CHAPTER III

MATERIAL AND METHODS

A) Plant material :

The seeds of groundnut variety SB-11 and Sesbania grandiflora were obtained from college of Agriculture, Kolhapur and local farmers respectively. The seeds were sown in acid free silica sand in culture pots of equal size. When the seedlings were well established (i.e. after 15 days) the salt treatments were started. The concentrations of NaCl used in the experiment were 0 (Control), 100 and 200 mM. The requisite quantity of NaCl to obtain the above concentrations was added in the Hoagland culture solution. The treatments were given twice a week, alternating with watering the plants with equal amount of water to avoid excess salt accumulation and loss of water due to evaporation in the medium. After 3 months of treatment the plants were analysed for various growth parameters, enzymes, organic constituents, inorganic constituents and root anatomy.

B) Methods :

1) Growth :

Length, number of leaves and leaflets per plant and number of gynophores were determined by usual methods.

2) Inorganic constituents :

i) Quantitative estimation of inorganic constituents :

For the estimation of inorganic constituents an acid digest from the oven dried plant material was used.

The material was digested following the method of Toth et al., (1948). 0.5 g of the oven dried powdered material was transferred to 100 ml beaker to which 20 ml concentrated HNO_3 were added. The beaker was covered with watch glass and was kept till the primary reactions subsided. It was then subjected to heating first at low temperature till the solid particles were completely dissolved. After cooling to room temperature 10 ml of per chloric acid (60%) were added to it and mixed thoroughly. It was then heated strongly and vigorously until a clear and colourless solution resulted. Heating was stopped when the volume of extract was reduced to approximately 2-3 ml. It was then cooled and transferred quantitatively to 100 ml volumetric flask and volume was made with distilled water. It was kept overnight and filtered through dry whatman No.1 filter paper next day. The filtrate was used for the estimation of inorganic constituents.

Na^+, K^+ and Ca^{2+} were estimated flame photometrically following the procedure standardized in our laboratory. P^{5+} was estimated following the method given by Sekine et al., (1965). Fe^{3+} was estimated according to the method described by Durie et al., (1965) while Magnesium was determined following the method described by Drowsdoff and Nearpass (1948). Chloride was estimated according to the method by Volhard (1956).

ii) "Free space" ("Donan" equilibrium) :

Two sets, one of each of roots of plant grown under saline and non-saline conditions were thoroughly washed well in distilled water for about half an hour. Roots were then transferred to 20 ml 50 mM NaCl solution and kept for one hour. Roots were then removed from the solution and blotted carefully with blotting paper. One set of such roots (1 g) was transferred to 30 ml distilled water and kept there for half an hour. Na^+ and Cl^- were then estimated from this medium.

Another set of roots (1 g) taken out from the above NaCl medium was subjected to 20 ml 50 mM KCl solution and was kept for one hour. Roots were then removed from this medium and surface blotted. These roots were immediately transferred to 30 ml distilled water and kept for half an hour. Na^+, K^+ and Cl^- were then estimated from this medium.

"Outer" or "Free" space in the roots was determined following the equation given by Epstein(1955) as follows :

$$\text{"Outer" space} = \frac{\{\text{Diffusible ions}\}}{\{\text{External concentration}\}}$$

iii) Root anatomy :

For root anatomy the roots of plants, both treated and untreated, selected were of maximum size in each case.

3) Organic constituents :

Titratable acidity (TAN), Chlorophylls and polyphenols and some enzymes, peroxidase, catalase, acid phosphatase and nitrate reductase were determined from the fresh material, while some other organic constituents such as total nitrogen, carbohydrates and proline and inorganic constituents namely sodium, chloride, calcium, potassium, iron, magnesium and phosphorus were estimated from the oven dried material.

Titratable acidity (TAN) was determined by the method of Thomas and Beevers (1949) while chlorophylls were estimated by the method of Arnon (1949). Carbohydrates were estimated following the method by Nelson (1944) polyphenol were estimated following the method by Folin and Denis (1915). Nitrogen was estimated colorimetrically by the method of Hawk et al., (1948) while the method of Bates et al., (1973) was followed for determination of free proline.

4) Enzymes :

All the operations i.e. isolation and assay of enzymes were carried out at 0 to 4°C temperatures. The peroxidase (E.C. 1.11.1.7) was determined following the method described by Maehly (1954), while catalase (E.C. 1.11.1.6) was determined by employing the method by Herbert (1955). The acid phosphatase (E.C. 3.1.3.2) was determined following the method of Leo and Sacher (1970). The enzyme nitrate reductase (in vivo) was studied by the method of Evans (1982).