MATERIALS AND METHODS

Source of seeds

Seeds of Oryza sativa L. var GR.11 (salt sensitive) were obtained from State Department of Agriculture, Gujarat.

Method of germination

The seeds were presoaked in distilled water for 2h and dehusked without damaging the embryo. They were then surface sterilized with 0 1 % mercuric chloride for 2 min followed by thorough washing in sterile distilled water. These seeds were blotted and a set of 25 seeds each were transferred to sterile 9 cm Petriplates containing Whatman No.1 filter paper moistened with 5 mL each of the test solutions for germination as given below:

a. Sterile double distilled water for control

b. 0.15M NaCl solution (The salinity level which inhibited growth by 50%) Table-1

c 10 ppm GA_3 solution. (The concentration which gave maximum stimulation of growth) Fig. 1A.

d. 0.15 M NaCl solution containing 10 ppm GA_3

e. 10^{-5}M putrescine solution (The concentration which gave maximum growth stimulation), Fig. 1B.

f 0.15 M NaCl solution containing 10^{-5} M putrescine

Each treatment had 5 replicates and the seeds were allowed to germinate at 30 ± 2°C in darkness. The seeds were germinated for a period of 120h. For growth measurements seeds were washed in water, blotted gently and extension growth of shoot and root systems were recorded. Endosperm and embryo axis were separated, dried at 80°C for 48h and their dry weight was determined. Standard error was calculated for all experiments.

Scanning Electron Microscopy

The rice seeds were germinated without dehusking in

a) distilled water and b) 0.15M NaCl solution

Seeds germinated for a period of 120h were collected and their radicle and plumule were cut very close to the scutellum. The seeds were then fixed in 2.5% glutaraldehyde in 0.05M phosphate buffer of pH 6.7. They were dehydrated in graded alcohol series. Before observing the specimens, they were mounted on brass stubs using glue and then coated with a gold layer of 400°A using JEOL FINECOAT JFC-1100 sputter coating unit. The surface of husk just near the scutellum was observed under JEOL JSM-35C scanning electron microscope. Photographs were taken at 25 kV operating voltage at different magnifications.

Energy dispersive X-ray microanalysis

After 5 days of germination in different test solutions, the seeds were harvested and their radicle and plumule were removed. The longitudinal

sections of the seeds were transferred to the cryostage of scanning electron microscope.

Elemental analysis with an electron microprobe was done to determine the ionic distribution in the selected tissues of 120h germinated rice seeds under the influence of NaCl, GA_3 and putrescine. A line scan was made across the longitudinal section to investigate the distribution of ions especially sodium and chloride. The scutellum, aleurone and endosperm tissues were scanned to trace the elements (Fig. 5).

A reasonably flat surface of the selected regions of the specimen were scanned. However the surface remained a limiting factor in standardization. The levels of different ions in the spectrum were compared. The electron microprobe detector was standardised by scanning frozen droplet of culture medium in which the ratios of Na^+ and Cl^- were known. The elemental data were displayed as a line scan by 'KEVEX' model 7000, Energy dispersive X-ray analyser at 15kV operative voltage and ~10nA beam current. The acquisition time was 10 sec.

Histochemistry

The samples were collected after 120h of germination. The embryo along with scutellum was separated and the region of grain just above scutellum was cut. Transverse sections of the seeds measuring 2 to 3 mm thickness were fixed

in 2.5 % glutaraldehyde in 0 05 M phosphate buffer, pH 7.2 for 4 h at 21°C. The tissues were washed in phosphate buffer and post fixed in 1 % Osmium tetroxide for overnight at 4°C. After washing in the buffer tissues were soaked in uranyl acetate for 30 min The samples were then dehydrated in graded acetone series and embedded in araldite (Glauert, 1975).

Longitudinal sections of 1 μm thickness were cut from embedded tissues with glass knife on a Reichert Jung Ultracut ultramicrotome. The sections were transferred to clean glass slides for localization of total polysaccharides, proteins and lipids as described below.

Total Polysaccharides: The sections were first treated with 1% periodic acid for 30 min. After washing in distilled water they were air dried and treated with Schiff's reagent for 30 min. Following washing and air drying the sections were mounted in DPX (Barger and DeLamater, 1948).

Proteins: The semithin sections were treated with ethanolic 2% NaOH for 5 min to remove the epoxy resin and thereafter with 10% hydrogen peroxide for 10 min to reoxidise the Osmium. The sections were then incubated with an acetic acid solution of Coomassie brilliant blue R. 250 at 40°C for 40 min (Fisher, 1968). After washing in 7 % acetic acid followed by distilled water, the sections were airdried and mounted in DPX.

Lipids: The sections were treated with ethanolic Sudan Black B for 1h at 60°C. They were washed in 70% alcohol, rinsed in distilled water and mounted in glycerine jelly (Bronner, 1975).

These sections were observed and photographed under a Zeiss research microscope.

Enzyme localization

The seeds were dehusked and germinated as previously described. The samples were harvested after 120h. The embryos were removed and seeds were dissected to obtain their aleurone layer (bran) for analysis. Small strips of the layer containing aleurone cells were pulled from the region of the germ with the help of fine forceps and used for enzyme localization (Doig et al., 1975).

Succinate dehydrogenase (E.C.1.3.99.1) : The aleurone layers were incubated for 15 min at 35°C in a freshly prepared reaction mixture containing 10 mL of 0.1 M phosphate buffer (pH 7.2), 5 mg EDTA (disodium salt), 81 mg sodium succinate, 4 mg sodium azide, 13 mg nitroblue tetrazolium (NBT) and 0.1 mL of 15 mg/mL phenazine methosulfate (PMS). For control the peels were incubated in reaction mixture without sodium succinate. After incubation the peels were washed thoroughly in distilled water and mounted in glycerine jelly (Sexton and Hall, 1978).

Glucose-6-phosphate dehydrogenase (E.C.1.1.1.49) : The reaction medium was prepared by mixing 10 mL of 0.05 M phosphate buffer of pH 7.8 containing 0.014 M glucose-6-phosphate (disodium salt), 0.0024 M NBT and 0.02 M sodium azide. The reaction mixture was kept in dark for 30 min prior to use. The aleurone strips were incubated for 30 min at room temperature. For control the peels were incubated in mixture without glucose-6-phosphate. The peels were picked up, washed thoroughly in distilled water and mounted in glycerine jelly (Gahan, 1984).

The peels were observed and photographed immediately under a Zeiss research microscope.

Validity of the enzyme localization results : The validity of the enzyme localization was obtained from the control minus substrate which did not contain the reaction product (Figs. 22A, 23A, 24A, 25A). Strips of the tissues were further stained with 0.5 % toluidine blue for 1 min to get better cellular contrast.

Ultrastructure

For transmission electron microscopic studies, the tissues were processed as described earlier and embedded in araldite. It was difficult to obtain good quality sections due to the extreme hardness or lack of plastic infiltration of the endosperm tissue. The quality of sections was improved by uniform sectioning for 1h at very low cutting speed. The sections usually had numerous wrinkles and lines presumably due to the friction of endosperm components and

hydration of starch granules. The ultrathin sections were stained with 2 percent uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). They were observed under Philips CM 10 Transmission Electron Microscope at 80 kV and photographed at different magnifications.