perchloric acid as described by McCready et al. (1950). The residue was suspended in 0.5 ml of distilled water, 6.5 ml of 52 per cent perchloric acid was added and stirred continuously for 5 minutes and occasionally thereafter for 20 minutes. The sample was then spinned at 6,000 r.p.m. for 15 minutes and the supernatant was collected. The residue was extracted again with perchloric acid and the volume of the pooled supernatant

was made upto 100 ml. The supernatant was dry filtered and the residue was discarded. 10 ml of the perchloric acid extract was hydrolysed with 10 ml of 1N HCl for 3 hours in a boiling waterbath. It was then neutralised with 2 N sodium carbonate and the volume was made upto 25 ml. An aliquot of this extract was used for the estimation of its reducing sugar content following the method of Folin and Malmrose (Umbreit et al., 1959). The estimated glucose content was then converted into starch equivalent by multiplying the glucose value with 0.9. Non-reducing sugar was calculated by substracting reducing sugar from total soluble sugar. Total carbohydrate was calculated from total soluble sugar + starch content.

h. Determination of ascorbic acid

Determination of ascorbic acid was followed by the method of Jayaraman (1981). 500 mg of fresh leaf material was ground with 15 ml of 5% metaphosphoric-10% acetic acid solution. The homogeneate was filtered. To a 12 ml aliquot of the filtrate 0.75 g of activated charcoal was added and was vigorously shaken. The sample was filtered. To a 4 ml aliquot of the filtrate in a tube 1 drop of 10% thiourea solution and 1 ml of 2, 4-dinitrophenylhydrazine reagent (2 g of reagent in 100 ml of 9NH_2SO_4 and filtered) was added. The tubes were placed at 37°C water bath for exactly 3 hours. An appropriate blank was maintained

without the reagent. The tubes were cooled in ice. 5 ml of 85% H_2SO_4 was added dropwise with constant stirring. After 30 minutes the colour development was read at 540nm. The ascorbic acid content was calculated from the standard curve.

i. Determination of titrable acid number

The method employed for the determination of acidity was essentially that of Thomas and Beevers (1949). One gram of leaf material was sliced and plugged into the 100 ml of boiling water. CO_2 was expelled from the extract by continuing the boiling for 15 minutes. The CO_2 -free extract was then titrated with centinormal NaOH, with a few drops of phenolphthalein as indicator. The result was expressed in centinormal NaOH required neutralize the acid extract of 10 g fresh material.

j. Chlorophyll determination

Chlorophyll content was extracted from the leaf with 80% acetone and determined by Arnon method (1949).

IV. Yield component studies

1. Yield parameters

Flowering data was recorded in both controls and treatments. Plants were allowed to mature. The yield components were measured as described by Yoshida (1976).

The yield components such as panicle length, number of panicle per plant, number of spikelets per panicle, number of filled and unfilled grains per panicle, fresh weight of filled and unfilled grains per panicle, dry weight of filled and unfilled grains per panicle, net grain yield per plant, straw weight per plant, biological yield, harvest index and 100 grain weight were analysed in all the nine rice varieties. Biological yield and harvest index were analysed as described by Donald and Hamblin (1976). All the measurements were taken from the five replicates each consisting of two plants.

2. Germination behaviour of harvested grains

Seeds of all the nine varieties were germinated as given in the germination studies (I) using distilled water. Germination percentage was recorded at 2, 3, 4 and 10 days after germination. Shoot and root length, fresh weight of shoot and root, and dry weight of shoot and root were studied at the 10th day after germination. All the measurements were taken from the five replicates each consisting of five plants.

3. Inorganic constituents of straw, harvested filled and unfilled grains

Straw, filled and unfilled grains were milled and powdered separately for the estimation of inorganic elements. Sodium, potassium and chloride were determined

as given (earlier) in the early growth studies (1.a). Calcium and magnesium were estimated as given above in the later growth studies (3). All determinations were repeated twice.

4. Organic constitutents of harvested grains

Husk was removed from the grain and powdered nicely for the estimations of carbohydrates, amino acids, total protein and total nitrogen. All the assays were repeated twice.

a. Carbohydrates estimation

Reducing sugars, non-reducing sugar, total soluble sugar, starch and total carbohydrate were estimated as given earlier in the later growth studies (5.f and g).

b. Amino acids estimation

The extract prepared for total soluble and reducing sugars were used for the determination of amino acids. The amino acid was estimated by ninhydrin method as given earlier in the early growth studies (2.b.).

c. Total protein determination

50 mg of dry powder was suspended in 0.3N KOH and was incubated for 18 hours at 37°C and the residue was suspended at 10,000 r.p.m. for 15 minutes. The supernatant

was collected and the residue was repeatedly washed till no more protein remained in it. The supernatants were pooled together, protein was precipitated using 5 ml of 10% TCA, centrifuged to save the residue. Once again, the protein was precipitated with 10% TCA. The residue was collected together, neutralized and dissolved in 10 ml of 0.3N KOH. An aliquot was used for the estimation of protein following the procedure of modified Lowry et al. method (Hartree, 1972) and compared with a standard graph established with bovin albumin (Sigma).

d. Total nitrogen determination

50 mg of dry powder was taken and estimated the total nitrogen as given earlier in the early growth studies (2.g).