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Effect of Naturally Occurring Soil Salinity on Chlorophyll Content in *Amaranthus viridis* L.

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Abstract

Soil salinity is a crucial environmental stress factor which has negative impacts on growth and development of plants, including the synthesis of photosynthetic pigments which are essential for photosynthesis and overall plant metabolism. This study investigates the effect of soil salinity on chlorophyll content in *Amaranthus viridis* L., a fast-growing leafy green vegetable known for its nutritional value. The samples of plant leaves and supporting rhizospheres saline soil supporting the plants were collected from three different salt-affected sites from Baramati Tahsil and an estimation of the pH and EC of soil and chlorophyll content in leaves was carried out. The levels of EC in soil ranged between 7 dS/m - 8 dS/m and the observed soil was saline in nature. To assess salinity impact on chlorophyll-a, chlorophyll-b, and overall chlorophyll levels. Findings indicated a significant decline in chlorophyll levels as salinity increased. Chlorophyll a and b contents decreased progressively, with the highest reduction observed at the highest salinity level. The study concludes that soil salinity impairs chlorophyll synthesis in *Amaranthus viridis* L., suggesting that salinity stress effect the plant's physiological process photosynthesis. The findings also highlight the high salt tolerant potential of *Amaranthus viridis* L. and future alternative crop for saline areas.

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Introduction

Irrigation water that drains through the soil becomes more salinized as irrigation efficiency rises. According to Childs and Hanks,[2] salinity is the state that results from the accumulation of different soluble salts present in a solution to a degree that prevents crop plants from growing and developing normally. On the other hand, a saline soil has a pH of less than 8.5, an ESP of about 15.0, an EC of more than 4.0 dS/m and a sodium adsorption ratio is lesser than the 13.0 (at 25^{o}C).[8,14,19]

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Soil salinity is refers to the presence of different water-soluble salts, includes sodium (Na+), potassium (K+), chloride (Cl−), and sulfate (SO_4^-). Some ions such as K+ and SO_4 also act as plant nutrients, while Na+ and Cl− are not plant nutrients.[16]

Seeman and Critchley[3] as well as Sharkey *et al*.[13] demonstrate that salinity has the potential to significantly alter the carbon metabolism of photosynthesis, leaf chlorophyll levels, and photosynthetic effectiveness. Under salt stress, plant tissues store proteins and carbohydrates, which are thought to aid in osmotic regulation.[9,4]

Baramati with 1,38,248 hectors total area is located on Eastern region of Pune district. Out of this total area, nearly 70% area is used for the agricultural practices. River water is the main source of agricultural practices as this Tahsil comes under rain shadow areas. Neera is the river on which “Veer dam” is constructed. The water from dam is used by different areas of Baramati through canal irrigation system which support the crop rotation. Agricultural Land of Neera River becomes salt affected because of growing monotype cropping system and use of harmful chemicals factors i.e. chemical fertilizer and irrigation water. Plants growing in dry and semi-dry areas are facing salinity stress which severely reduce growth and yield.[6,20] Data on the tolerance of *Amaranthus viridis* L. to salinity stress are lacking. So it is very important to find salt tolerance mechanism of *Amaranthus viridis* L.

The *Amaranthus viridis* L included to family Amaranthaceae distributed Worldwide. The plant commonly known as slender Amaranth and Math bhaji in Marathi. According to reports, Amaranthus can withstand modest amounts of salt and is comparable to other vegetable crops like mustard and cowpeas.[11] Amaranth has emerged as a key vegetable crop that is climate change resistant and effective for meeting human dietary demands because of its capacity to tolerate severe abiotic environments.

Materials and Methods

Selection of the Sampling Area and Sample Collection

The plants material, rhizospheres and non-rhizospheres saline soils was collected from three different salt affected areas (Songaon, Khandaj and Mekhali) from Baramati Tahsil. The three samples taken for each measurement.

Sample Preparation and Laboratory Analysis

After collection the soils were dried and mix well and these soils are subjected for further analysis. The plant material was washed thoroughly and these fresh plant material used for further analysis.

Assessing pH and Electrical Conductivity (EC)

For each sample, 20 g of soil was mixed with 50 ml of deionized water and mixed by keeping on a shaker for 30 min. The soil solutions were then filtered by filter paper. The filtered liquid sample were used to measure pH and electrical conductivity (EC) by a pH meter and EC meter respectively.

Estimation of Chlorophyll Content

Chlorophyll a, chlorophyll b, and overall chlorophyll were obtained and measured from fresh leaves, adhering to the standard procedure by Arnon.[1] One gram of chopped fresh leaves was taken and crushed with 20 – 40ml of 80% acetone. The sample was centrifuged at 5000–10000 rpm for 5 minutes. The supernatant was removed and the process was repeated until the residue turned colorless. The solution's absorbance was measured at 645nm and 663nm using the solvent (acetone) blank. The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation.

Chlorophyll a*: 12.7 (A663) – 2.69 (A645)
Chlorophyll b*: 22.9 (A645) – 4.68 (A663)
Total Chlorophyll*: 20.2 (A645) + 8.02 (A663)

Results

Results show that, the EC of soil samples collected from non-rhizosphere soil was better in comparison with rhizosphere soil. Comparatively, the EC value in Songaon is higher than soil collected from Khandaj and Mekhali, which clearly shows that reduction of the chlorophyll content in *A. viridis* L. Finding of accumulation of photosynthetic pigments in leaves of *A. viridis* collected from three different sites showed that, chlorophyll contents in leaves of Songaon sites is minimum.

Furthermore, the trend of synthesis of chlorophyll in leaves of *A. viridis* is observed in the following sequence - Control > Khandaj > Mekhali > Songaon.

The observations show a negative relationship between salinity and chlorophyll pigment synthesis. Nevertheless, further scientific research is required to generalize the relationship between synthesis of chlorophyll and salinity in salt-tolerant species.

Table 1: pH and EC of soil samples.

Sites	pH (NR)	pH (R)	E.C (NR) (dS/m)	E.C (R) (dS/m)
Mekhali	8.45 ± 0.023	8.25 ± 0.127	6.59 ± 0.596	5.79 ± 0.3516
Khandaj	8.21 ± 0.012	8.17 ± 0.159	5.706 ± 0.312	4.7 ± 0.331
Songaon	8.50 ± 0.047	8.64 ± 0.092	6.75±0.685	6.16 ± 0.406

(Each Value Represents Mean±S.E.of three observations).
Where R= Rhizosphere soil, NR= Non-rhizosphere soil, E.C= Electric Conductivity.

Table 2: Chlorophyll content in Amaranthus viridis L. growing on saline soils.

Sites	Cha a*(μg g^{-1} FW)	Chl b* (μg g^{-1} FW)	Total Chl* (μg g^{-1} FW)
Control	305.21±1.17	153±2.14	458.21±1.17
Mekhali	290.57 ± 3.62	135.45 ± 1.78	426.02 ± 3.27
Khandaj	304.36 ± 1.13	141.66 ± 1.68	446.02 ± 1.25
Songaon	280.43 ± 3.25	138.23 ± 1.78	418.66 ± 2.26

(Each Value Represents Mean±S.E.of three observations).

Discussion

The findings of our study revealed notable reductions in amount of chlorophyll a* and chlorophyll b* levels in response to salt stress, corroborating the earlier findings of Turan *et al*.,[18] regarding *P. vulgaris* L. and Taffouo *et al*.,[17] concerning *Vigna subterranean* L. The reduction of chlorophyll content in plants subjected to salt stress is recognized as a common indicator of oxidative stress.[15] This phenomenon has been linked to the suppression of chlorophyll production, as well as the enhancement of its breakdown facilitated by the enzyme chlorophyllase.[12] The decrease in chlorophyll levels, attributed to either a slow synthesis rate or rapid degradation, suggests the presence of a photoprotection mechanism that mitigates light absorption by lowering chlorophyll content.[5] It was observed that high levels of salinization induced a significant decrease in the content of chlorophyll a and chlorophyll b and consequently the total chlorophyll in plants. When E.C of soil decreases, the chlorophyll content in leaves of *Amaranthus viridis* L. increases. Our findings concur with those of Kaya *et al*.,[7] and Noreen *et al*.,[10] who noted a decrease in chlorophyll concentration in plants under salt stress.

Conclusion

Our findings suggest that, the *Amaranthus viridis* L. is posses salt tolerant plant and grow in higher saline conditions (Electric conductance upto 7 dS/m). When salinity increases, the plant reduces growth and chlorophyll content because of osmotic and toxic effects of salts. The *Amaranthus viridis* L. is future plant in salt affected soils and also used as bio-remediation purposes.

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Not Applicable

Author Contributions
- **Vikram Sanjay Patil:** Survey, Data Collection, Analysis, Writing – Original Draft
- **Bhagawan Shankar Mali:** Resources, Supervision and Writing – Review & Editing

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