Short communication
J. Adv. Nutri. Sci. Tech. 4(1-2): 35-40, 2024 **DOI:10.15228/ANST. 2024.v04.i01-2. p04**

Microscopic Examination of Biosynthesis of Anthocyanins in Leaves of *Vigna radiata* under Saline Stress: a New Insight

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Abstract
The irrigation water used for crop cultivation contains soluble substances, such as Cl^-, SO_4^{-2}, and CO_3^-, referred to as salts, which are necessary for plant growth. Salinity generally affects soil structure and inhibits plant growth, reducing crop yields when present in excess. The current article aims to monitor the visual accumulation of anthocyanin in the leaves of the plant *Vigna radiata* cultivated under salinity in a half-strength Hogland solution. Anthocyanins extracted from plants are generally used as natural colours in cosmetics, dyes, and food additives. The relationships between salt stress and anthocyanin content in leaves of *Vigna radiata* were investigated under a photo camera microscope. The experiment was conducted after 2 days of germination in the dark and then shifted into the nutrient media viz 0, 15, 20, and 25 ppm NaCl. Visual monitoring revealed a direct relationship between the reduction in plant growth and the increase in salinity. The leaves were harvested after 15 days, washed, and then cleaned before being subjected to monitored anthocyanin synthesis under a photo camera microscope. It was observed that the red and purple regions in leaves appeared under salinity conditions and increased as the NaCl concentration increased. Anthocyanin coloration specifically reflects anthocyanidin glycosides. The highest anthocyanin content (red and purple regions in leaves) was observed under high salinity conditions (25 ppm). Microscopic evaluation of leaves suggests that further research is needed to enhance anthocyanin content under salinity in anthocyanin-rich fruits and vegetables that may be extracted for antioxidant, anti-inflammatory, pharmacological, and other dietary purposes, including use in jams, jellies, candies, fresh sausages, and yogurts.
Keywords: salinity, plants, anthocyanin, leaves

Highlights

- Microscopic study of anthocyanin under salinity
- red and purple regions showed the synthesis of anthocyanin
- Anthocynine synthesis increases as salinity increases

1. Introduction

Anthocyanin pigments play a role in developing the colour of plants' fruits, flowers, leaves, and roots. Its biosynthesis signifies a substantial metabolic asset for the development of plant cells. The biosynthesis of anthocyanin implies the plant's protective system against stress and the development of tolerance mechanisms stimulated by excess reactive oxygen species (ROS). They act as ROS scavengers, protecting against oxidative damage and enhancing their sustainability (Brodribb et al., 2010; Borghesi et al., 2011; Xu et al., 2017). Salinity refers to the presence of salt in the soil that limits plant water availability; consequently, high electrical conductivity is observed. This is a problem caused by the presence of high amounts of salt near the roots of the plants, which prevents them from absorbing water from the soil (Al-Busaidi & Cookson 2003). Although water preservation in the soil is not increased in saline surroundings, salt in the water stimulates plants to expend more energy in extracting water from the soil. Soil water salinity also increases as plants take up or lose water through transpiration, thereby increasing salt concentration in the remaining soil water (Li et al., 2022).

Osmosis is the process by which water moves into the plant from the roots. If the salt concentration in the soil water is high, osmosis slows down; consequently, dehydration occurs, leading to a decline in growth or the plant's death. Salinity affects the growth of plants due to specific ions, such as chloride, which are lethal to plants when present in excess. The leaves of most higher plants are green in color, indicating the presence of chlorophyll and other light-harvesting pigments that facilitate photosynthesis (Nishio, 2000). Anthocyanins are associated with improved resistance to environmental stresses, including both abiotic and biotic stresses. The colour and stability of anthocyanin pigments are influenced by pH, light, temperature, and structure. It appears red in acidic soil and blue under alkaline soil conditions (Farooq et al., 2022). It was reported by Al-Busaidi et al. (2003) that the pH of the soil decreases as the salinity of the soil increases, possibly due to the inverse correlation between soil pH and the enhanced solubility of Ca^{2+} ions in saline soil under typical atmospheric CO_2 concentrations, which leads to the release of hydrogen ions. Another report described a 2-fold increase in saline treatment in the plant Sun Black, which enhanced the synthesis of anthocyanins in fruits (Borghesi et al., 2011). The use of anthocyanins in the food and pharmaceutical industries is widespread, extracted from various types of fruits and vegetables.

Moreover, anthocyanins exhibit antidiabetic, anticancer, anti-inflammatory, antimicrobial, and anti-obesity properties, and they also assist in preventing cardiovascular diseases. Consequently, anthocyanins derived from edible plants are promising pharmaceutical compounds (He et al.,2011). Therefore, the present investigation was undertaken to examine the leaves of *Vigna radiata* cultivated under diverse saline conditions, visually inspecting anthocyanin contents under a microscope, and to determine whether salinity may enhance anthocyanin content in anthocyanin-rich fruits and vegetables.

2. Materials and methods

Chemically sterilized seeds of Vigna radiata were washed thoroughly with distilled water and dried on filter paper. These seeds were transferred to sterilized petri plates and placed in the dark for two days. The two-day-old germinated seeds were placed in a half-strength Hoagland solution. Control plants were grown using a half-Hoagland solution without NaCl. All plants were placed in a natural environment where saline treatments were conducted at 0.0, 15, 20, and 25 ppm. The solution was patterned daily to check the plants for further additions of the half-Hoagland solution during 15 days of growth. After 15 days, the harvested plants were weighed, and their length was recorded. The leaves were then cleaned according to the Koga & Kobayashi (1980) method to observe the synthesis of anthocyanins using a photo camera microscope.

3. Results and Discussion

Leaf clearing is valuable for numerous ecological, physiological, and taxonomical aspects of research (Bruzzese & Hasan, 1983). The primary function of leaves is to harvest solar radiation to synthesize glucose. Its further polymerization is related to plant growth, which is affected by environmental conditions and may be used to observe the ecological impact on primary reactions due to reduced plant growth (Farooq et al.,2022; Brodribb et al., 2010).

The biosynthesis of anthocyanins was inspected microscopically under salinity in the leaves of *Vigna radiata*. It was observed that the growth of the plants was affected under salinity, as shown in Fig. 1).

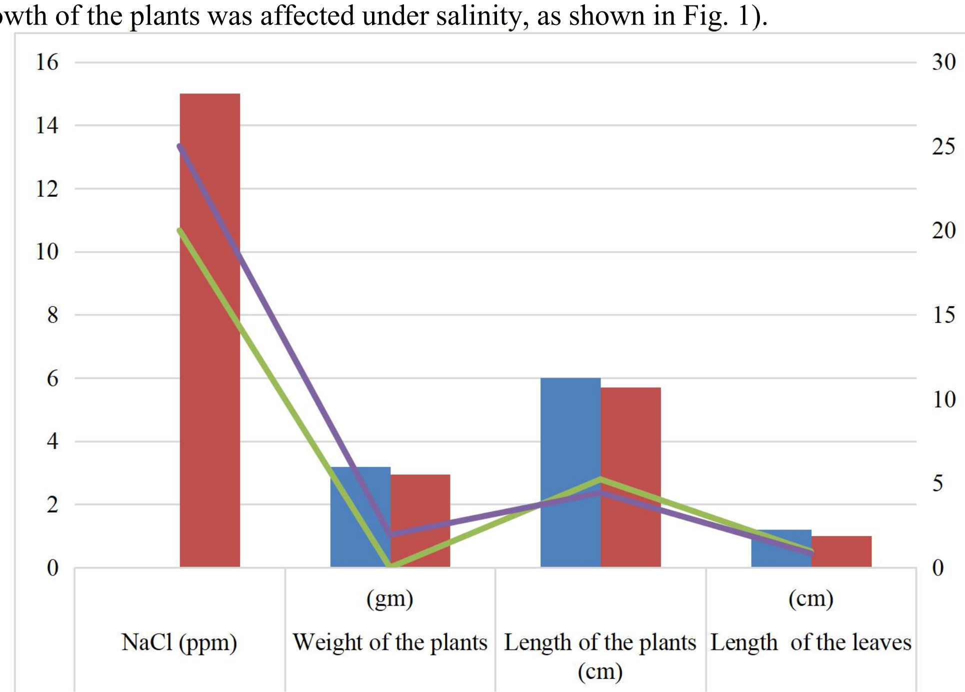

Figure 1. Reduction in the growth of *Vigna radiata* under different saline applications

It was reported that the yield and quality of crops were reduced under salt stress, similar to the current study, which threatened the supply of food to achieve the target of the population due to the higher dry matter accumulation in plants directly correlated with the increased synthesis of anthocyanin content in wheat that enhance salt tolerance (Mbarki et al., 2018). Salinity significantly impacts plant cell membranes' permeability, which is responsible for physiological drought in plants, photosynthesis, respiration, growth, and yield (Kim et al., 2022). It was established that salinity limits the growth of the plants, consequently limiting the yield (Fig.1). The harvested leaves, after clearing, were placed under a microscope where no anthocyanin contents (Fig. 2) were observed in control plants, indicating the normal metabolism of the plants.

Figure 2. No anthocyanin contents in *Vigna radiata* in control plant

The leaves of the *Vigna radiata* in low salinity (Fig.3) exhibited a shallow red colour, indicating the synthesis of anthocyanins underway in the trichomes (Zhang et al. 2016) under salinity, followed by fading of the green colour in this region.

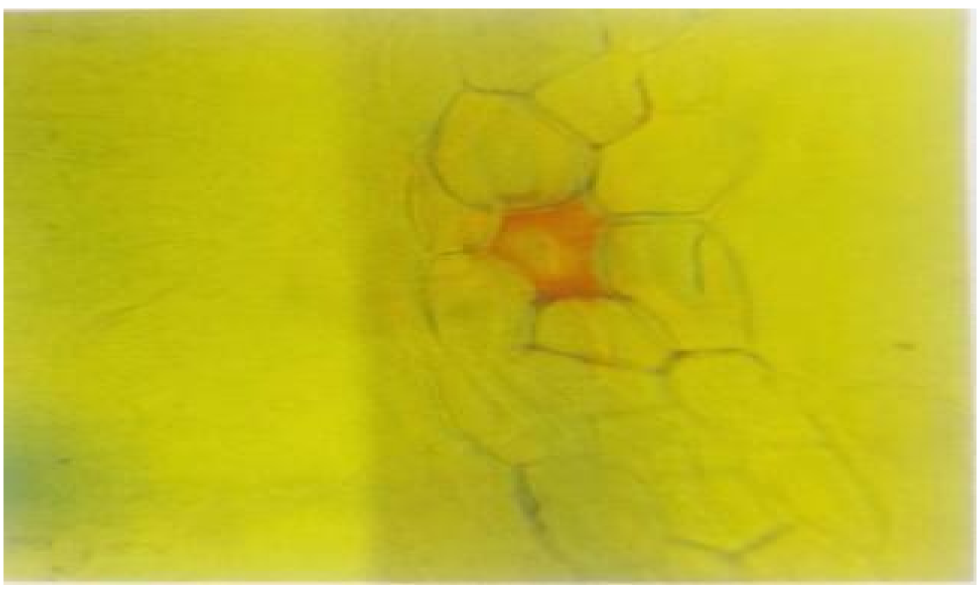

Figure 3. Synthesis of Anthocynine under 15ppm of NaCl in *Vigna radiata*

Microscopic examination of leaves grown under 20 and 25 ppm exposed red and purple regions with less green regions display the synthesis of anthocyanins directly related to enhanced saline conditions (Fig. 4 &5) compared to control plants (Fig.2).

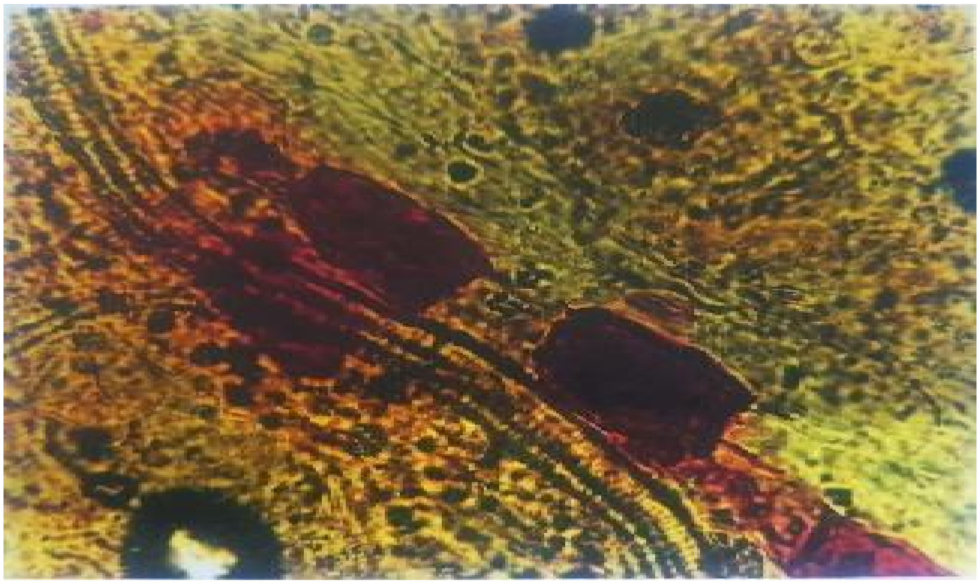

Figure 4. Synthesis of Anthocynine under 20ppm of NaCl in *Vigna radiata*

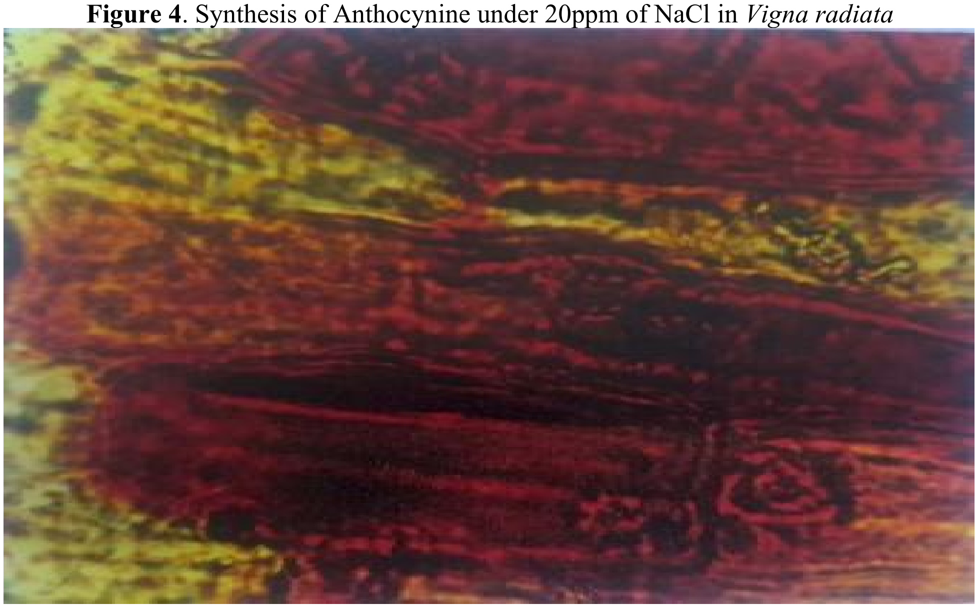

Figure 5. Synthesis of Anthocyanin under 25ppm of NaCl in *Vigna radiata*

Zhang et al. (2016) reported that synthesis occurs in the cytosol, a platform for cellular metabolic processes and reactions (containing approximately 70% water), and is then transported to the vacuole region, where it is found to be enhanced as salinity increases. This is also supported by earlier reports, which indicate that a large number of anthocyanin trichomes contribute to the red region, potentially serving as a protective mechanism for photosystem 1 (Zhang et al. 2016). Plant physiology and metabolism are disrupted under salinity through oxidative and osmotic stress, ion toxicity, and nutrient imbalance, leading to cellular damage, reduced growth and yield, and even plant death (Ma et al., 2021; Muchate et al., 2016). This may also lead to the modification of diverse anthocyanin derivatives, which are then transported to the vacuoles. Zhao et al. (2023) reported more than 200 unique anthocyanins, and several enzymes are involved in their modification; however, these late modification pathways are primarily unidentified (Zhao et al., 2023). It increases the capability of the antioxidant system to overcome the salinity. Furthermore, anthocyanins have been shown to help plants cope with salt stress under nitrate-deficient conditions, a finding similar to the present research project, where major red and purple regions indicate the scavenging capability of ROS by the anthocyanin pigment. Gould's report also supports the microscopic examination of the leaves. (2004), who established that red pigment in wounded leaf peels under the Microscope may be involved in eliminating H_2O_2 significantly higher than green cells. He also stated that scavenging of H_2O_2 may occur primarily by the red tautomers of anthocyanin found inside the cell vacuole or by the colourless tautomers in the cytosol, both of which showed good antioxidant capability. Neill, & Gould. (2003) conducted an in vitro study where they observed that colourless tautomers proved effective in scavenging the oxidative species, contributing to developing a defence system against ROS. High salinity inhibits the absorption of essential mineral nutrients and disrupts the dynamic balance of K^+ and Na^+ in new and old leaves

(Ismail and Horie, 2017). ROS is generally generated under conditions of salinity, biotic, and abiotic stresses, which negatively affect chlorophyll content (Fig. 3-5) and photosynthetic rate (Kim et al., 2022). The function of anthocyanins is to regulate the ROS species as they may interact with proteins to augment protein activity through the ROS reaction. The current investigation demonstrates that anthocyanin biosynthesis is enhanced under salinity conditions. Still, it is not usually a requirement for protection from oxidative stress. Microscopic examination of Fig. (4 & 5) showed the red and purple pigments, similar to earlier reports of Shi and Xie (2014 who established that anthocyanin (purple pigment) played a significant role in plant protection from UV light, ROS, and resistant to abiotic stress, and also involved in attracting pollinators (Khan et al.,2020). Besides all these, it was also recommended that salinity might be beneficial for the extraction of anthocyanins, which can be used as antioxidant, anti-inflammatory bioactive compounds, and for other uses in the cosmetics, food, and beverages industries, if saline stress is applied to anthocyanin-enriched fruits and vegetables (Jaakola 2013).

4. Conclusion

Anthocyanins have been used as pigments in various foods, such as jams, jellies, candies, and beverages, due to their antioxidant capacity and antimicrobial properties. The current search provides new insights into salinity and anthocyanin accumulation, which may aid in extracting organic anthocyanins from plants grown under different stress conditions. Based on current findings, plants enriched in anthocyanins should be tested and grown under saline conditions to enhance their anthocyanins. Therefore, further investigation is recommended at an advanced level to enhance the concentration of anthocyanins in plants grown in saline soil and to develop safe extraction methods, enabling their application as a green compound for food industrial purposes.

Acknowledgment

The author is grateful to the National Nematode Research Center for providing the facilities of the camera and the Microscope, and to the Department of Botany, Jinnah University for Women, Nazaimabad, for conducting this study.

Conflict of Interest: NA

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Received: March 11th 2024 **Accepted: May 22nd 2024**