

CHAPTER II

**MATERIAL AND
METHODS**



Effect of NaCl on growth of local P. aureus variety



Effect of NaCl on growth of Pusa valshakhi variety of mung

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MATERIAL AND METHODS

A) MATERIALS :

For the present investigation attempts have been made to study the effect of salinity (NaCl treatment) on the two Phaseolus aureus varieties as local and Pusa vaishakhi (Fig.3)

The local variety is obtained from market while seeds of pusa vaishakhi obtained from Nimbkar seed distributors at Karad from Panjabrao Krishi Vidya^apeet. There are so many varieties of Phaseolus aureus but the pusa vaishakhi is taken for present investigation because it is improved variety and it gives more yield than others and is disease resistant.

B) METHODS :

1) SALT TOLERANCE STUDIES :

The healthy seeds of Phaseolus aureus i.e. local and improved pusa vaishakhi were sorted out and surface sterilised with 0.1 % HgCl₂ solⁿ. they were sown in earthen pots (size 30 cm x 45 cm) filled with garden soil supplemented with farm yard manure in the propor-



tion of 3:1 in the second week of June. After one month of establishment the plants were thinned to five healthy plants per pot and two replicates or sets were kept for each cultivar. Then plants were subjected to 0.1 and 0.2% sodium chloride. The control plants were maintained without NaCl treatment but watered regularly. At the time of heading stage the plants were harvested and employed for analysis of effect of NaCl treatment on growth parameters, organic and inorganic constituents and activity of few enzymes.

(a) Physical Properties of the Leaves :

The physical properties of the leaves were studied by the method Linacre (1964). The green and senescent leaves of control and NaCl treated of both varieties of Phaseolus aureus were taken for consideration. Then the average leaf are were measured as well as thickness and leaf weight also measured. From these average volume and density is calculated by using following formula.

Volume = Average leaf area x Average leaf thickness

Density = $\frac{\text{Average leaf wt.}}{\text{Average leaf area} \times \text{Average leaf thickness}}$

(b) Growth Parameters :

From each set 5 plants were carefully uprooted, cleaned well with distilled water, surface dried and used for analysis. The parameters selected for growth analysis were total plant height, shoot length, root length, shoot/root ratio.

The total yield of the plant is also determined by measuring pod length and counting the average no. of seeds per pod.

(c) Organic Constituents :

(i) Moisture and Relative water content (RWC)

Moisture :

The green and senescent leaves of control as well as stressed plants were taken and cleaned well with distilled water, surface dried and weighed accurately. Then the leaves were dried at 80°C in an oven till constant weight obtained. The moisture percentage can be calculated by using following formula :

$$\text{Moisture Percentage} = \frac{\text{Fresh wt} - \text{Dry wt}}{\text{Fresh wt}} \times 100$$

(ii) R.W.C. (Relative Water Content) :

The clean washed and surface dried green and senescent leaves of control as well as stressed plants were taken. The leaf discs were prepared by punching leaf lamina and weighed. Then the same leaf discs were kept for 4 hours in distilled water and weighed. Later on these discs were dried at 80°C in an oven till constant weight obtained. The RWC can be calculated by using the following formula.

$$\text{RWC} = \frac{\text{Fresh wt} - \text{Dry wt}}{\text{Turgid wt} - \text{Dry wt}} \times 100$$

(iii)

ii) TITRATABLE ACID NUMBER (TAN) :

TAN was estimated by the method of Thomas and Beevers (1949). The plant leaf material (green and senescent) was cut into small pieces and weighed for 1 gm. material was boiled with distilled water for half an hour, then cooled and aliquot was titrated against N/40 NaOH using phenolphthalein as an indicator.

NaOH was standardised against N/40 oxalic acid using the same indicator. Titratable acid number (TAN) represents the no. of ml. of decinormal NaOH required to neutralize the acids present in 100 g.. of fresh tissue. It was estimated by using following formula.

$$\text{TAN} = \frac{\text{Volume of oxalic acid taken for titration}}{\text{Titration reading of NaOH}} \times \frac{\text{Total Vol}^m \text{ of extract}}{\text{wt. of plant material ing.}} \times \frac{\text{Extract titration x reading}}{\text{Vol}^m \text{ of extract taken for titration}} \times \frac{100}{4}$$

iii) TOTAL POLYPHENOLS :

Polyphenols were estimated by the method of Folin and Denis (1915). Polyphenols from sebescebt and green lvs. of phaseolus aureus were extracted in 80 % acetone and filtered through whatman filter paper No. 1 using Bachner's funnel under suction. Polyphenols were extracted repeatedly from the residue. The Vol^m of filterate was made to 50 ml. 0.5 ml. of filterate was taken in a 50 ml. marked Nessler's tube. In other such tubes the different concⁿ eg. 0.5, 1, 2, 3, and 4 ml. of standard polyphenol solⁿ (Tannic acid, 0.1 mg. mt¹) were taken. Then 10 ml. of 20%

Na₂ Co₃ were added to each take to make the medium alkaline. 2 ml. of Follin - Denis reagent (100 g. of sodium tungstate and 20 g. of phosphomolybdic acid dissolved in 200 ml. distilled water were mixed with 25% phosphoric acid. This was refluxed for 2.5 hours then cooled to room temp. and diluted to 1 litre with distilled water) were then added to each Nessler's tube and finally the volume was made to 50 ml. with distilled water. A blank was prepared without polyphenols. The ingredients were allowed to mix thoroughly well. After some time the optical density (ox) of each mixture was read at 660 nm, polyphenols were calculated from the calibration curve of standard tannic acid.

iv) CHLOROPHYLLS :

The Chlorophylls were estimated by the method of Arnon (1949). Chlorophylls were extracted in 80% acetone from 1 g. of the plant material. The extract was filtered through Buchner's funnel using Whatman filter paper no.1. Residue was washed repeatedly with 80% acetone. Collecting the washings in the same filtrate, the vol^m of the filtrate was made to 100 ml. with 80% acetone. Extraction was carried out in dark

and in cold conditions the absorbance of the filterate was read at 663 nm and 645 nm.

Chlorophylls in mg. 100 g^{-1} Fresh tissue were calculated by using following formula :

$$\text{Chl. 'a'} = 12.7 \times A_{663} - 2.69 \times A_{645} = X$$

$$\text{Chl. 'b'} = 22.9 \times A_{645} - 4.68 \times A_{663} = Y$$

$$\text{Total Chls.} = 8.02 \times A_{663} + 20.2 \times A_{645} = Z$$

$$\begin{aligned} &\text{Chl. 'a' or 'b'} \\ &\text{or total Chlorophylls} = \frac{X/Y/Z \times \text{Vol}^m \text{ of extract} \times 100}{1000 \times \text{wt. of the pt. material}} \\ &(\text{mg } 100 \text{ g}^{-1}) \qquad \qquad \qquad \text{in g.} \end{aligned}$$

v) CAROTENOIDS :

Carotenoids were extracted by crushing the fresh lvs. in 80% acetone. Procedure is similar to that of chlorophylls described earlier and carotenoids were estimated spectrophotometrically at 480 nm by following the method of Kirk and Allen (1965). Total carotenoids were estimated using the following formula of Liaaen - Jensen and Jensen (1971).

$$C = D \times V \times F \times \frac{10}{2500}$$

where,

C = Total carotenoids in mg.
D = Optical density
V = Total Vol^m in ml.
F = Dilution Factor.
2500 = Average extinction.

C) INORGANIC CONSTITUENTS :

i) PREPARATION OF ACID DIGEST (EXTRACT) :

The leaf material of random sampling was taken, cleaned well in distilled water and dried at 80°C in an oven till constant weight obtained. This oven dried material was taken for the estimation of different inorganic elements by following the method of Toth et al (1948)

0.5 g. of oven dried powdered material was transferred to a 150 ml. beaker to which 20 ml conc. HNO₃ were added. The beaker was covered with watchglass and kept till the primary reaction subsided. It was then subjected to slow heating to dissolve solid particles completely.

After cooling to room temperature 10 ml. of 60% perchloric acid were added and mixed thoroughly. It was then heated strongly and vigorously until a deam and colourless solution reduced to about 2-3 ml. While heating the liquid was made to 100 ml. with distilled water and kept overnight. Next day it was filtered through a dry whatman filter paper No. 44 (ashless) and the filtrate was used for the estimation of different inorganic elements.

ii) ESTIMATION OF SODIUM AND POTASSIUM :

Sodium and potassium were estimated Flame photometrically following the standard procedure The standard solutions of known concⁿ in parts per million (ppm) were used for Na⁺ in NaCl (1 to 10 ppm) and k⁺ in kcl (1 to 50 ppm) were used for calibration curves. From these calibration curves the unknown concⁿs of Na⁺ and k⁺ in the acid digest samples were calculated.

iii) ESTIMATION OF CALCIUM, MAGNESIUM, IRON COPPER
ZMC AND MANGANESE :

The acid digest extract was used to estimate

Ca^{2+} , Mg^{2+} , Cu^{2+} , Zn^{2+} and Mn^{2+} elements on atomic absorption spectrophotometer (Perkin - Elmer Model 3030) using acetylene air flame. The light source employed was hollow cathode lamp. The concⁿs of Ca, Mg, Fe, Cu, Zn and Mn were read at 422.7 nm, 285.2 nm, 248.3 nm, 213.9 nm and 279.5 nm respectively (Perkin - Elmer 1973).

iv) ESTIMATION OF CHLORIDES :

For estimation of chlorides, method described by Imamul Hug and Larher (1983) with slight modification was used. Estimation was done using chapmen and Pratt's (1961) method. The chlorides were extracted in boiling distilled water. After cooling the extract was filtered through a layer of cheese cloth. The filtrate was collected in 25 ml. volumetric flask and final vol^m was made with distilled water. From this 10 ml of extract was taken for titration against standardised 0.05 N AgNO_3 .

A few drops of 25% acetic acid solution were added to the filtrate until the pH of the solⁿ

was 6 to 7. Then a few drops of 1% potassium chromate solution were added and titrated against standardized 0.05 AgNo3 (Dissolve 8.5 g. Ar grade AgNo3 in 1000 ml. distilled water) until the first permanent reddish--brown colour appears and noted the burette reading.

(1 ml of 0.05 N AgNo3 = 1.775 mg of cl)