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Efficacy of Gibberellic Acid in promoting salt stress resilience in pea (*Pisum sativum* L.)

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Abstract

This study aimed to evaluate the effect of gibberellic acid on the growth, physiological, and biochemical attributes of pea plants under salt stress. Two pea cultivars were examined to determine their comparative performance in saline conditions. An experiment was conducted at the Old Botanical Garden, University of Agriculture, Faisalabad, using two pea cultivars, Pea 2009 and Green Pea. Plants were grown in pots arranged in a completely randomized design with three replications. Salt stress was applied using sodium chloride at two concentrations (0 and 100 millimoles), 21 days after sowing. Gibberellic acid was applied as a foliar treatment at three levels (0, 5, and 10 parts per million) fourteen days after salt application. Data on morphological and physiological traits were collected fourteen days after hormone treatment. Salt stress caused a noticeable reduction in shoot and root length, number of leaves and branches, and fresh and dry weights of shoots and roots. However, gibberellic acid significantly improved these parameters, even under salt stress. It enhanced chlorophyll content, carotenoids. Among the two cultivars, Pea 2009 exhibited better performance in both growth and stress tolerance. The application of gibberellic acid mitigated the adverse effects of salt stress in pea plants by improving morphological growth and enhancing key physiological responses. Pea 2009 showed greater resilience to salinity, making it more suitable for cultivation under saline conditions.

Keywords: Carotenoids, Chlorophyll content, Cultivar comparison, Ion accumulation, Morphological traits.

Introduction

Salinity is one of the most critical abiotic stresses affecting global agricultural productivity. It interferes with plant metabolism by altering physiological and biochemical processes (Kousik *et al.* 2023). The extent of damage caused by salinity depends on the severity and duration of exposure (Atta *et al.* 2022). High concentrations of sodium (Na^+) and chloride (Cl^-) ions accumulate in plant cells, leading to ion toxicity and osmotic stress. This disrupts ionic balance by inhibiting the uptake of essential ions such as potassium (K^+) eventually resulting in growth suppression and, under prolonged stress, plant death (Riffat and Ahmad 2018). Seed germination is a crucial and sensitive phase in the plant life cycle and often determines the success of crop establishment under salt-affected conditions (Riffat and Ahmad 2016; Reguera *et al.* 2020).

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Pea (*Pisum sativum* L.) is an economically and nutritionally important legume cultivated in various regions of the world. It is valued for its high protein, carbohydrate, fiber, and vitamin content (Wu *et al.* 2023). Although considered moderately salt-tolerant, yield losses of up to 50% have been observed at 100 mM NaCl (Navarro-Torre *et al.* 2023). In Pakistan, particularly in Punjab, pea is extensively grown and accounts for approximately 72-73% of national production. It is a cost-effective source of dietary protein and is widely consumed with rice, wheat, and other cereals (Rehman *et al.* 2015).

To improve crop productivity under stress, plant growth regulators have been increasingly utilized. Gibberellic acid plays a key role in enhancing seed germination, vegetative growth, and various physiological functions (Castro-Camba *et al.* 2022). The application of gibberellic acid has been shown to mitigate the negative impacts of salinity stress in several crop species (Attia *et al.* 2022; Zhuo *et al.* 2024). Other growth regulators such as kinetin and morphactin have also been studied for their roles in improving seedling establishment under saline conditions (Bueno and Cordovilla 2021). Salt stress often results in reduced seedling vigor, but growth regulators are known to counter these effects through hormonal and osmotic regulation (Riffat *et al.* 2023).

In addition, seed priming has been widely reported as an effective pre-sowing technique for enhancing germination and crop performance under salinity. Osmopriming and hormonal priming using gibberellic acid have demonstrated positive effects on seedling growth and salinity tolerance (Ma *et al.* 2018; Gueridi *et al.* 2024). These methods are simple, cost-effective, and practical for field application. However, the need remains to explore such approaches in a crop- and region-specific context.

Therefore, the present study is undertaken to evaluate the role of gibberellic acid in improving the physiological traits, growth, and yield of pea under salt stress conditions. Given the increasing salinization of soils and the importance of pea as a protein-rich food legume, this research aims to identify effective treatments that can enhance its tolerance to salinity and support sustainable production.

Methodology

Experimental Site and Design

The experiment was conducted in the Old Botanical Garden, University of Agriculture, Faisalabad. A total of 36 plastic pots were filled with homogenized, air-dried soil. The experimental design followed a Completely Randomized Design (CRD) with factorial arrangement and three replications. The study aimed to assess the effect of gibberellic acid on the growth and physiological attributes of pea (*Pisum sativum* L.) under salt stress conditions. Two cultivars, Pea 2009 and Green Pea, were used in the study.

Treatments and Stress Application

Seeds were sown directly into the pots. Salt stress was imposed by irrigating the soil with sodium chloride (NaCl) solution at two concentrations: 0 mM (control) and 100 mM, 21 days after sowing. Foliar application of gibberellic acid was conducted 14 days after the salt treatment at three levels: 0 ppm (control), 5 ppm, and 10 ppm.

Growth and Yield Parameters

The following morphological parameters were recorded 14 days after gibberellic acid application:

Shoot Length (cm): Measured using a measuring tape from the base to the tip of the main stem. Mean values were calculated from five randomly selected plants per treatment.

Root Length (cm): Plants were uprooted carefully, and root length was measured using a measuring tape. Mean values were recorded.

Shoot and Root Fresh Weight (g): The freshly harvested root and shoot from each plant was weighed immediately using a digital analytical balance.

Shoot Dry Weight (g): Shoots were placed in a hot air oven at 75°C for 72–96 hours until a constant weight was achieved, then weighed on a digital balance.

Root Dry Weight (g): Roots were oven-dried at 75°C for 72–96 hours and weighed similarly.

Number of Leaves per Plant: Counted manually on each plant and averaged per treatment.

Number of Branches per Plant: Counted and averaged from all plants per treatment.

Physiological Attributes

Estimation of Chlorophyll and Carotenoid Content

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were determined using the method described by Arnon (1949), with modifications. Fresh leaf samples (0.1 g) were taken from each treatment and cut into small pieces. 0.1 g of fresh leaf tissue was placed in a test tube and homogenized in 10 ml of 80% acetone. The samples were stored in the dark overnight to allow complete pigment extraction. After incubation, the samples were centrifuged at 10,000 rpm for 5–7 minutes. The supernatant was collected and absorbance was measured using a UV-Vis spectrophotometer (IRMECO UV-Vis Model U2020) at wavelengths of 663 nm, 645 nm, and 480 nm.

Chlorophyll and carotenoid contents were calculated using the following formulas:

Chlorophyll a (mg/g) = (12.7×OD663)–(2.69×OD645)(12.7 × OD_{663}) – (2.69 × OD_{645})(12.7×OD663)–(2.69×OD645) × V / (1000 × W)
Chlorophyll b (mg/g) = (22.9×OD645)(22.9 × OD_{645})(22.9×OD645) × V / (1000 × W)
Total Chlorophyll (mg/g) = Chlorophyll a + Chlorophyll b
Chlorophyll a/b Ratio = Chlorophyll a / Chlorophyll b
Carotenoids (mg/g) = OD_{480} × V / (1000 × EM × W)

Where:
OD = Optical density at specified wavelength, V = Volume of the extract (ml), W = Fresh weight of the leaf sample (g), EM (Extinction coefficient) = 2500

Statistical Analysis

The recorded data were subjected to analysis of variance (ANOVA) using CO-STAT software (Steel and Torrie 1996). Treatment means were compared using the Least Significant Difference (LSD) test at a 5% probability level to determine significant differences among treatments.

Results and Discussion

Number of Leaves per Plant

The number of leaves is a key morphological indicator of vegetative growth. In the present study, a decline in leaf number was observed under 100 mM NaCl stress in both pea cultivars, with a more pronounced effect in Green Pea. However, foliar application of gibberellic acid significantly increased the number of leaves across all treatments, particularly at 10 ppm (Figure 4.1). Pea 2009 consistently produced more leaves than Green Pea under similar conditions. These results indicate that salt stress reduces leaf production by interfering with cell division and expansion, while gibberellic acid mitigates this effect by stimulating leaf initiation and expansion, as previously reported by Vetrano *et al.* (2020) and Liu *et al.* (2023).

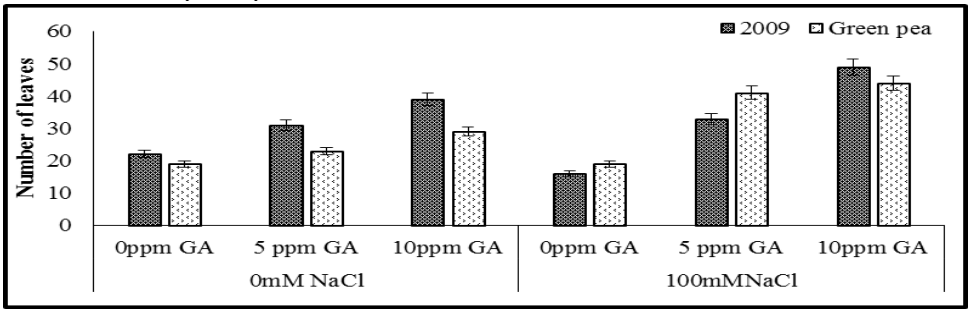

Figure 1: Number of leaves of two cultivars germinate under NaCl salinity stress conditions with gibberellic acid foliar spray

Root Length (cm)

Root elongation is essential for water and nutrient acquisition. As shown in Figure 4.2, root length was significantly reduced under salt stress, with minimum values observed in untreated plants exposed to 100 mM NaCl. Application of gibberellic acid enhanced root length under both control and saline conditions, with the highest values recorded in Pea 2009 treated with 10 ppm GA_3. This enhancement may be attributed to the role of gibberellic acid in promoting cell elongation and tissue differentiation, as also suggested by Miceli *et al.* (2019).

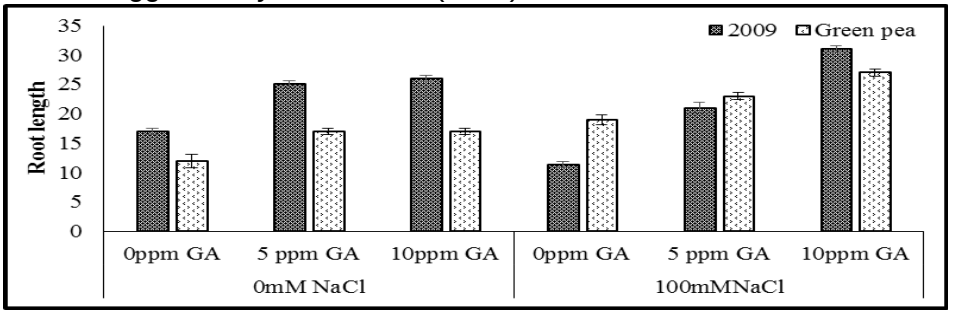

Figure 2: Root length of two cultivars germinate under NaCl salinity stress conditions with gibberellic acid foliar spray

Shoot Length (cm)

Salt stress inhibited shoot elongation in both cultivars, as seen in Figure 4.3. However, gibberellic acid application alleviated the negative effects of NaCl, resulting in significantly longer shoots. The greatest increase in shoot length was observed in Pea 2009 at 10 ppm GA_3 without salt stress. These results are consistent with Fu *et al.* (2023), who reported that gibberellic acid counteracts shoot growth suppression by improving cell elongation and reducing oxidative damage in stressed tissues.

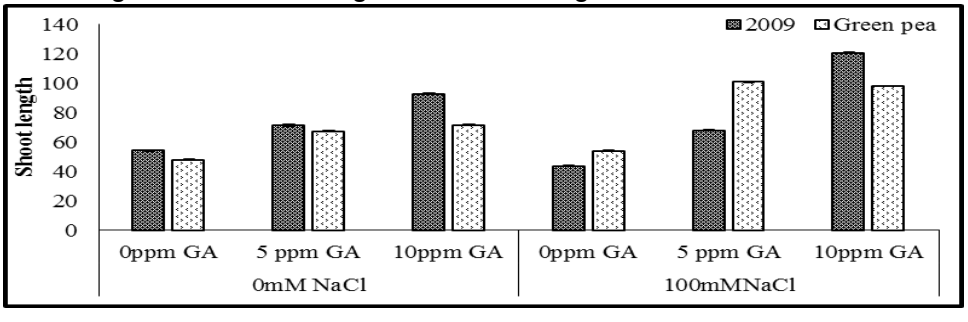

Figure 3: Shoot length of two cultivars germinate under NaCl salinity stress conditions with gibberellic acid foliar spray

Root and Shoot Fresh Weight (g)

Root and shoot fresh weights were significantly reduced in plants subjected to salinity without gibberellic acid treatment (Figures 4.4 and 4.5). Gibberellic acid at 10 ppm led to substantial improvements in both shoot and root fresh weights, particularly in Pea 2009. The observed increase may be due to improved water uptake and cell turgidity, as gibberellic acid promotes vascular development and delays senescence under salt stress (Seleiman *et al*. 2021). These findings corroborate the physiological basis of enhanced biomass accumulation in GA_3-treated plants.

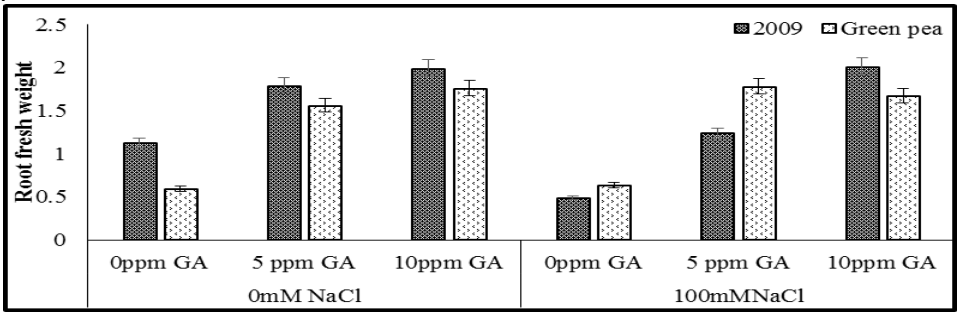

Figure 4: Root fresh weight of two cultivars germinate under NaCl salinity stress conditions with gibberellic acid foliar spray

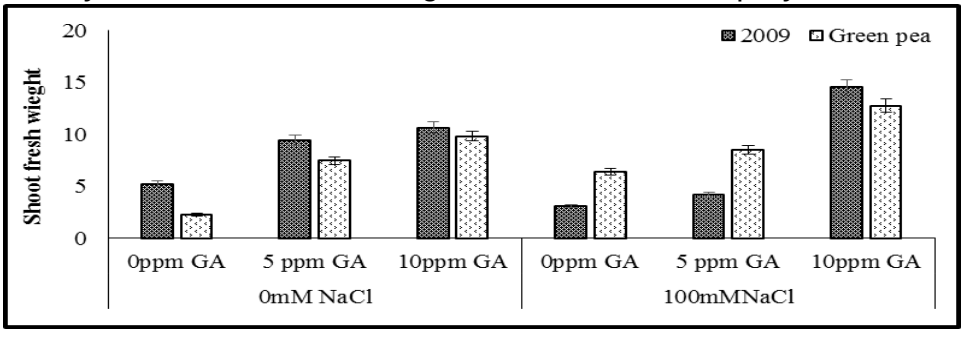

Figure 5: Shoot fresh weight of two cultivars germinate under NaCl salinity stress conditions with gibberellic acid foliar spray

Root and Shoot Dry Weight (g)

Dry weights followed trends similar to fresh weights, with reductions under NaCl and recoveries upon gibberellic acid application (Figures 4.6 and 4.7). The highest root and shoot dry weights were recorded in Pea 2009 treated with 10 ppm GA_3 under non-saline conditions. These results are consistent with the findings of Sarwar *et al.* (2023), who reported improved dry biomass due to GA_3-mediated enhancement of nutrient assimilation and photosynthetic activity.

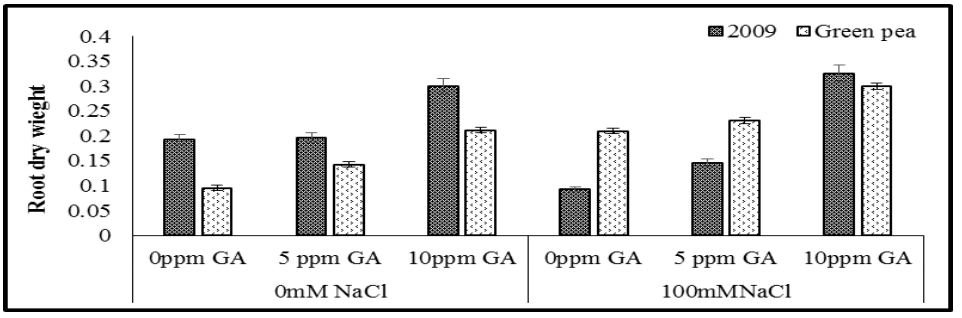

Figure 6: Root dry weight of two cultivars germinate under NaCl salinity stress conditions with gibberellic acid foliar spray

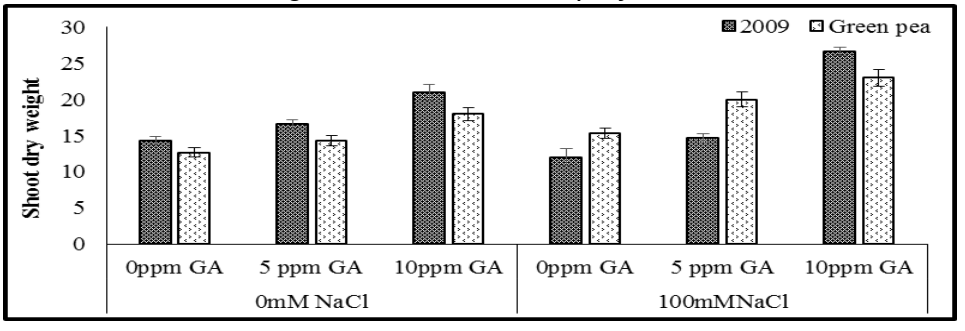

Figure 7: Shoot dry weight of two cultivars germinate under NaCl salinity stress conditions with gibberellic acid foliar spray

Chlorophyll a and b Content (mg/g FW)

Salt stress significantly reduced both chlorophyll a and b contents in untreated plants, indicating damage to the photosynthetic apparatus (Figures 4.8 and 4.9). However, application of gibberellic acid increased chlorophyll concentrations, with 10 ppm treatment showing the most pronounced effect. Pea 2009 exhibited higher pigment retention than Green Pea under all treatments. The increase in pigment content may be attributed to GA_3-mediated upregulation of chlorophyll biosynthesis enzymes and protection against chlorophyllase activity, aligning with the findings of Muhammad *et al.* (2021) and Sherin *et al.* (2022).

Carotenoid Content (mg/g FW)

Carotenoids serve as antioxidants and photoprotective pigments. As shown in Figure 4.10, carotenoid content declined under salinity stress but improved with gibberellic acid treatment. The highest accumulation occurred in Pea 2009 under 10 ppm GA_3 and non-saline conditions. This improvement suggests that gibberellic acid enhances antioxidant capacity, which supports the work of Rady *et al.* (2021) and Salehi-Sardoei *et al*. (2024), who noted increased carotenoid content as a stress-buffering mechanism in hormone-treated plants.

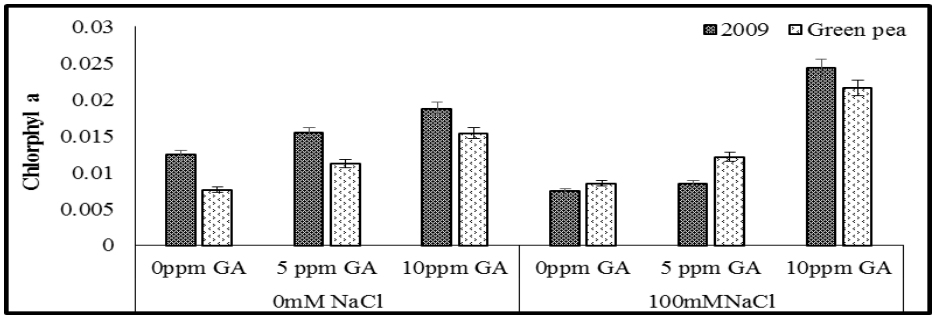

Figure 8: Chlorophyll b of two cultivars germinate under NaCl salinity stress conditions with gibberellic acid foliar spray

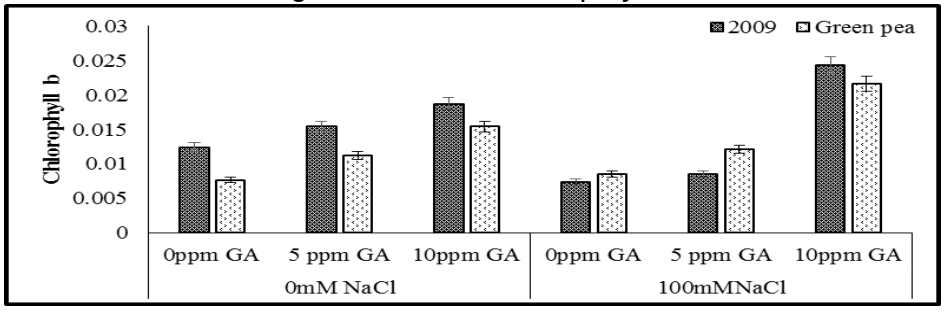

Figure 9: Chlorophyll b of two cultivars germinate under NaCl salinity stress conditions with gibberellic acid foliar spray

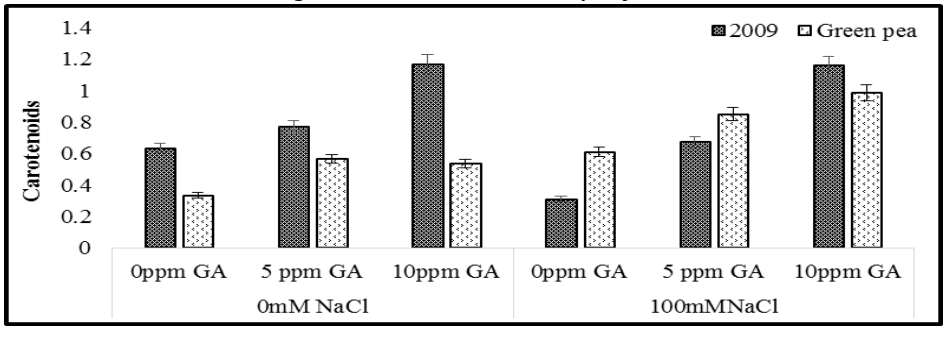

Figure 10: Carotenoids of two cultivars germinate under NaCl salinity stress conditions with gibberellic acid foliar spray

Comparative Performance of Cultivars

Overall, Pea 2009 outperformed Green Pea across all morphological and physiological parameters. This suggests a stronger genetic potential for stress tolerance and hormonal responsiveness. These cultivar differences may be due to variations in root architecture, hormone sensitivity, or osmotic regulation efficiency, as reported by Wahab *et al.* (2022).

Conclusion

The present study demonstrates that gibberellic acid significantly improves the morphological and physiological performance of pea (*Pisum sativum* L.) under salt stress. Foliar application of gibberellic acid, particularly at 10 ppm, effectively mitigated the adverse effects of 100 mM NaCl on shoot and root growth, biomass accumulation, and chlorophyll and carotenoid content. Among the two cultivars tested, Pea 2009 exhibited superior tolerance to salinity and a stronger response to gibberellic acid application compared to Green Pea. These findings suggest that gibberellic acid can serve as a practical and low-cost strategy to enhance pea growth and productivity in salt-affected soils. Future field-based trials and molecular studies are recommended to validate these findings and further explore the underlying mechanisms of hormonal regulation under salinity stress.

Author's contribution statement

Komal Shahzadi conducted the experimental work, collected and analyzed the data, and prepared the initial draft of the manuscript. Muhammad Sajid Aqeel Ahmad provided guidance throughout the experimental design, supervised data interpretation, and contributed to the scientific development of the study. Alia Riffat and Wasifa Ranicritically reviewed, corrected, and formatted the manuscript, ensuring clarity, consistency, and compliance with academic standards. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest Statement

The authors declare no conflict of interest.

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