



## EFFECT OF CHLOROPHYLL CONTENT AND PROLINE METABOLISM IN GREEN FLAVOUR SPINACH CULTIVAR

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Communicated : 09.08.2023

Revision : 26.08.2023 & 13.09.2023  
Accepted : 22.09.2023

Published : 30.10.2023

### ABSTRACT:

Spinach vegetable is highly consumed by Asian countries people. Increased soil salt concentration decreases the productivity and changes the physiological and chemical properties of plants. In this experiment, the effect of salt stress was studied with respect to chlorophyll estimation and proline accumulation in spinach-Green Flavour cultivar. The above parameters were studied under the control conditions as well as in presence of various levels of salinity i.e. 0.4%, 0.8%, 1.2%, 1.6% and 2% for 42 days. In these parameters, Green Flavour cultivar performs well. Salt tolerance of Green Flavour cultivar was indicated in chlorophyll estimation which decreases as the salt concentration increases. Further, Increased proline accumulation was observed more in Green Flavour cultivar.

**Keywords:-** Green Flavour Spinach, Chlorophyll, Proline.

### INTRODUCTION :

Globally, salinity is the significant factor that constraints the productivity potential of agricultural land, especially for decrease in plant growth and development and leads to decrease in yield and quality changes in different plant species. The accumulation of soluble salts into the soil layers is a limiting factor that affects the growth of crop which is important for our food.

Spinach (*Spinacia oleracea* L.) is an annual herb belongs to family Chenopodiaceae. It is native to South-West Asia and widely distributed and cultivated through the world including Iran as vegetables for its high nutritious value. Spinach is a good source of minerals (iron, copper, phosphorous, zinc, selenium), vitamin B complex (niacin and folic acid), ascorbic acid, carotenoids (Beta-carotene, lutein, zeaxanthin), phenols (flavonoids, p-

coumaric acid) and omega 3 fatty acids. It is a relatively quick-growing vegetable and easy to maintain. Also, the whole plans medicinally important and are used in traditional medicine for numerous therapeutic effects because of the presence of biological tannins and phenolics active phytochemicals such as alkaloids, flavonoids, steroids, glycosides and terpenoids. It is used to treat diabetes, leprosy, asthma, urinary diseases, lung inflammation, joint pains, thirst, scabbies, vomiting, ringworm, sore eyes, cold, sneezing, fever and the diseases related to brain and heart.

Proline accumulation is one of the adaptations of plants to salinity and water deficit (Salinity stress responses in two genotypes of mulberry studied by Ramanjulu *et al.*, (1998). However, proline accumulation cannot be regarded as marker for salt tolerance, as it accumulates under various conditions of stresses such as



temperature, drought and starvation (Liang *et al.*, 2013) whereas in many salt stress plants its level decreases according to Ondrasek *et al.*, (2022).

Salt stress also induces a decrease in stomata conductance and transpiration. Under saline condition, stomata closure helps to maintain high leaf water content i.e. thick leaves may be observed; however this leads to decrease in leaf carbon dioxide assimilation rate (Maggio *et al.*, 2007). Moreover, stress induced chlorosis and necrosis of mungbean (*Vigna radiate* L.) leaves due to decreased chlorophyll and mineral nutrient deficiency by salinity (Sehrawat *et al.*, 2015).

## MATERIALS AND METHODS

### Plant Material and Treatment

#### Plant Material:

Spinach seeds were selected for the experiment, i.e. *Green Flavour*.

#### Treatment:

This experiment was conducted on open terrace in pots during February to April 2021. *Green Flavour* variety of Spinach was taken. The experiment was done for fourteen replications per salt concentration (%) for respective variety. Plants were irrigated with salt solution of different concentrations such as control, i.e. 0%, 0.4%, 0.8%, 1.2%, 1.6% and 2.0% respectively. Spinach cultivar was subjected to salinity treatment once in a day or as required for 42 days. Then chlorophyll estimation and proline accumulation were studied for further analysis of effect of salinity stress in *Green Flavour* spinach variety.

## BIOCHEMICAL PARAMETERS

### Estimation of Chlorophyll Content

#### Procedure:

1. 1 g of finely cut and well-mixed representative sample of leaf tissue was weighed and taken into a clean mortar.
2. Tissue was grind to a fine pulp with the addition of 20 ml of 80% acetone.

3. Centrifuged (5000 rpm for 5 min) and transferred the supernatant to a 100 ml volumetric flask.

4. In residue addition of 10ml of acetone, centrifuged again and transferred the supernatant to the same volumetric flask.

5. Procedure was repeated until the residue appears to be colorless. Mortar and pestle was washed thoroughly with 80% acetone and collected in the volumetric flask.

6. Make up volume to 100ml with 80% acetone.

7. Read the absorbance of solution at 645, 663 nm against the solvent (80%acetone) blank.

#### Formula:

$$\text{Chlorophyll a (mg/g)} = 12.7(A_{663}) - 2.69(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b (mg/g)} = 22.9(A_{645}) - 4.68(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll (mg/g)} = 20.2(A_{645}) + 8.02(A_{663}) \times \frac{V}{1000 \times W}$$

Where, A = absorbance at specific wavelengths;  
V = final volume of chlorophyll extract in 80% acetone;

W = fresh weight of tissue extracted.

### Estimation of Proline Content

Amount of proline concentration in control and stress treated seedling was estimated by Ninhydrin method (Bates *et al.* 1973).

#### Procedure:

Leaves (0.5g) from each replication were individually grinded in 5ml 3% (w/v) sulphosalicylic acid in chilled mortar and pestle. The crude extract was passed through what man filter paper. Add 2ml ninhydrin reagent and 2ml of glacial acetic acid into 2ml of the filtered extract. The mixture was incubated 95°C in water bath for 1 hr. Then placing tubes on ice terminated the reaction. To this 4ml toluene was added and tubes were vortexes. The toluene layer was separated and the intensity of red color was measured at 520nm on UV

spectrometer. A series of standards L-Proline (10-100µg/ml) were prepared in 3% sulfosalicylic acid the standard graph was prepared. The amount of proline was calculated from standard curve and following formula was used to determine the concentration of proline in it sample. Proline content was expressed on fresh weight basis as follows;  
 (115.5 is the molecular weight of proline).

$$\text{Proline } (\mu\text{moles/ml}) = \frac{\mu\text{g proline/ml}}{115.5} \times \text{ml toluene} \times \frac{5}{\text{g sample}}$$

## RESULTS AND DISCUSSION :

### RESULTS:

#### Biochemical Parameters

#### Estimation of Chlorophyll Content (mg/g)

The estimation of chlorophyll content of both the varieties of spinach were carried out using 1 gm of plant sample per concentration on 42nd day of the treatment.

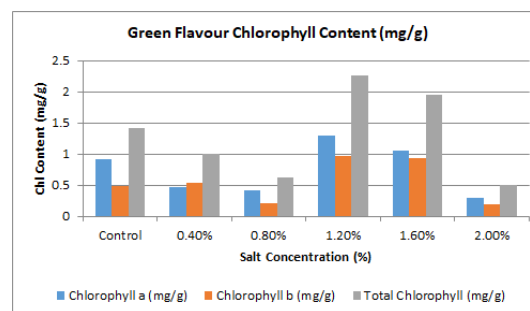


Fig. Variation in leaf color in Green Flavour Variety's Chlorophyll Estimation.

#### Green Flavour Variety's Chlorophyll

#### Estimation

Treatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll a+b (mg/g)
Control	0.92	0.48	1.42
0.4%	0.47	0.53	1.00
0.8%	0.41	0.21	0.62
1.2%	1.29	0.97	2.26
1.6%	1.05	0.94	1.96
2.0%	0.30	0.20	0.50



In *Green Flavour* variety, chlorophyll a, b and total chlorophyll content was reduced in lower and higher concentration of salinity treatment whereas it was found to be increased at 1.2% and decreased in 2.0% conc. Treatment compared with control condition.

#### Estimation of Proline Content (umoles/ml)

The estimation of proline content using ninhydrin method for *Green Flavour* variety of spinach was carried out using 0.5 gm of plant sample per conc. of salinity treatment.

#### Standard Proline reading:

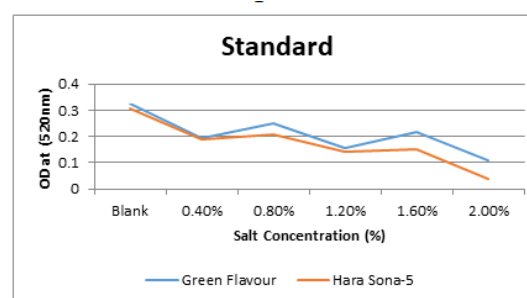


Fig. Graph showing Standard Proline readings O.D. at 520nm.

#### Green Flavour's Proline

The above graph depicts the amount of proline content in *Green Flavour* cultivar. In *Green Flavour* variety, there is slight decrease in the proline content with respect to control.

#### DISCUSSION:

Chlorophyll is main component of plant and when there is effect on chloroplast different mechanisms of plant get imbalanced and causes harmful effect on the yield of plant. In *Green Flavour* variety, chlorophyll a, b and total chlorophyll content was reduced in lower and higher concentration of salinity treatment

whereas it was found to be increased at 1.2% and 1.6% conc. treatment compared with control condition.

Accumulations of the proline in plant stress that help in response to growth and also help in maintain the yield of the plant. Mishra and Gupta (2005) studied that effect of salt stress on proline metabolism in two high yielding varieties of green gram. They found that the magnitude of increase in free proline accumulation was higher in the tolerant variety than in the sensitive variety. In this experiment, increase in proline accumulation was observed in Green Flavour variety with the increase in salt as compared to control. Therefore, we can analyze that *Green Flavour* Variety is a salt tolerant variety which justifies the Mishra and Gupta's study.

#### CONCLUSION:

From the above experiment, we can conclude that *Green Flavour* variety is more salt tolerant variety. By studying the biochemical parameters such as estimation of proline and chlorophyll content, all these parameters gives us an idea that of *Green Flavour* variety is responsive to salinity than that. Role of proline accumulation in any stress condition (here in salinity stress) in a plant decipher the maintenance of turgor pressure by accumulation of osmoprotectants such as proline and there the resistance it creates for any plant or variety to survive in adverse condition. In this experiment, the physiological parameters were in consistent with the accumulation of proline content. There were slight alterations in certain parameters but still they were in compatible with respect to the control condition. Through the above discussion, it is further confirmed that salt tolerance in *Green Flavour* variety.

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