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Role of Salicylic Acid in the Alleviation of Salt Stress on Pea Cultivars Using Growth, Biochemical and Physiological Attributes

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Abstract

Salinity stress is the crucial abiotic stress that limits crop production worldwide. A plant growth promoter, salicylic acid (SA), was used as a foliar application (FA) to allay the toxic influence of NaCl (salt) on growth of winter vegetable pea. Salt stress with two doses (0 and 100 mM NaCl) was applied through the rooting growth medium, while two levels of SA were used through exogenously. Salt in the growth medium impaired the shoot fresh (13.25%) and dry weight (21.20%) as well as the root fresh (22.12%) and dry weight (6.65%) in the pea cultivars "RSK 510" and "Lena Pak." Foliar SA reduced salt-induced effects while increasing the dry and fresh weights of the shoot as well as of the root. Besides these growth attributes, salt accumulation also limits the rate of photosynthesis (35%) and transpiration (13.21%), sub-stomatal CO_2 concentration (14.78%), and the stomatal conductance (5%) also, and in the pea cultivars "RSK 510" and "Lena Pak." Here, the foliar spray of SA overcomes these gas exchange parameters and enhances their values. The SA treatment showed higher performance in the cultivar "RSK-510" while lower in the "Lena Pak" under salt stress. Photosynthetic pigment values like the chlorophyll a (15.47%) and b (24.10%) decreased in the cultivars "RSK-510" and "Lena Pak," which were improved by foliar SA treatment. The values of MDA contents were also lessened due to salt accumulation in the plant cell, and that lessened effect was controlled by the foliar spray of SA in both pea cultivars. Overall, it was noted that the cultivation of pea cultivars "RSK-510" and "Lena Pak" showed their growth in regard to biomass production, photosynthetic rate, and transpiration rate, and also that the photosynthetic pigments and foliar spray of SA promoted these growth attributes.

Keywords: Biogas; Salt Stress; Salicylic Acid; Pea; Gas Exchange

1. Introduction:

Climate change is posing a threat to the environment and attracting the attention of crop growers, researchers, and policymakers due to its negative impact on agriculture (Genc *et al*., 2019; Wang *et al*., 2021). Abiotic stresses involve in the 50% limitation of crop production (Verma *et al*., 2013; Hernandez, 2019; Neila *et al*., 2022). Abiotic stress, including salinity, drought, high or low temperature, an increase in heavy metals, and flooding are serious challenges to the growth and development of the crop and its productivity (Hasanuzzaman *et al*., 2014). The salt of NaCl is the most damaging abiotic stress, affecting crop production at a rate of 1.5 million ha per year due to the adding of salt in the soil and water (Neila *et al*., 2022). Increased salt concentrations on agricultural land have damaging effects on worldwide crop production.

Salt accumulation in agricultural land limits 50% of crop production globally (James *et al*., 2011). The problem is getting worse as a result of an

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environment that is gradually changing as a result of serious climate change. The presence of sodium and chloride ions in excess in the growth medium is the main reason for salt stress. High salinity levels in the growing media result in ionic and osmotic stress, which eventually kills plants. (Gopalakrishnan *et al*., 2020; Soliman *et al*., 2020). Salt-induced effects include membrane injury, an imbalance in the nutrients, enzymatic inhibition that inhabits photosynthesis, and ultimately declined crop productivity (Hasanuzzaman *et al*., 2012). The toxic ROS like hydroxyl radicals and singlet oxygen are produced in response to salinity stress, which damages cell organelles like mitochondria and chloroplasts and further suppresses the biochemical and physiological activity of the crop cell (Borsani *et al*., 2001: Abdi *et al*., 2022).

Various plant growth enhancers have a potential role in crop productivity by affecting various biochemical and physiological processes when plants are subjected to sodium chloride (Iqbal *et al*., 2022; Khan *et al*., 2012). Among the various growth promoters, salicylic acid is the most important because it involves various biochemical and physiological attributes that enhance plant growth (Athar *et al*., 2010; Jahan *et al*., 2019). Salicylic acid stimulates plant growth by increasing stomatal conductance (Aldesuquy *et al*., 1998), proper ion absorption and transport (Tohma and Esitken, 2011), photosynthetic activity (Tohma & Esitken, 2011), and chlorophyll synthesis (Jahan *et al*., 2019). Behind this, it also enhances growth (Arfan *et al*., 2007) and uplifts the antioxidant defense system (Faghih *et al*., 2019).

1.1. Objectives:

- To study aims to lower the deleterious response of salt on peas via foliar application of the growth promoter salicylic acid.

2. Materials and Methods:

To access the response of salicylic acid in the alleviation salt stress on two pea cultivars "RSK-510" and "Lana Pak," an experiment was completed in the Botany Department, University of Gujrat, Pakistan. The seeds of RSK-510" and "Lana Pak (pea cultivars) were collected from the local market Gujranwala, Pakistan. Fresh river sand from the Chenab River in Gujrat was used as the growth medium. To remove the contamination from the river sand, it was washed with tap water before being placed in the plastic pot, which weighed 12.5 kg. Eight seeds from each pea cultivar were sown in the pots. The Hoagland's nutrient solution with full strength was given to each replicate to irrigate the growth medium. The moisture contents of the sand growth medium were maintained using tap water (100 ml) on each day. After 10 days of seed germination, a salt treatment with two levels (0 and 100 mM NaCl) was applied through the rooting medium. Plants were thinned to maintain four healthy plants in each pot. After two weeks of salt application, salicylic acid was applied at two levels (0 and 1.0 mM) through foliar spray. At the vegetative stage, data of the following parameters were collected.

2.1. Biomass Production:

One plant was uprooted from each replicate and detached into its shoot and root to determine the fresh and dry weight of the root and shoot. The fresh weight of shoots and roots was measured using an electrical balance, and samples were placed in an oven at 60^{o}C to measure their dry weight.

2.2. Chlorophyll Contents:

For the estimation of chlorophyll contents, the procedure of Arnon (1949) was followed with minor modifications.

2.3. Malondialdehyde Contents:

The malondialdehyde contents were determined using the protocol of Carmark & Horst (1991).

2.4. Gas Exchange Parameters:

Various gas exchange parameters including rate of net CO_2 assimilation, rate of transpiration, stomatal conductance, and sub-stomatal CO_2 concentration were noted using an open-system LCA-4 ADC infrared gas analyzer. The readings of the attributes were taken at 10 a.m. at light with full intensity with the following adjustments and specifications: The atmospheric pressure was 99.9 kPa, the water vapour pressure into the chamber (7.0 to 9.8 mbar), the PAR at the leaf surface was maximum at 1812 mol m^{-2} s^{-1}, the leaf temperature (26.2 to 34.6 ^{o}C), and the ambient temperature (20.3 to 24.7 ^{o}C), ambient CO_2 conc. was 352 µmol mol^{-1}.

2.5. Water Use Efficiency:

The values of water use efficiency were calculated with the help of following formula:

Water use efficiency: Net photosynthetic rate divided by transpiration rate.

2.6. Statistical Analysis:

A completely randomized design with three replications was used for the current experiment. For the analysis of all parameters the COSTAT computer package was used (MSTAT Development Team, 1989). Microsoft Office was used to make the graph.

3. Results:

The addition of salinity (100 mM NaCl) in the growth culture of the experiment decreased the biomass (fresh and dry weight of shoot) in RSK-510 and the Lana Pak pea cultivars. Beside this, a clear difference was documented in both cultivars. Application of salicylic acid by foliar treatment resulted in a decrease in the shoot's dry and fresh weight. Less reduction was observed in the cultivar "RSK-510" than in those in the "Lena-Pak" under salt treatment, and salicylic acid enhanced the values in these attributes (Fig. 1).

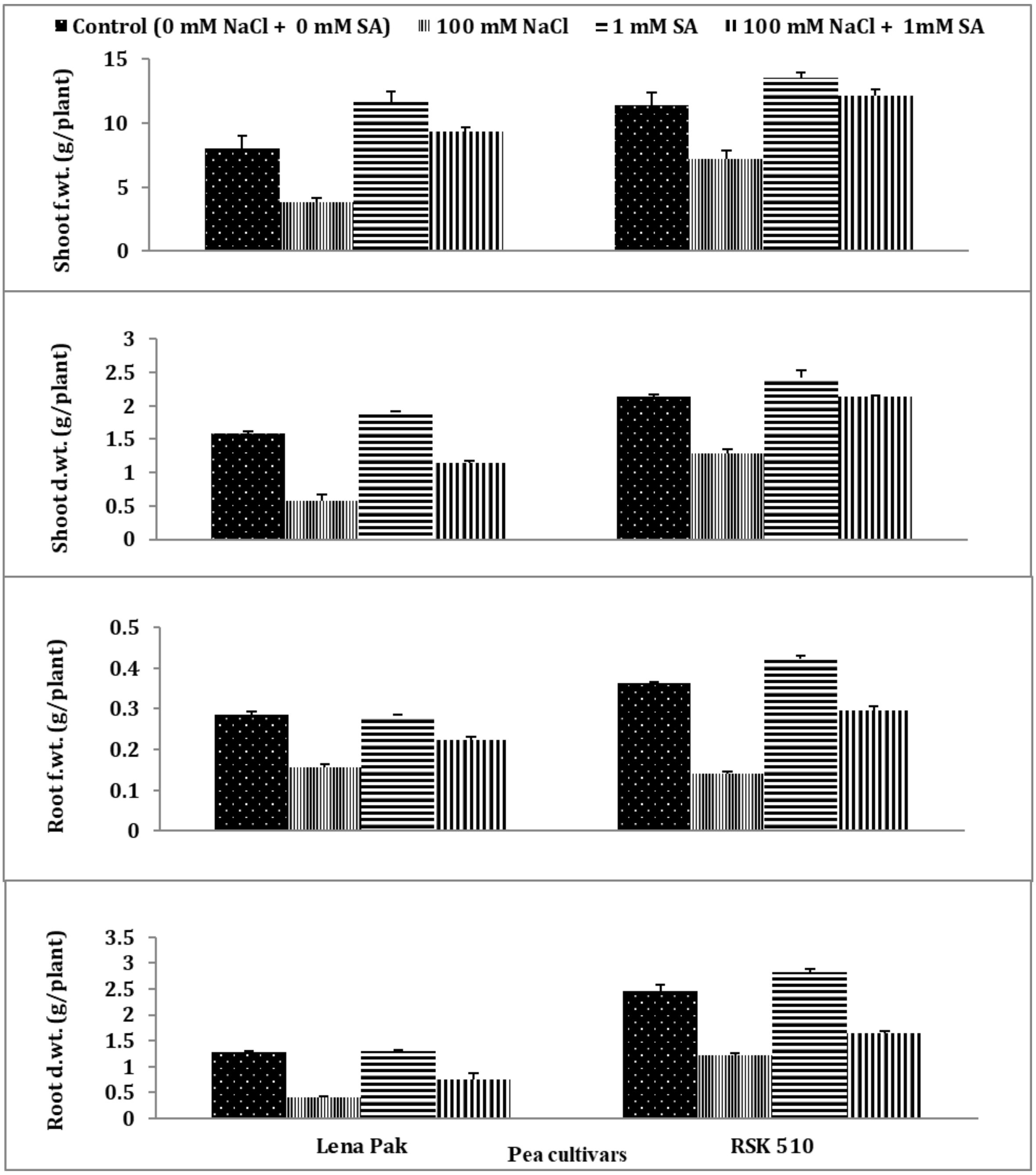
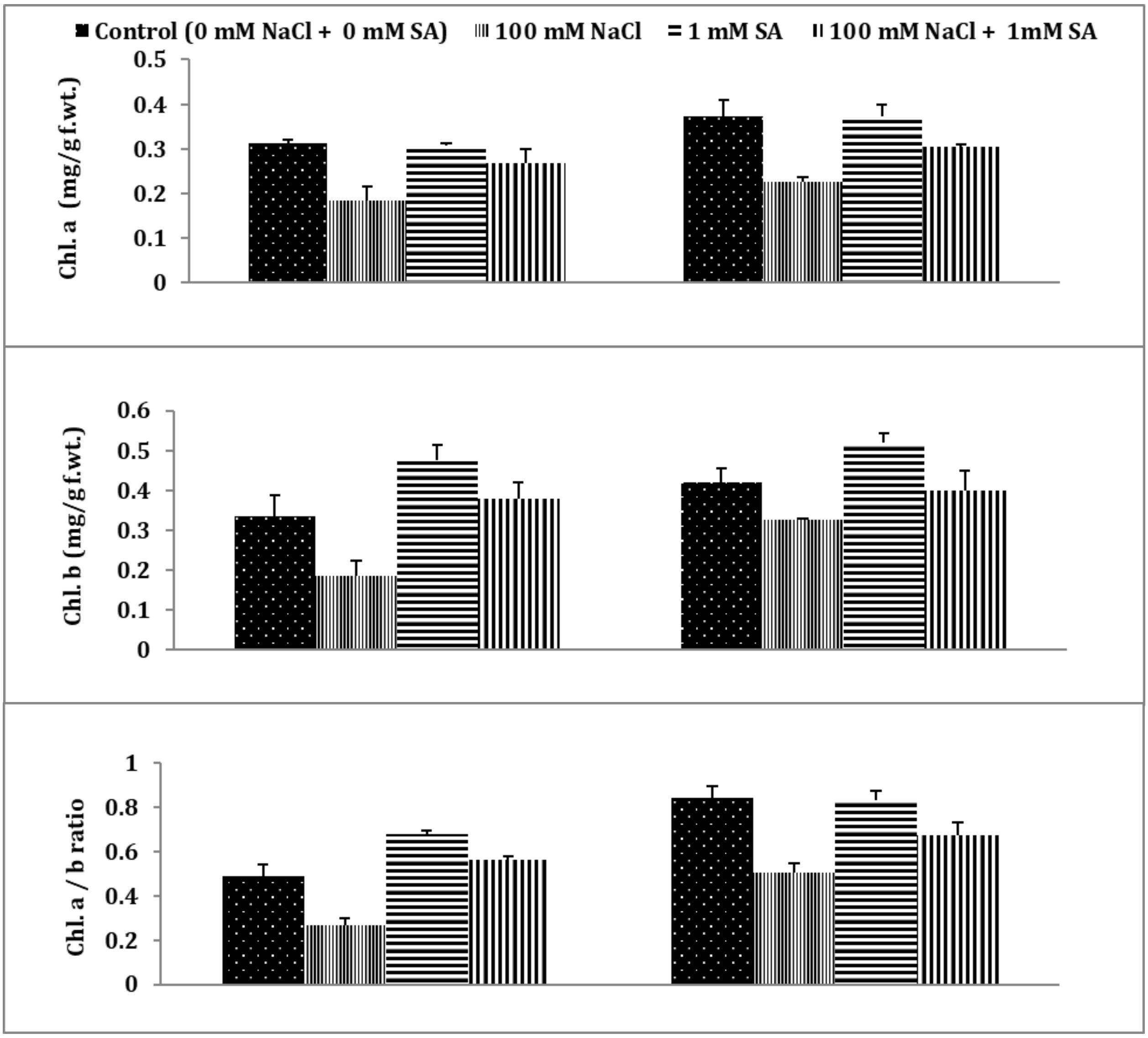


Figure 1: Effect of salt stress on the fresh and dry weights of shoots and roots of two pea cultivars under foliar applied salicylic acid

Salt accumulation in the growth culture limits the growth of fresh and dry root weight in both the RSK-510 and Lana Pak pea cultivars. When comparing the root fresh and dry weight under the salt regime, less variation was found between "RSK-510" and "Lana Pak" in the root fresh weight but more in the root dry weight. In the root dry weight, a higher reduction was found in the cultivar "Lena Pak" while a lower reduction was found in the cultivar "RSK-510." Intake of salicylic acid via the foliar method overcame salt-induced reduction and enhanced their values (Fig. 1). In both pea cultivars the values of chlorophyll a b, and a/b ratios decreased when salt treatment was given via rooting medium. A moderate response was noted in chlorophyll a content under saline growth medium, while a clear variation was observed in chlorophyll b in the both cultivars "Lena Pak" and "RSK-510." A potential plant growth regulator, salicylic acid, showed an alleviating effect and enhanced the synthesis of chlorophyll b under salt stress. It was also perceived that the application of salicylic acid also boosted the values of chlorophyll contents in the control conditions than those to saline conditions. The value of malondialdehyde (MDA) content was also decreased under the salt treatment in the pea cultivars "RSK-510" and "Lena Pak." On the basis of the comparison, a very slight difference was noted in regard to MDA content in the saline growth medium. Salicylic acid, which has a stimulating effect, increased the MDA content in both the salt treatment and the control plants (Fig. 2).



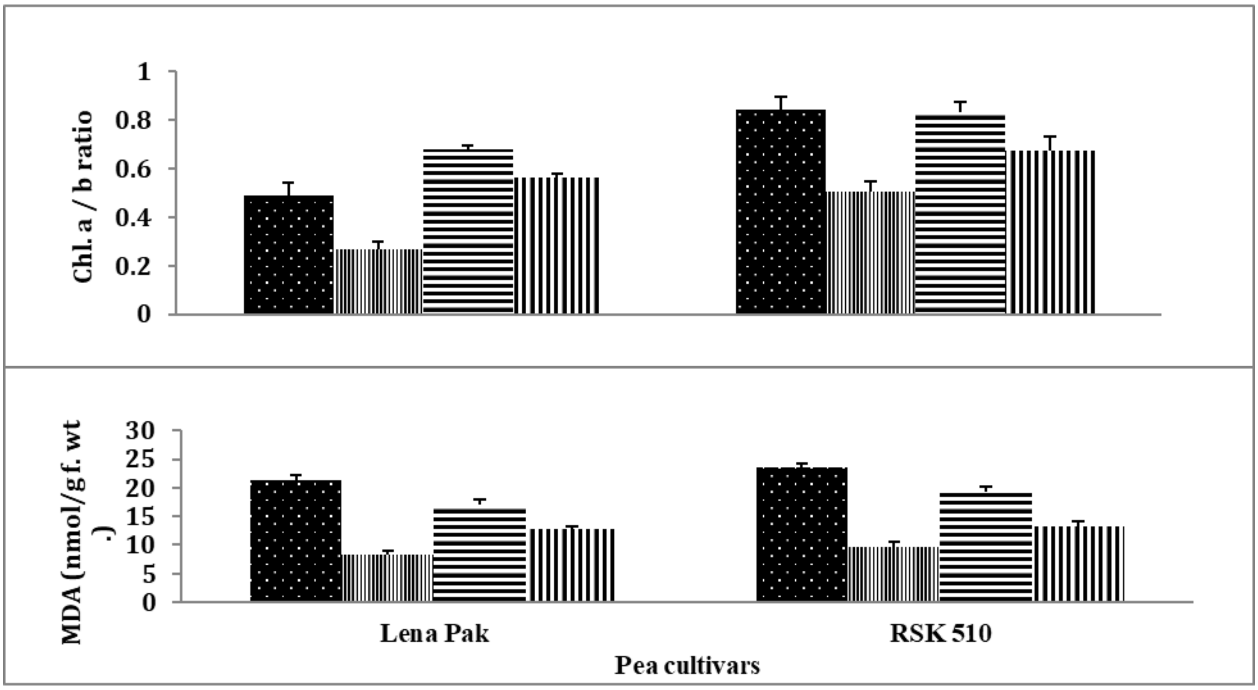
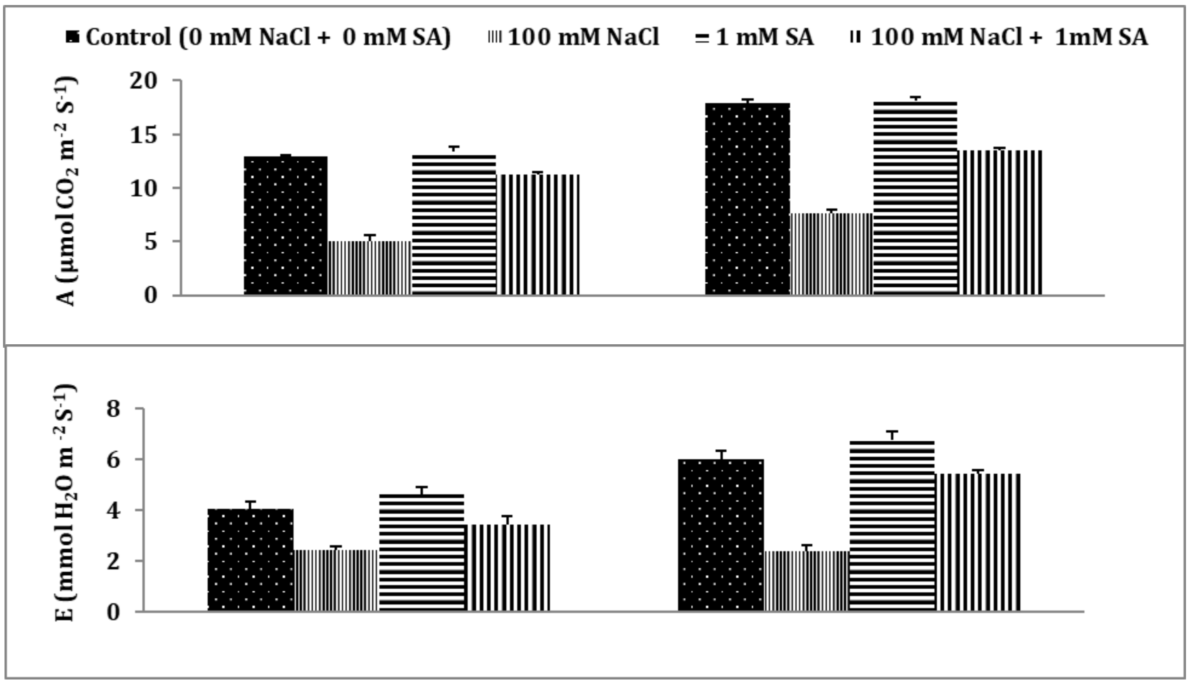


Figure 2: Effect of salt stress on chlorophyll a, b, a/b ratio and malondialdehyde content of two pea cultivars under foliar applied salicylic acid

The photosynthetic as well as transpiration rates were also decreased under salt accumulation in the growth medium of "RSK 510" and "Lena Pak" pea cultivars. In terms of photosynthetic rate, "RSK-510" had a lower reduction and "Lena Pak" had a higher reduction in saline growth medium. On the other hand, in the transpiration rate, a lower reduction was noted in the "Lena Pak" and a higher one in the "RSK-510" under salt application. Treatment of salicylic acid through foliar application mitigated the salt-induced reduction and enhanced the transpiration rate as well as the photosynthetic rate (Fig. 3). The values of stomatal conductance and sub-stomata CO2 conc. showed the declining response under the salt regime in the two pea cultivars. A higher value of stomatal conductance was noted in the cultivar "RSK-510" while the value was lower in the cultivar "Lena Pak," and no clear variation was observed in the attribute sub-stomatal CO2 conc. Intake of foliarly applied salicylic acid promotes these gas exchange attributes and enhances their growth (Fig. 3).



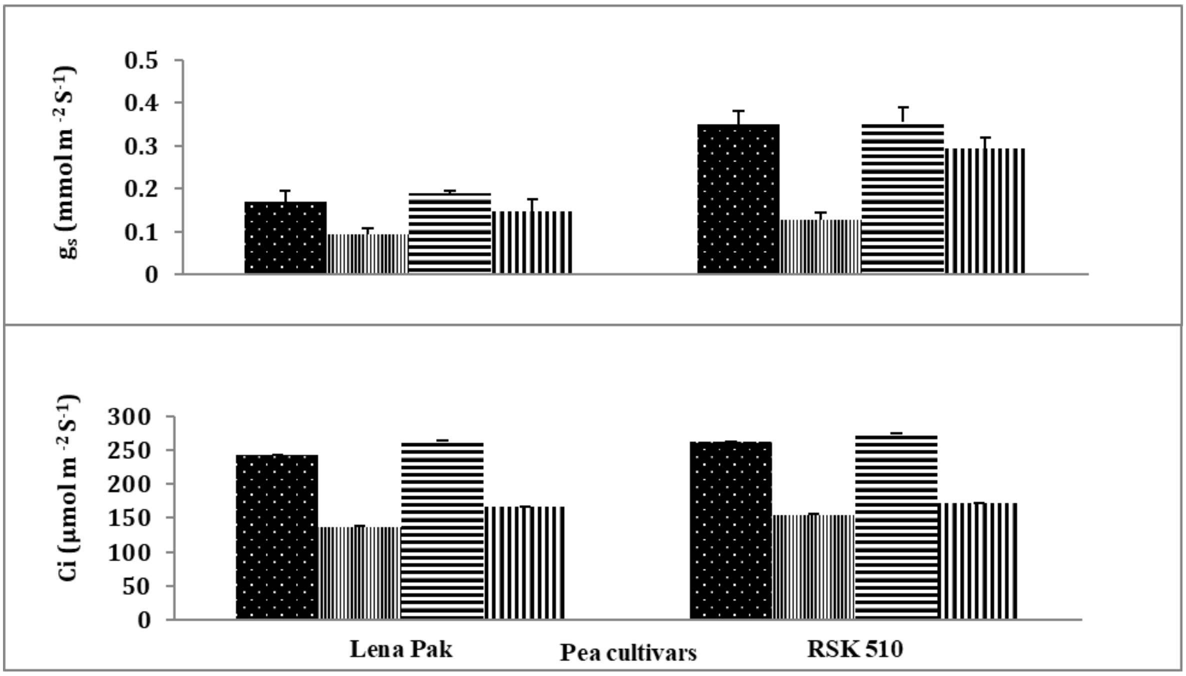


Figure 3: Effect of salt stress on rate of photosynthesis (A), rate of transpiration (E), stomatal conductance (gs) and sub-stomatal CO_2 conc. (Ci) of two pea cultivars under foliar applied salicylic acid

Salt application significantly decreased the water usage efficiency (WUE) of both pea cultivars. Under the salt treatment, the cultivar "RSK-510" had a greater WUE value than the cultivar "Lena Pak," which had a lower value. The values of WUE were recovered under the salt treatment with the application of salicylic acid. Salicylic acid treatment also enhanced the values of WUE in the non-saline environment as compared to the control (Fig. 4). Accumulation of sodium and chloride in the root zone had lowered the values of Ci/Ca ratio in the pea cultivars “Lena Pak” and “RSK 510”. A clear difference was observed in the two pea cultivars in regard to the Ci/Ca ratio. In this case, salicylic acid foliar treatment was used to overcome salt-induced limitation.

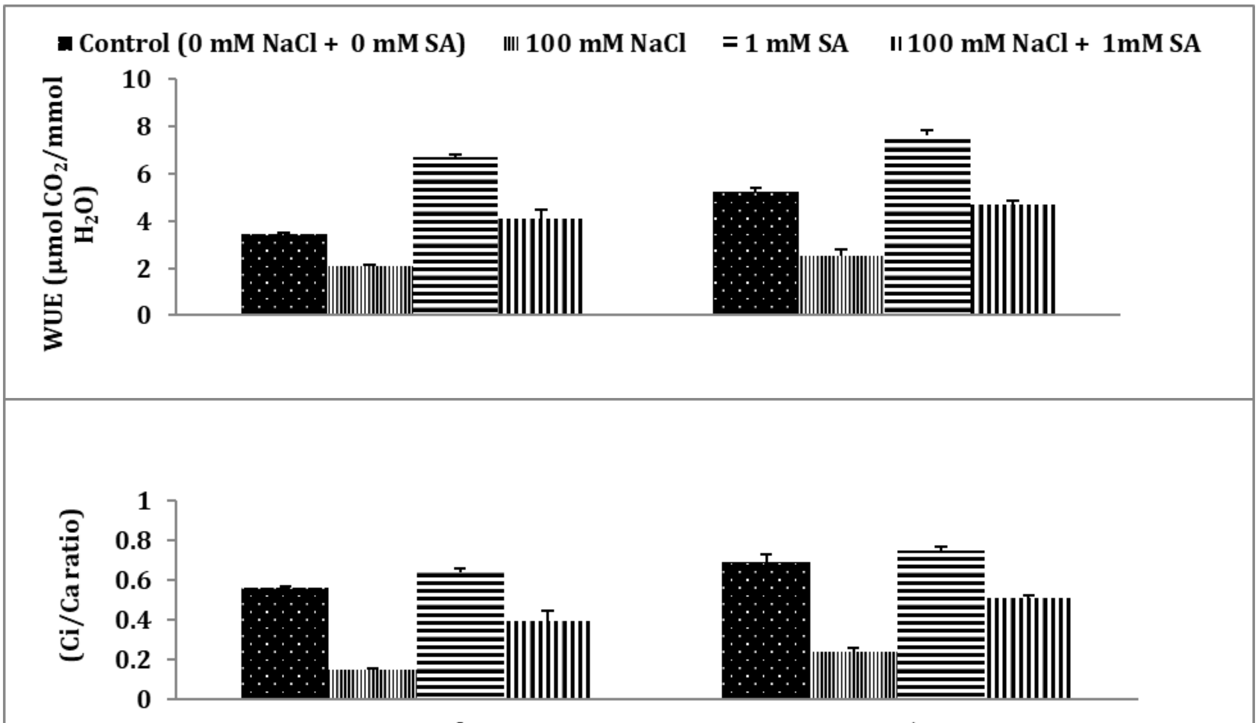


Figure 4: Effect of salt stress on WUE and the Ci/Ca ratio of two pea cultivars under foliar applied salicylic acid

4. Discussion:

Phenolic substances salicylic acid is a crucial antioxidant and a unique plant growth regulator that has the ability to counteract the effects of salinity on a variety of crop (Ahmed *et al*., 2022). Our results regarding pea cultivars are very close with the findings of Noreen *et al*. (2009), where addition of salinity in the rooting zone limited the uptake of water and nutrients, reduced the growth process and ultimately declined the growth of shoot and root fresh and dry weight. Here accumulation of salt in the tissues of root and shoot alters physiological process, halt the activity of indole-3-acetic acid, inhibit cell division of shoot and root cells and overall decreased the biomass production. Moreover, the foliar application of salicylic acid activates the synthesis of indole-3-acetic acid, induce cell division in the root and shoot tissues and increase their values. According to the findings of the current study, both pea cultivars' shoot fresh and dry weight decreased as a result of the salinity of the growing medium, where salt-induced growth reduction was recovered by the treatment of SA through foliar spray. The results of the current investigation are very close to those of Noreen *et al*. (2009), who demonstrated that saline growing medium decreased the shoot fresh and dry weight and that this loss was only slightly mitigated by the topically applied SA. Afzal *et al*. (2006) reported their work on the barley plant, where salicylic acid boosted growth under saline circumstances; Tayeb (2005) also validated similar findings.

In the current investigation, fresh and dry root weight values similarly decreased under saline circumstances. The research on rice by Jini & Joseoh (2017) which found that root zone salinity decreased root fresh and dry weight and was counteracted by exogenously applied salicylic acid, provided support for our findings on root fresh and dry weight. Examples of photosynthetic pigments that are necessary for the photosynthetic process, which enhances growth, development, and yield, include chlorophyll a and chlorophyll b. In the current study, foliar salicylic acid spray has overcome the salt-induced decline in the synthesis of chlorophyll a, b and it's a/b ratio. These findings were in line with the work of Fariduddin *et al*. (2003), which discovered that foliar salicylic acid treatment up to 30% mitigated the inhibition of chlorophyll a and b production caused by 120 mM NaCl in the growing medium. Malondialdehyde (MDA) levels were decreased by salt stress in pea cultivars, although there was less variance in this response across the two cultivars. The foliar application of salicylic acid in this parameter reduced the negative effects of the salt-induced impact. The findings of the current investigation support a previous finding made in wheat by Shagufta *et al*. (2014) that plant growth regulators were effective in increasing lipid peroxidation values.

According to the literature, photosynthetic activity is crucial for promoting growth, development, and overall yield. The results of the current study demonstrated that pea cultivars' photosynthetic activity and transpiration rate were decreased by salty growing medium, and both characteristics were improved by exogenously applying salicylic acid. Our current research confirms earlier studies by Noreen and Ashraf (2008) on sunflower who reported that the novel plant growth regulator salicylic acid was applied topically to sunflower to mitigate the negative effects of salinity on photosynthetic activity and transpiration rate. Salinity in the root zone decreased stomatal conductance and sub-stomatal CO_2 concentration as well. SA applied topically overcame this salt-induced inhibitory impact. The gas exchange attributes of the present study showed results reliable with the study of Khan *et al*. (2003) on the maize and soybean cultivars, foliar applied SA overcome the salt injury and enhanced the values of gas exchange parameters.

5. Conclusion:

In conclusion, it is found that salt-induced alteration in the growth, biochemical, and physiological parameters is alleviated using salicylic acid as a foliar application on two pea cultivars.

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