CHAPTER - II

Material & Methods

A) PLANT MATERIAL :-

i) Cultivation of Sansevieria in Pot (culture)

The plants of Sansevieria trifasciata lourentii were raised in the pots as well as in the garden soil by using plantlets for the growth studies. However the growth being plantlets for the growth studies. However the growth being very slow, only growth pattern was studied in these plants. For the full development up to flowering; Sansevieria requires about 2 to 1 1/2 years.

To overcome this difficulty during present investigation the Sansevieria plants raised in the garden of Krishna Mahavidyalaya, Rethare (Bk.) were used. About 20 - 25 plants as same age (2 1/2 years maturity) were selected. These plants were provided with adequate a mount of water in specific intervals. The required plant material i.e. leaves were taken from these selected plants and used oftenly during the experimental studies.

ii) Sampling method :-

From healthy and insect free Sansevieria plant matured (fully expanded) leaves were carefully selected from identical position during the summer, rainy and winter months. The selected leaves were first washed and cleaned with tap water following with distilled water. Then they were blotted dry and subsequently cut into small pieces. These pieces were mixed randomly and this leaf material was taken for the different analytical experiments as follows.

The meteorological reading such as soil, moisture, maximum and minimum temp, humidity, relative humidity, evaporation, soil temp, at various depth. Light intensity of light were also recorded at the time of each harvest.

To determine soil moisture percentage. The soil was collected from the root zone of selected plants of Sansevieria trifasciata raised in pots and in the garden. The collected soil was mixed thoroughly and known amount of soil (10 gms) was kept in oven at 70^{oC} for 8 - 10 days for drying, till a constant weight. Loss in wt. per 100 gms was expressed as moisture percentage.

B) Methods :-

I) Inorganic Constituents :-

i) Preparation of Acid digest :-

The plant material mentioned above was kept in oven 70^{oC} for 8 days till a constant weight was obtained. The oven dried material was powdered.

For the estimination of different inorganic constituents an acid digest was preferred following the method of Toth; et al., (1948).

500 mgs of oven-dried powdered material was transferred to 150 ml capacity beaker to which 1 ml. concentrated. HNO_3 were added. The beaker was covered with watch glass and was kept till the primary reaction subside. It was then heated slowly to dissolve solid

particles. After cooling to room temp. 10ml. perchloric acid (60%) were added and mixed thoroughly. It was then heated strongly until a clear and colourless solution (about 2 to 3 ml.) obtained. While heating the liquid was not allowed to dry. It was then cooled and transferred to 100 ml capacity volumetric flask diluted with distilled water and kept over night. Next day it was filtered through dry whatman. No. 44. (ashless). Filter paper and the filtrate was used as the source different inorganic constituents.

ii) Estimation of Sodium, Potassium, Calcium Magnesium, Iron, Copper and Phosphate –

Sodium and potassium were estimated flame photometrically following the procedure standardized in our laboratory. Stock solution of known concentrated in parts per million (ppm) in NaCl (1-10 ppm), K in KCl (10 - 15 ppm) were used for calibration curve from these calibration curves the concentrated sodium and potassium in the acid digested samples were calculated.

Calcium, Magnesium and Iron were estimated with the help of computarised atomic absorption photospectrometer. In the E.P.R.F. Sangli. Phosphate is estimated by Ammonium molybadate method. Take the 5 ml. sample in Nesslers tube. Make the volume 50 ml. with distilled water. Add 2 ml. of Ammonium molybadate and four drops of Stanous chloride ($SnCl_2$) after proper mixing. Seen on spectrophotometer at 690 nm.)

For estimation of copper by calorimetry (ISI)

method is followed water. Add 2.5 ml. Ammonium hydroxide then add 2.5 ml. Diethly dithiocarbonate solution. After proper mixing seen on spectrophotometer at 457 nm.

II) Organic Constituents :-

i) Moisture Percentage of leaf :- The matured leaves were taken as described earlier from the selected plant. The leaves were washed, bloted dry and cut into small pieces. These pieces were thoroughly mixed and 10 gm. leaf material was kept in oven at 70^{oC} for drying, till a constant weight obtained. The loss in weight per 100 gms. was expressed as moisture percentage.

ii) Organic acid status (Titrable acid numbers) :-

TAN was estimated by the method of Thomas and Beevers, (1949). TAN was estimated from mature leaves in each month of season. The fresh leaf material was cut into small pieces and weighted amount (2 mg) was immersed in boiling water and boiled for half an hours. It was cooled and filtered through cheese cloths. The final volume of filtrate was made to 50 ml. with distilled water. This served as plant extract for determination of TAN. It was titrated against N/40 NaOH using phenolpthalin as an indicator. NaOH was standerdised against N/40 Oxalic acid using the same indicator. Titrable acid number (TAN) represent the number of ml. of decinormal NaOH required to neutralized the acid present in 100 gm. of fresh tissue. It was estimated by applying following formula.

$$TAN = \frac{\text{Volume of oxalic acid taken for titration}}{\text{Oxalic acid titration reading}} \times \frac{\text{Total volume of extract}}{\text{Wt. of plant material in g}} \times \frac{\text{Extract titration reading}}{\text{Volume of extract taken for titration}} \times \frac{100}{4}$$

iii) Carbohydrates :-

Carbohydrates were estimated according to the method described by Nelson (1944). These were estimated from mature leaves in each month of season. Five hundred mgs fresh leaf material was homogenised in mortar with pestle and extracted with 80% alcohol. It was filtered through Buchner's funnel using whatman No.1 filter paper. The filtrate was used for the estimation of soluble sugars while the residue was used for starch determination. The filtrate thus obtained was condensed on a water bath till the volume was about 3 ml and treated with lead acetate and potassium oxalate (1:1) to decolourize it. To this distilled water was added and filtered. It was again washed with distilled water for 2-3 times collecting the washing in the same filtrate. This filtrate was used for estimation of reducing sugars (A). A known volume of this extract was hydrolysed with concentrated HCL in pressure cooker at 15 lbs pressure for half an hour. The contents were cooled, neutralised with Na_2CO_3 and filtered. The filtrate was used for the estimation of total (reducing + non-reducing) sugars (B).

The residue obtained in the first filtration (alcohol extract) was transferred to a conical flask with

50 ml water and 2.5 ml. of concentrated HCl. This was hydrolysed, neutralized and filtered as stated earlier. This filtrate contains reducing sugars produced as a result of hydrolysis of starch. The sugars so available were estimated to determine the starch present in the tissue (C).

The requisite quantity (preferably 0.1 ml) of the above filtrates A, B and C was taken separately in 10 ml. marked test tubes. In other such test tubes different concentrations (0.1, 0.2, 0.3, 0.4 ml) of standard glucose solution (0.1 mg ml^{-1}) were taken. One ml of Somogyi's alkaline copper tartarate solution (4g $CuSO_4$, $5H_2$; 24g unhydrous Na_2CO_3; 16g Na, K, tartarate (Rochelle salt) and 180 g unhydrous Na_2SO_4 dissolved in 1000 ml distilled water) was added to each test tube. All the reaction mixtures were subjected to boiling water bath for about 10 minuted. After cooling to room temperature 1 ml of Arsenomolybadate reagent (25 g Ammonium molybadate in 450 ml. distilled water to which were added 21 ml concentrated H_2SO_4. To this was then added 3% sodium arsenate, Na_2SO_4, $7H_2O$; dissolved in 25 ml distilled water. All ingredients were mixed well and the solution was placed in each reaction mixture. The contents of each test tube were then diluted with distilled water to a volume (10 ml.). A blank was prepared by the same way but without sugar solution. After 10 minuted the absorbance of reaction mixture was read at 560 nm.

With the help of glucose standard curve, the amounts of these carbohydrate fractions were determined.

iv) **Pigments :-**

(a) Chlorophylls :

Chlorophylls were estimated following the method of Arnon (1949). Fresh plant material (mature leaves) was used for estimation of chlorophylls in each month of season. Chlorophylls were extracted in 80% chilled acetone from 0.5 g of fresh leaf material in dark. This extract was filtered through whatman No. 1 filter paper using Buchner's funnel. Residue was washed repeatedly with 80% acetone, collecting the washings in the same filtrate. The volume of the filtrate was made to 50 ml with 80% acetone. The absorbance was read at 663 and 645 nm for chlorophylls a and b respectively.

Chlorophylls (mg $100g^{-1}$ fresh tissue) were calculated using the following formulae. :

Chlorophyll a = (12.7 X A 663) - (2.69 X A 645 ... X

Chlorophyll b = (22.9 X A 645) - (4.68 X A 663 ... Y

Total chlorophylls = (8.02 X A 663) + (20.2 X A 645)... Z

$$\text{Chlorophyll a or Chlorophyll b or Total Chlorophylls (mg } 100^{-1} \text{ g fresh tissue)} = \frac{X/Y/Z \times \text{volume of extract} \times 100}{1000 \times \text{weight of material (g)}}$$

III) **Crassulacean Acid Metabolism Studies :-**

1) Study of diurnal fluctuations in Titratable Acid Number (TAN) during different months of season

Monthly variations in diurnal fluctuations in titratable acidity in the mature leaves of Sansevieria trifasciata were studied. For this purpose the leaf material from the leaf material from the same plant was sampled at 6-00 a.m., 12-00 noon, 6-00 p.m. and 12-00 night and TAN was estimated according to the method described earlier.

2) Study of diurnal fluctuations in Carbohydrates during different seasons :

The diurnal fluctuations in carbohydrate contents during different months of were studied in mature leaves Sansevieria trifasciata at different hours of day (6-00 a.m., 12-00 noon 6-00 p.m. and 12-00 night). The carbohydrates were estimated according to the method described by Nelson (1944) which has been described in details in earlier section.

The values of various organic and inorganic constituents and stomatal parameters, presented in Chapter "Results and Discussion" represent average of three independent determinations.