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Effect of salinity stress on physiological and biochemical aspects of *Vigna radiata*

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Abstract:

Among abiotic stress salinity is the main stress that affects the morphology, and physiology of the plant which finally leads to a decrease in the crop yield. An experiment was carried out to look into the effects of salt stress on *Vigna radiata* (mung bean) plants. A few characteristics were examined in both the control and stressed plants, including root length, leaf length, leaf breadth, shoot length, root weight, proline, total carbohydrates, and flavonoids. The experiment's findings demonstrated a noteworthy distinction between stressed and healthy plants. The shoot height was the highest healthy plant compared to the stressed plant. The amount of chlorophyll was much reduced in the stressed plants. The amount of carbohydrates, proline, and flavonoids was higher amount in stressed plants as compared to the healthy plants.

Keywords- Proline, carbohydrates, flavonoids

1. INTRODUCTION:

Any negative environment or chemical that interferes with or hinders a plant's ability to grow, develop, and metabolize is considered stress (Lichtenthaler, 1996). Plant stress causes some adverse effects on the physiology of the plant which is induced due to the sudden transition from the normal condition or optimal condition to the un optimal condition. Plant stress can be of two types stress Abiotic and Biotic stress. Abiotic stress mainly includes cold, drought, salt, and heavy metals that largely affect plant growth and productivity. Biotic stress includes infection by microbes, herbivores, injuries, etc. Because they are sessile, plants are unable to survive in unfavorable environmental conditions. Abiotic stress is the primary factor affecting plant growth and productivity because of the continuously changing environment. Abiotic stress is a significant factor in agricultural yield limitation as well as plant dispersal under various environmental circumstances. There are occasions when these pressures coexist, which affects plant productivity and growth. Drought, excessive temperatures, and salt in the soil are examples of abiotic stress.

Vigna radiata: The mung bean is a diploid grain legume that belongs to the Fabaceae family. It self-pollinates and grows quickly (in less than 60 days). Through a symbiotic relationship with the nitrogen-fixing Rhizobium bacteria, mung beans are able to fix nitrogen. Nutrients such as minerals, fiber, carbs, protein, and antioxidants including phenolics and flavonoids are abundant in mature seeds (Allito et al., 2015, Bangar et al. 2019). According to Basu et al. (2016), mung beans grow best in rain-fed circumstances with high temperatures (27–30 °C), low humidity, and moderate rainfall (60–80 cm). In comparison to other leguminous crops, it needs comparatively less water to grow. *Vigna radiata* is a good source of protein and has a low oligosaccharide content. Because of its short lifespan and ability to fix nitrogen, *Vigna radiata* is one of the most valuable crops.

Salt stress: - A variety of physiological and metabolic processes are altered by salt stress, and ultimately, the stress's duration and intensity can lower agricultural productivity. (Gupta and Huang 2014). Soil salinity in agricultural soil refers to the high concentration of soluble salts in the soil moisture of the root zone. Plant growth is impacted by these high osmotic pressure concentrations of soluble salts because they restrict the roots' capacity to absorb water.

Crop productivity is hampered by salinity stress, which causes modifications in physiological and metabolic functions that depend on the intensity and duration of the stress (Bindumadhava et al., 2016). Plants experience hyper-ionic and hyperosmotic stress as a result of salinity's detrimental effects on the homeostasis of vital nutrients. The salinity stress may lead to membrane damage, nutrient damage, nutrient imbalance, alternation of growth regulator levels, enzyme inhibition, etc.

Secondary Metabolites: Secondary metabolites in plants are defined as substances that do not contribute fundamentally to the upkeep of biological processes but are crucial for defense and adaptation in response to environmental changes in the plant. They play a significant role in plants' defense against environmental stressors, herbivores, and infections. The physiology and development of the plant determine the extremely low production of secondary metabolites

Effect of salinity on the secondary metabolites: Plants under salt stress frequently experience osmotic and ionic stress, which can cause certain secondary metabolites to accumulate or decrease. Salt stress is known to cause an increase in 13 anthocyanins (Parida et al., 2005 Ramakrishna et al., 2011). On the other hand, in salt-sensitive species, anthocyanin levels were reduced by salt stress (Daneshmand et al., 2010 & Ramakrishna et al., 2011). Salt-tolerant alfalfa plants increased their proline concentration in their roots fourfold in a short amount of time, but plants that were sensitive to salt increased their proline content more slowly (Petrusa et al., 1997 and Ramakrishna et al., 2011). This was shown by Petrusa and Winicov. Plant polyamines have been connected to the way plants respond to salt (Mutlu et al., 2007 Ramakrishna et al., 2011).

2. REVIEW OF LITERATURE

The reaction of the cereal crop to salinity stress revealed that the early stages of reproduction and the vegetative stage are more vulnerable to stressful conditions. Even the soil, water, and climate have an impact on a plant's ability to tolerate stress. (Maas, 1990).

Amira (2011) investigated the impact of salt stress on the development and metabolism of the bean plant *Vicia faba* by exposing it to varying amounts of NaCl. A few characteristics were taken into account, including growth, osmotic potential, protein content, and chlorophyll concentration. As the concentration rose, they noticed a few modifications in the plant's morphology as well as a rise in both fresh and dry weight and a decrease in osmotic potential. Subsequently, the amounts of chlorophyll also decreased.

The effects of salt stress were investigated in Vigna radiata during two summer and spring seasons (Nirmala et al., 2015). They investigated how salt stress affected the plant's morphology and physiology at various stages of growth. Growth stages include 1) vegetative; 2) flowering; and 3) pod filling. They noticed that the summer had a far greater impact on the plants than the spring did (Nirmala et al., 2015).

Measurements of height, biomass, leaf area index, relative water content, seed production, chlorophyll a & b, proline, and ions content were part of a study on the biochemical and physiological effects on *Brassica napus*. they discovered that all cultivars exhibited considerably lower levels of relative water content, leaf area index, chlorophyll a and b, K+, K+/Na+ ratio, height, biomass, and seed production as well as increased levels of Na+ and proline content due to the salinity stress. They deduced from the data that plants with a low leaf area index are better suited to flourish in salt stress.(Rezaei et al, 2017)

A study examining the effects of varying salt concentrations on *Schizonepeta tenuifolia* was carried out, and observations were made on alterations in plant development, antioxidant capacity, and other related factors. Following the investigation, they came to the conclusion that growth and secondary metabolism are impacted by salt stress. (Zhou et al, 2018)

The dry weight, fresh weight, relative water content, & photosynthetic pigments (carotenoids and chlorophyll) of the seedlings all drop as a result of the salt stress. Proline, protein, total soluble sugar, lipoxygenase activity, and electrolyte leakage are also increased by it. It was also seen that osmolytes were building up. (Alharby et al., 2019)

3. MATERIALS AND METHODS –

3.1 Measurement of Shoot length

The measurement of the shoot (devoid of root) was taken using a scale to study the comparative account between the control and salt-stressed plant.

3.2 Measurement of Root Length

The measurement of root (devoid of shoot) was taken using a scale to study the comparative account between the control and salt-stressed plant.

3.3 Measurement of Root Biomass

The biomass of the root is here inferred as the total fresh weight of the plant (devoid of shoot). The weight of the control and salt-stressed plant was taken separately.

3.4 Estimation of total chlorophyll content in plants –

The total chlorophyll content calculation procedure shown below was taken from Kamble *et al.* (2015).
Principle: The chlorophyll from different leaf samples is extracted using an organic solvent (acetone). Then the concentration of chlorophyll in different samples is estimated by measuring their absorbance values in a spectrophotometer at 645 and 663nm.

3.5 Estimation of Carbohydrates –

The below protocol for the estimation of carbohydrates was obtained from Dubois et al (1956) Krishnaveni et al (1984)

Principle: To make hydroxymethyl furfural, glucose is dehydrated in a heated, acidic solution. When combined with phenol, this produces a green product that reaches its maximum absorbance at 490 nm.

The standard graph of glucose concentration (µg/mL) vs absorbance (at 490 nm) (Y-axis) was used to calculate the quantity of carbohydrates present per gram of the plant samples.

3.6 Estimation of proline –

The below proline estimation protocol was derived from the paper by Abraham et al, 2010.

Principle: Proteins precipitate as a compound during selective extraction with aqueous sulpho-salicylic acid. It is also likely that other disruptive substances are eliminated through absorption into the protein sulpho-salicylic acid complex. In an acidic environment (pH 1.0), the extracted proline is allowed to react with ninhydrin to generate the chromophore, which is red in color and read

Standard graph: A standard graph of Proline concentration (µg/mL) (X-axis) versus Absorbance (at 520nm) (Y-axis) was plotted, which was used to find out the amount of proline present in the leaf extracts used in the experiment.

3.7 Estimation of Phenolics by Folin - Ciocalteu method –

The below protocol for the estimation of phenolics was obtained from the paper on Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. Extracts Milan S. Stanković. (2011)

Principle: Using the FC reagent, one can determine the concentration of phenolics—aromatic benzene ring compounds having one or more hydroxyl groups—in a plant sample. Phenolics are primarily produced by plants to guard against stress. A combination of tungstates and molybdates, the Folin-Ciocalteu reagent operates on the oxidation-reduction reaction mechanism. The phenolic compounds can only react with the Folin-Ciocalteu reagent under very specific circumstances, which are adjusted by adding sodium carbonate solution. The dissociation of the phenolic molecule to form a phenolate anion has been shown to occur under basic conditions. This generates a blue-colored solution and decreases the Folin-Ciocalteu reagent, which is a mixture of molybdates and tungstates. The absorbance measurements can be used to calculate the color intensity of the generated blue chromogen with a spectrophotometer.

A standard graph of Phenolics concentration (µg/mL) (X-axis) versus Absorbance (at 650nm) (Y-axis) was plotted, which was used to estimate the number of phenolics present in the leaf extracts used in the experiment.

3.8 Estimation of Flavonoids

The protocol for the estimation of flavonoids Milan S. Stanković. (2011)

After thoroughly mixing the solution once more, absorbance at 510 nm was measured in relation to a blank. Based on the calibration curve and the following linear equation, the flavonoid content was determined.

4. Results and Observation

4.1 Shoot length

The length of the shoot of control and salt stress was measured after removing the root. The measurement was as follows:

Plant Sample		Shoot Length	Average
Control	Plant-1	22	22.4
	Plant-2	22.7	
	Plant-3	22.5	
	Plant-1	21.8	

Salt	Plant-2	21.6	21.8
	Plant-3	22	

Table 1- Measurement of Shoot length

4.2 Root length

The length of the root of control and salt stress was measured after removing the shoot. The measurement was as follows:

Table 2- Measurement of Root length

Plant Sample		Root length	Average
Control	Plant-1	15	14.47
	Plant-2	15.4	
	Plant-3	13	
Salt	Plant-1	11.3	10.96
	Plant-2	10.9	
	Plant-3	10.7	

4.3 Root Biomass

The weight of the entire root of the control and salt-stressed plant was measured. The measurement was tabulated as following

Table 3- Measurement of Biomass of Root

		Root Biomass(gm)	Average
Control	Plant-1	0.251	0.251
	Plant-2	0.250	
	Plant-3	0.252	
Salt	Plant-1	0.249	0.249
	Plant-2	0.248	
	Plant-3	0.252	

4.4 Estimation of Chlorophyll content

At 645 and 663 nm, the absorbance of the leaf extracts from the salt-stressed and control groups was observed. The following value was tabulated:

Table 4.1- Absorbance value

Plant sample	**Absorbance**	
	645	**663**
Control	0.432	0.693
Salt stressed	0.274	0.521

The "total chlorophyll content, chlorophyll a content, and chlorophyll b content of the plants were calculated using the following formulas:

- mg of Chlorophyll a/gram of tissue = $12.7(A_{663}) – 2.69(A_{645})$ x V/1000xW
- mg of Chlorophyll b/gram of tissue = $22.9(A_{645}) – 4.68(A_{663})$ x V/1000xW
- mg of Total Chlorophyll/gram of tissue = $20.2(A_{645}) + 8.02(A_{663})$ x V/1000xW

where V is the chlorophyll extract's final volume, A is the absorbance at specific wavelengths, and W is the weight of the tissue used to make the" extract.

The Calculations of chlorophyll content in the plant's samples are given below:

Control

i. mg of chlorophyll a/gram of tissue=12.7(0.693) -2.69(0.432) X 20/1000

=8.8011 -1.162 X0.02
=8.8011 – 0.0232=**8.778 mg/g**

ii. mg of chlorophyll b/gram of tissue = 22.9(0.432) – 4.68(0.693) X20/1000
=9.892 – 3.2432 X0.02
=9.892 -0.065 =**9.827 mg/g**

iii. total chlorophyll/g of tissue = 20.2(0.432) + 8.02(0.693) X20/1000
=8.726 + 5.558 X0.02
= 8.726 +0.111=**8.837 mg/g**

Plant under salt stress

i. mg of chlorophyll a/gram of tissue =12.7 (0.521) -2.69(0.274) X20/1000
=6.616-0.737 X0.02=**6.601 mg/g**

ii. mg of chlorophyll b/gram of tissue= 22.9(0.274) - 4.68(0.521) X20/1000
=6.275 – 2.438 X0.02
=6.275 -0.044=**6.23 mg/g**

iii. Total of chlorophyll/g of tissue=20.2 (0.274) +8.02(0.521) X20/1000
=5.535- 4.178 X 0.02
=5.535 – 0.084=**5.451 mg/g**

A comparative result of the above calculations is as follows

Table 4.2- Amount of Chlorophyll present

Plant sample	Chlorophyll a content(mg/g)	Chlorophyll b content(mg/g)	Total Chlorophyll content(mg/g)
Control	8.778	9.827	8.837
Salt	6.601	6.23	5.451

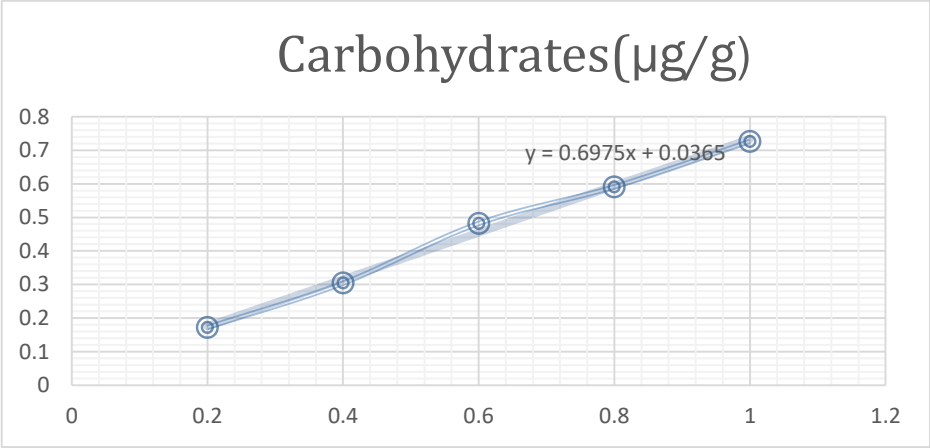
Chlorophyll a, b, and total chlorophyll were thus highest in the control plant and lowest in the plant under salt stress, as can be observed from the above result.

4.5 Estimation of Carbohydrates

The amount of carbohydrates in control and salt-stressed was estimated. The absorbance value(490nm) was tabulated as following:

Table 5-Absorbance value

Plant Sample	Absorbance (490 nm)
Control	0.277
Salt Stressed	0.521



Graph 5-Standard Graph of Carbohydrate

Plotting the above absorbance values on a standard carbohydrates graph allowed us to determine the amount of carbs present in the plants. The outcomes are listed below:

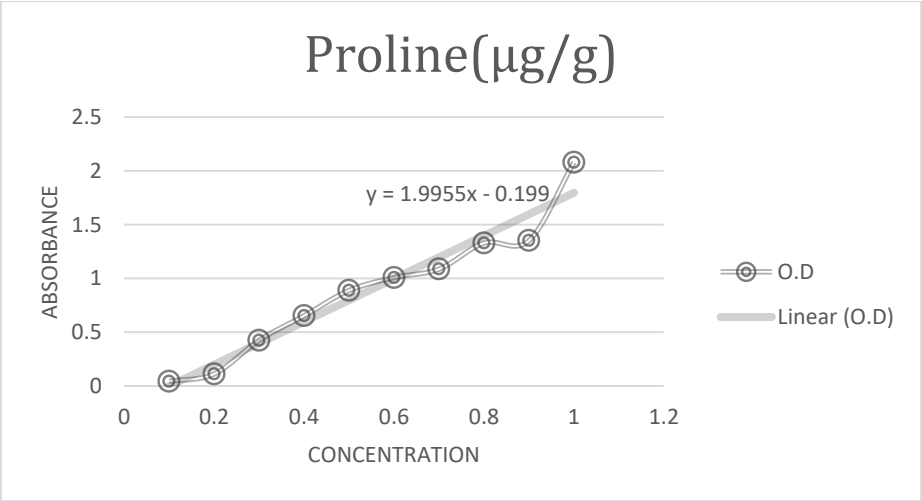
i.Amount of carbohydrates in control plants =**345 µg/g** of the leaf tissue
ii.Amount of carbohydrates in salt-stressed plants = **695 µg/g** of the leaf tissue

4.6 Estimation of Proline

The amount of Proline in control and salt stress was estimated. The absorbance value(520nm) was tabulated as following:

Table 6-Absorbance value

Plant sample	Absorbance(520nm)
Control	0.025
Salt	0.185



Graph 6 -Standard graph of Proline

Plotting the above absorbance values on a typical Proline graph allowed for the determination of the amount of Proline present in the plants. The outcomes are listed below:

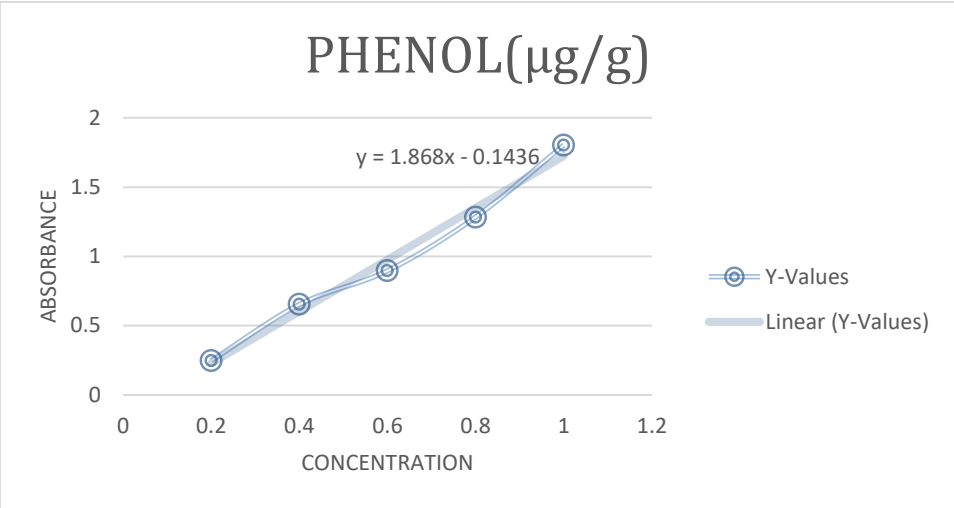
i. The concentration of Proline in control plants = 11**µg/g** of the leaf tissue.
ii. Concentration of Proline in plants under salt stress = 19**µg/g** of the leaf tissue.

4.7 Estimation of Phenolics

The amount of Phenol in control and salt stress was estimated. The absorbance value(650nm) was tabulated as following:

Table 7-Absorbance value

Plant Sample	**Absorbance(650nm)**
Control	0.624
	0.626
Salt	0.793



Graph 7 -Standard graph of Phenol

The amount of Phenolic compounds in the plants was obtained by plotting the above absorbance values on a standard Phenolics graph. The results are given below

i. Amount of Phenolics in control Plants,
   - Plant – 1 = 41**µg/g** of the leaf tissue.
   - Plant – 2 = 42**µg/g** of the leaf tissue.

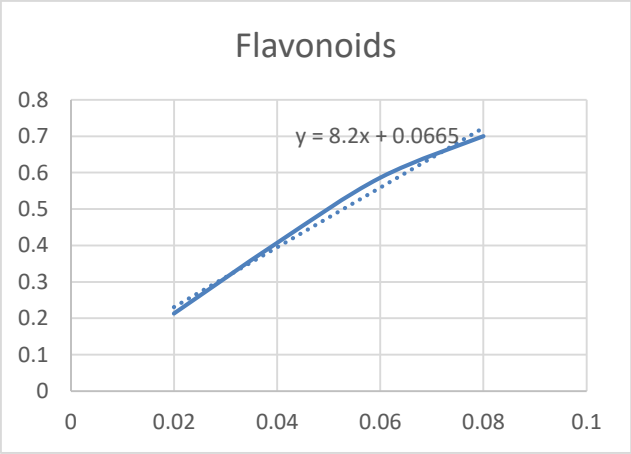
ii. Amount of Phenolics in Plants under salt stress=50**µg/g** of the leaf tissue.

4.8 Estimation of Flavonoids

The amount of Flavonoids in control and salt stress was estimated. The absorbance value(650nm) was tabulated as following:

Table 8-Absorbance value

Plant Sample	**Absorbance (510 nm)**
Control	0.453
Salt	0.509



Graph 8- Standard graph of flavonoids

Plotting the absorbance readings on a typical quercetin graph allowed for the determination of the amount of flavonoids present in the plants. Below are the results:

Amount of Flavonoids in control plant-**47 µg/g** of the leaf tissue.
Amount of Flavonoids in salt-stressed plant=**54 µg/g** of the leaf tissue.

5. Discussion

Abiotic environmental factors such as salt are major stresses that affect plant development and productivity ultimately leading to decreased agricultural yield.

The plant root system modifies its morpho-physiological traits in response to salt stress in order to absorb nutrients (Hasegawa et al., 2000). In order to avoid or reduce the absorption of salt, plants adapt by shrinking their roots (Hasegawa et al., 2000). Salt stress reduces the extracellular water potential and bioavailability of water in the root zone, which prevents plants from developing and generating biomass at a rate that is sufficient. It also hinders plants capacity to absorb nutrients and water.

Mung bean yield is significantly decreased by salinity stress due to a decrease in root and shoot lengths, as well as a decrease in seed germination. Long-term salt damage can cause necrosis, chlorosis, and a reduction in chlorophyll concentration (Hanumantha et al., 2016).

The growth of the stressed plant was conspicuously reduced. The root length shrank as the concentration of salt stress increased. Plant growth is inhibited in saline conditions due to the suppression of cell division and expansion (Zahra et al., 2018). A reduction in leaf length and width was seen in stressed plants, which has an impact on photosynthetic rate and lowers biomass. NaCl was used to apply salt stress to the plant, which lowers the roots' ability to absorb water by raising the osmotic pressure in the root (Zahra et al., 2018).

In comparison to the control, the stressed plant has reduced amounts of total, a, and b chlorophyll. The breakdown of chlorophyll by chlorophyllase or the instability of the proteins in the photosynthetic apparatus are the two main causes of the decrease in chlorophyll content during salt stress.

The stressful condition increases the amount of carbohydrates in the plant because of decreased carbohydrate metabolism. (Rosa *et al*, 2009). The carbohydrate content was higher in the salt-stressed plant. Control has a lesser amount of carbohydrates than the stressed plants.

The fact that stressed plants have higher concentrations of proline indicates that proline is among the first molecules to create tolerance in salt-stress environments. Plant cells have a greater water potential when they include proline, which has a higher osmosis potential than saline soil. Proline can also boost enzyme activity because it is a chaperon molecule shielded from protein structures (Ashraf and Foolad, 2007; Szabados and Savour'e, 2010). The sensitive cultivar Sarigol's higher proline concentration raises the possibility that proline may not be as important in controlling osmosis in brassica plants. To increase their resistance to salinity stress, cultivars that yielded a lot of seed under salinity stress were combined with extra osmoprotectants.

The amount of flavonoids is higher in the salt-stressed plants and is lower in control. Flavonoids in higher plants serve as antioxidants in response to stressful conditions.

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