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Responses of dragon fruit (*Selenicereus undatus*) to NaCl-Induced salinity stress

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ABSTRACT

Dragon fruit, an obligate CAM species, demonstrates high water efficiency and drought resistance. A pot experiment assessed its response to saline conditions using water with 0, 25, 50, 75, and 100 mM salt concentrations. Under salinity stress, dragon fruit showed changes in shoot and root growth. New shoot production was stable up to 75 mM salinity, with a slight reduction at 100 mM. Compared to the control, shoot production decreased by 13.33% at 75 mM and 33.33% at 100 mM. Increasing salt stress reduced total plant fresh weight, with the highest and lowest values at 0 mM and 100 mM, respectively. A similar trend was observed for plant dry weight, peaking at 0 mM and lowest at 75 mM. Salinity stress significantly decreased chlorophyll content and NDVI in dragon fruit. Plant mortality varied with salinity, reaching 40% at 100 mM and 20% at 50 mM. Salt stress also delayed cutting sprouting by 4-10 days. Higher salinity levels reduced shoot and root biomass, though new shoot formation persisted up to 75 mM, and shoot girth remained unaffected. Notably, root elongation occurred under saline conditions. While salt stress negatively impacted some growth aspects, other indicators showed positive responses. Therefore, investigating genetic variability within dragon fruit populations to identify salt-resistant genotypes is essential.

Keywords: Chlorophyll, mortality, root biomass, salinity, stress tolerance,

INTRODUCTION

Salinity, a significant abiotic stress factor, profoundly affects agriculture and human development globally. Rising salinity threatens arable land, agricultural productivity, plant growth, biodiversity, freshwater wetlands, land and water resources, and ecosystem functions, potentially leading to desertification (Herbert *et al*., 2015; Canedo-Arguelles *et al*., 2013). Climate change, characterized by global warming and altered precipitation patterns, exacerbates salinization, expanding saline soil areas due to population growth and development. Increased temperatures enhance evaporation, raising soil salt concentrations, while changes in rainfall patterns may prevent adequate salt leaching or cause waterlogging, further contributing to soil salinization (Bannari and Al-Ali, 2020; Hassani *et al*., 2020). Population growth and continuous development exacerbate the problem. Intensive farming methods, such as irrigation without proper drainage, lead to waterlogging and increased saline groundwater reaching the soil surface. Irrigating with saline water worsens this issue. Urban development disrupts natural water flow and drainage, causing soil salt build-up. Deforestation and land use changes reduce natural salt leaching, heightening salinization risks. Salinization is categorized as natural or anthropogenic. In arid and semi-arid regions, natural or primary salinization occurs due to low precipitation and high evaporation rates. Anthropogenic or secondary salinization results from human activities like excessive irrigation, poor drainage, and using low-quality water for irrigation (Bannari and Al-Ali, 2020; Ahamad *et al.,* 2023). Consequently, soil salinity, saline groundwater, and water scarcity due to limited resources or declining quality from salinization pose serious challenges in these areas. These issues significantly affect water quantity and quality globally and in India, impacting plant growth and development (Pandey *et al.*, 2014; Sarkar *et al.*, 2024).

Salt stress negatively impacts plant growth and development through multiple mechanisms, including osmotic stress reducing water availability, ionic stress from ion toxicity, nutritional imbalances, and oxidative stress from reactive oxygen species (ROS) (Carillo *et al.*, 2011; Zhou *et al.*, 2024). Specifically, salinity stress causes water deficits and harmful Na^+ and Cl^- ion accumulation in cells, leading to physiological and biochemical changes such as reduced chlorophyll, leaf water content, photosynthesis, respiration rates, and carbohydrates, while sometimes increasing proline and polyamines. These changes collectively impair plant growth and development (Shafieizargar *et al.*, 2015; Alam *et al.*, 2020). High salt concentrations near the roots can severely hinder plant growth and development.

Dragon fruit, an obligate CAM plant, demonstrates exceptional water use efficiency and drought resistance (Wang *et al.*, 2019). Some studies highlight its ability to thrive in high-salinity environments, while others note sensitivity to salt stress. Dragon fruit shows potential for growth in saline environments, but its sensitivity to salinity in certain scenarios necessitates further research and careful cultivation practices. The inconsistent results regarding its performance under salt stress highlight the need for comprehensive studies to clarify these findings. Despite its drought resistance reputation, limited research has explored its ability to thrive in high-salinity conditions, resulting in a lack of data on saline water irrigation or cultivation in salt-affected soils within arid and semi-arid regions. The exact salinity tolerance thresholds for dragon fruit remain unclear or poorly documented, with divergent claims from previous studies emphasizing the need for further investigation, particularly in regions facing salinity challenges. Earlier studies classifying it as salt-sensitive were based on seedling observations, which are generally considered less robust than commercial cuttings (Kakade *et al.*, 2019; Kakade *et al.*, 2024), underscoring the importance of conducting additional research on dragon fruit plants propagated through stem cuttings. Although some genotypes have shown favourable responses to salinity (Barcenas-Abogado *et al.*, 2002; Ortiz *et al.*, 2014), comprehensive examinations of responses under salt stress are crucial for more successful cultivation. This study was conducted to evaluate the morphological alterations and assess the physiochemical changes in dragon fruit cuttings exposed to various salinity levels.

MATERIALS AND METHODOLOGY

A pot experiment was conducted from January 2023 to May 2024 at ICAR-National Institute of Abiotic Stress Management, Baramati, India. During the experiment, monthly mean temperatures ranged from 21.8 to 30.2 °C, with the maximum temperature peaking at 38.6 °C in April and the minimum temperature ranging from 13.8 (January) to 22.3 °C (May). Morning relative humidity varied between 59 (April) and 85% (January). Local white dragon fruit cuttings, representing *S. undatus* are subjected to NaCl-induced salinity stress. Mature, disease-free cuttings (25 ± 4 cm long, 15 ± 2 cm girth) were taken from the mother block orchard, kept in shade for ten days, and treated with 0.25% copper oxychloride. Cuttings were potted in a mixture of black soil and well-decomposed farmyard manure, with each pot receiving fertilizers biweekly. The potted cuttings were irrigated with water containing different salt concentrations (0, 25, 50, 75, and 100mM) prepared using laboratory-grade NaCl. From January to March, cuttings were irrigated weekly with 500 ml of water per pot, which increased to 750 ml per pot per week in April and May. New sprouts, the girth of new cladodes (cm), total shoot length (cm), total plant biomass (g), primary root length (cm), shoot-to-root ratio, plant mortality (%), and days to sprout cuttings were measured. Chlorophyll content (µg ml^{-1}) was extracted using N, N-dimethylformamide (Inskeep and Bloom, 1985) and absorbance was measured at 647 and 664 nm. Normalized difference vegetation index (NDVI) was determined using a green seeker. Cladode moisture content (%) by oven drying the cladode samples. The membrane stability index (MSI) was measured using the protocol developed by Sairam (1994). Water use efficiency (WUE) was also assessed by dividing biomass produced per litre of water applied. Soil EC and pH were measured by mixing 10 g of soil with 25 ml of distilled water, stirring, and using a pH meter and EC meter. Soil moisture was determined gravimetrically. The study employed a completely randomized block design with five salinity levels, replicated four times with four cuttings per replication. Morphological, physiological-biochemical, and soil chemical properties, data were collected. Two-way ANOVA and least significant difference tests were conducted using 'R' studio (Versions 4.1.1 and 1.4.1417; $P < 0.05$).

RESULTS AND DISCUSSION

Dragon fruit exhibited notable alterations in shoot and root development when exposed to salinity stress. New shoot production remained stable up to 75 mM salinity, with a slight decrease at 100 mM. Compared to the control, shoot production declined by 13.33% at 75 mM and 33.33% at 100 mM (Fig. 1A). Salinity stress also impacted shoot length, with the longest shoots observed at lower salinity levels. At 75 mM and 100 mM, shoot length decreased by 28.74 % and 17.96 %, respectively, relative to the control (Fig. 1B). However, shoot girth was not significantly affected, ranging from 10.43-11.47 cm across treatments (Fig. 1A). As salinity stress intensified, total plant fresh weight decreased, with the highest and lowest values recorded at 0 mM and 100 mM, respectively. A similar pattern was observed for total plant dry weight, with the maximum at 0 mM and minimum at 75 mM (Fig. 1B). The shoot-to-root ratio was highest at 0 mM salinity, comparable at 25 and 100 mM, and reduced at 50 mM and 75 mM. Plant mortality varied under different salinity conditions, with 40% mortality at 100 mM and 20% at 50 mM (Fig. 1A). Salinity stress also delayed sprouting in cuttings by 4-10 days (Fig. 1A). Salinity stress adversely affects crop growth and development (Anjum, 2008; Kakade *et al.*, 2014). Research on the salinity response of dragon fruit is sparse, with some studies labeling it as salt-sensitive and others identifying it as salt-tolerant. This inconsistency necessitates further investigation considering drought tolerance, clonal propagation robustness, and genotypic diversity (Wang *et al.*, 2019; Tomaz de Oliveira *et al.*, 2020), potentially revealing genotypes with salinity tolerance. The findings of this study are vital for promoting dragon fruit cultivation in saline environments. In this study, salinity stress reduced the shoot and root biomass of dragon fruit but promoted root elongation. Previous studies by Cavalcante *et al.* (2007) and De Sousa *et al.* (2021) reported adverse effects of salinity on the growth metrics and biomass of dragon fruit seedlings. Pandey *et al.* (2014) have observed that salinity has resulted in to decrease in plant growth, leaf production and leaf area in mango grown under salinity. Furthermore, they also observed a decrease in the fresh and dry weights of plants with increasing salinity. Irrigation with 75, 100, and 150 mM salt resulted in shorter plants, decreased stem width, lower plant dry weight, fewer flowers, smaller leaf area, and reduced fruit yield in cherry tomatoes (El-Mogy *et al*., 2018). These observations are consistent with our findings. Salinity stress alters water relations, causing osmotic stress and water deficits, and affecting cell turgor and water use efficiency (Munns, 2002). Water stress affects growth during short-term exposure and induces stomatal closure, reducing CO_2 availability and photosynthetic efficiency (Chauhan *et al*., 2023). Combined osmotic and ionic stress leads to excessive reactive oxygen species (ROS) production, causing oxidative damage to macromolecules and redox imbalance (Kesawat *et al*., 2023). Therefore, the combination of these stresses affects physiological and biochemical processes, such as photosynthesis and protein synthesis, ultimately impairing plant growth and development (Dexana *et al*., 2022). In the present study, salinity stress affected growth parameters, but shoot length and shoot girth remained unaffected, indicating some positive responses of dragon fruit to salt stress. Dragon fruit is known for its drought tolerance and ability to grow in degraded lands, and has mechanisms to combat various stresses (Jinger *et al*., 2024). Identifying specific genotypes and responses is crucial for assessing the performance of dragon fruit under salinity stress. Interestingly, root length increased under salinity stress compared to the control.

The greatest root length was observed at 75 mM salinity, which was comparable to other treatments, while the control showed the shortest roots. Root length increased by 18.19 % to 30.06 % across treatments compared to the control (Fig. 2A). The highest below-ground fresh biomass was recorded at 50mM salinity, similar to other treatments except for 100mM (Fig. 2A). No significant differences were found in below-ground dry biomass. Roots, the primary organs encountering salt stress, undergo changes similar to shoots, but play a more crucial role in adaptation. The effects of salt stress on roots include decreased quantity, length, and biomass, as reported in previous studies (Cavalcante *et al*., 2007; Alam *et al*., 2020; De Sousa *et al*., 2021). Our study noted reduced root biomass under salt stress, although root elongation increased with increasing stress levels. Enhanced root biomass and elongation may improve water uptake and performance under stress conditions (Zou *et al*., 2022). Thus, dragon fruit plants attempt to grow deeper roots to mitigate salt stress. However, another hypothesis suggests that increased root mass might lead to greater absorption of harmful ions, intensifying salt stress (Fernández-Ballester *et al*., 2003). Glycophytes show reduced growth and yield, and halophytes thrive and reproduce under saline conditions. In the present study, although plants showed potential salt tolerance, plant mortality questioned their tolerance capacity. Plant survival is an important indicator of salt tolerance (Munns, 2002). Goodman *et al*. (2012) found soil salinity 1.5 times higher where tree cactus plants were dead compared to where they were alive. Consequently, white-fleshed dragon fruit plants failed to sustain growth as stress severity increased.

The chlorophyll content in dragon fruit significantly decreased with salinity stress. The highest chlorophyll content (4.68 to 5.66 µg/ml) was observed at lower salinity concentrations, followed by the control. It decreased to 3.64 µg/ml at 75 mM and 3.49 µg/ml at 100 mM salinity stress (Fig. 2B). Similar trends were noted for chlorophyll b and total chlorophyll content. The highest NDVI (0.55) was reported in the control, decreasing under various salinity stresses, with the lowest NDVI (0.32) observed at 100 mM (Fig. 2B). Water use efficiency (WUE) was found highest in the control treatment, showing minor reductions under various salinity stress conditions. Salinity stress led to a decrease in WUE by 22.00 to 37.03% across different treatments (Fig. 2B). Under salinity stress, cladode moisture content remained relatively stable, ranging from 87.48 to 89.84 %, with a slight decrease to 84.4 0% at 100mM salinity stress (Fig. 3A). Membrane stability index (MSI) peaked at lower salinity stress levels and in the control, while reaching its lowest point in the 75 and 100 mM salinity stress treatments (Fig. 3A). Previous studies have reported that salinity decreases plant growth, net photosynthetic rate, stomatal conductance, transpiration rate, water use efficiency and chlorophyll concentration in avocado (Musyimi *et al*., 2007). Al-Gaadi *et al*. (2024) also reported reduced stomatal conductance, photosynthesis, leaf chlorophyll content under salinity stress. They observed variations of 5%, 9%, and 5% in photosynthesis, stomatal conductance, and chlorophyll content of leaves, respectively, between medium- and high-salinity trials. This decrease might be attributed to the inhibition of enzymes, impairment of pigment protein complexes, Fe^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+} deficiency, and chlorophyll pigment destruction under salt stress (Gao *et al*., 2024). High salt concentrations in soil disrupt the water extraction capacity of roots from soil because of reduced soil water potential, which causes difficulties in the uptake of water by plants, eventually leading to physiological drought conditions (Lu and Fricke, 2023), resulting in reduced cladode moisture. However, this reduction was marginal, which may be because dragon fruit is a cactus, have succulent and waxy stems, and keeps stomata closed during day time, which helps in storing water for longer periods under both normal and stressful periods (Nobel and De la Barrera, 2002). Degradation of chlorophyll content and deficit water-induced stomatal closure might have resulted in the overall reduction in photosynthesis and thus reduced NDVI under salinity stress in the present experiment.

Salinity stress significantly affected soil pH, with higher salinity levels corresponding to increased soil pH. The control group exhibited the lowest soil pH at 7.49, while the 100mM salinity stress treatment resulted in the highest pH of 8.05 (Fig. 3B). Soil electrical conductivity (EC) followed a similar pattern, with the control group showing the lowest EC at 1.50 dS/m and the 100 mM salinity stress treatment producing the highest EC at 9.49 dS/m (Fig. 3B). Irrigation with higher salt concentrations enhanced the soil pH and EC compared to the control because the presence of NaCl increases the soil pH and dissociation of NaCl into Na^{+} or Cl^{-} when dissolved in water, enhancing the ability to conduct electricity, which enhances the EC of the soil and makes the soil saline. Thus, increased soil pH and EC could be associated with Na^{+} and Cl^{-} ion exclusion mechanisms and the retention of these ions in the soil. Salinization due to greater accumulation of either Na^{+}, Cl^{-}, or both, in the root zone or plant cells leads to deficiencies or toxicities of nutrients and disturbances in ion homeostasis, ultimately affecting plant growth and development (Munns, 2002; Munns and Tester, 2008).

CONCLUSION

The current research revealed significant changes in dragon fruit's shoot and root development when subjected to salt stress. As salinity levels increased, the plants demonstrated decreased shoot and root biomass production. Nevertheless, new shoot formation persisted up to 75 mM salt concentration, and shoot girth remained unaffected by salt stress. Interestingly, root elongation was observed under saline conditions. While salt stress negatively impacted certain growth and developmental traits of dragon fruit plants, some growth parameters showed positive responses. Consequently, there is a need to explore the genetic diversity within dragon fruit populations to identify salt-tolerant genotypes or varieties. This approach could potentially enable the expansion of dragon fruit cultivation into saline environments.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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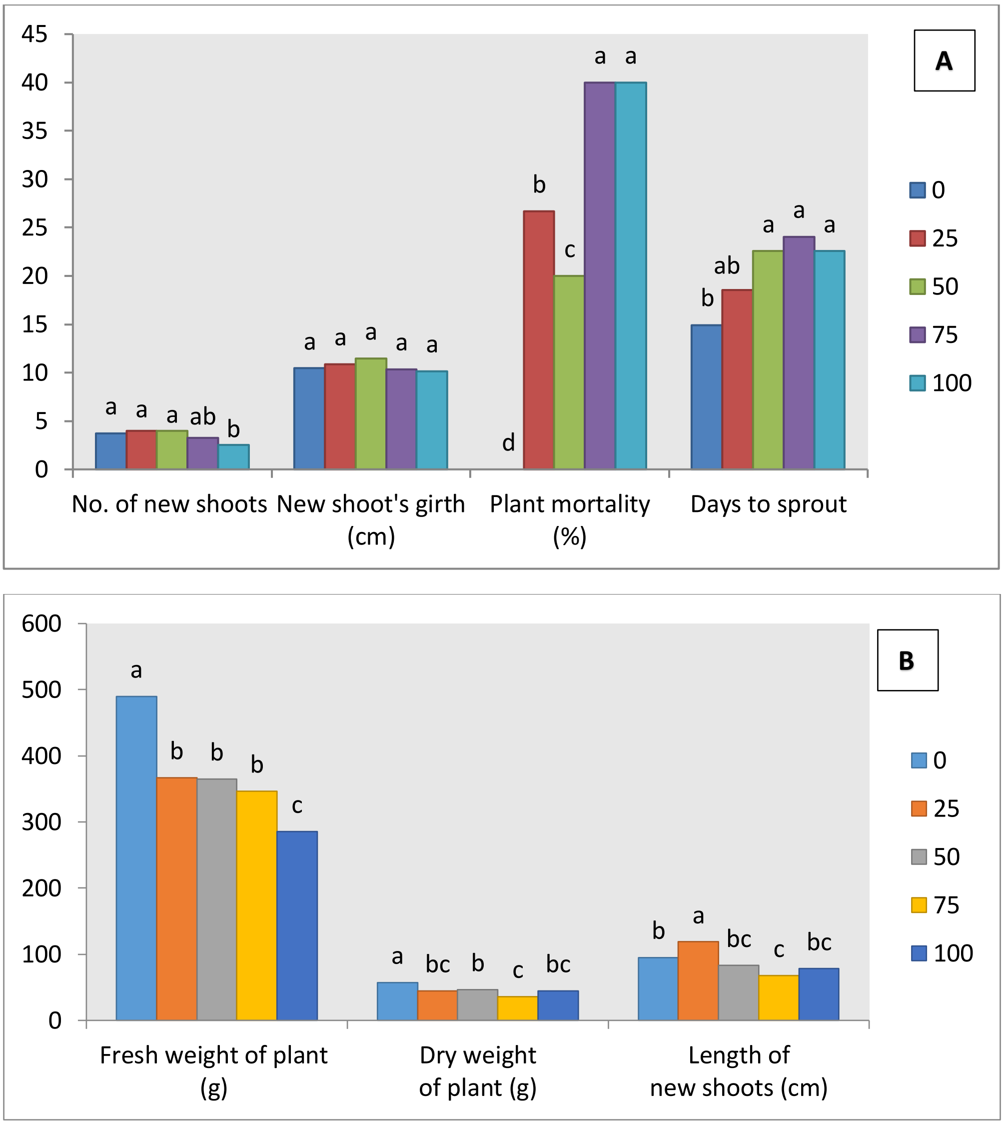
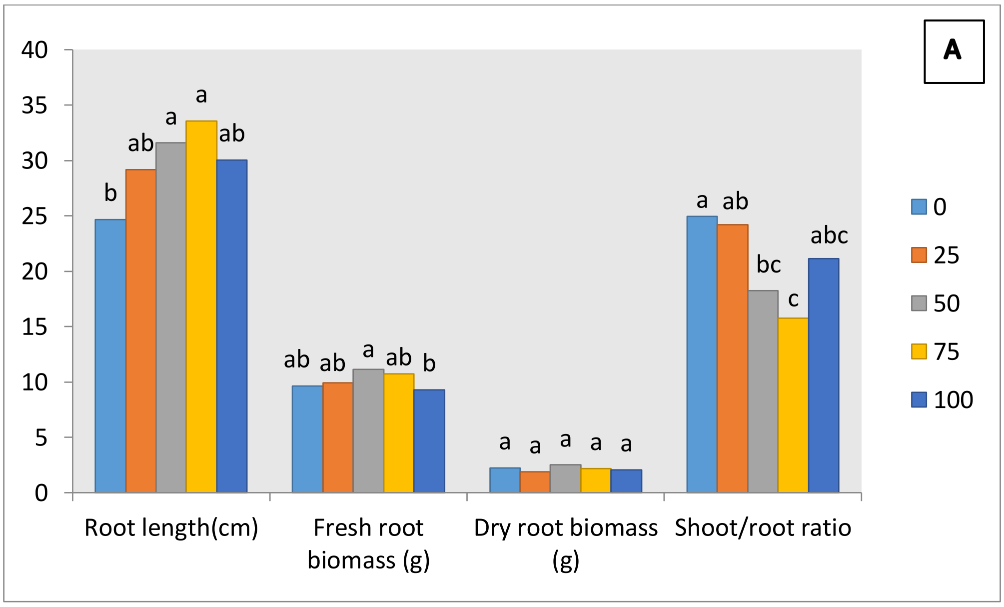


Fig. 1: Effect of different saline water levels on different plant growth traits (A & B) of dragon fruit



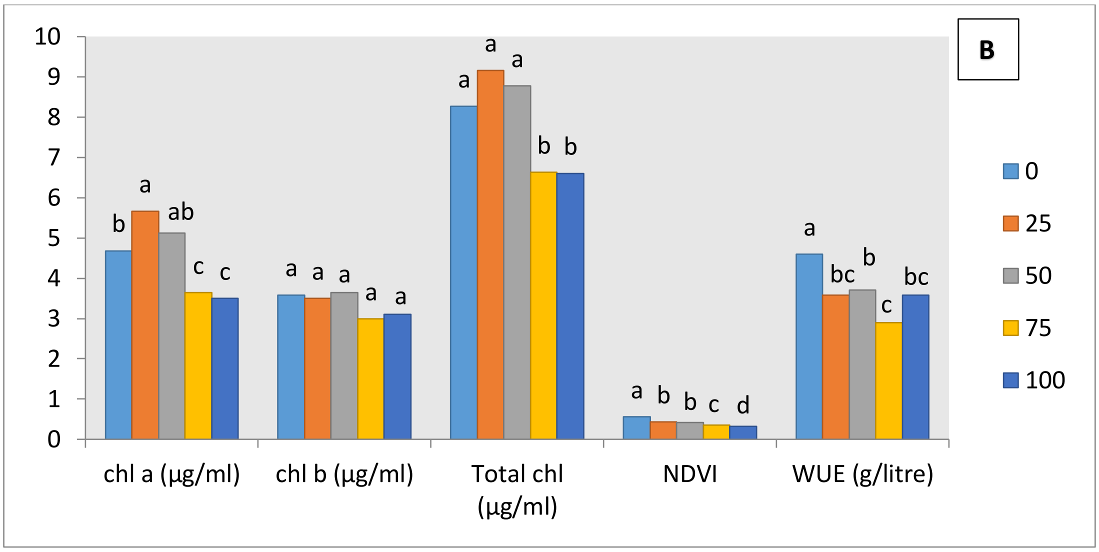


Fig. 2: Effect of different saline water levels on root growth (A) and physiological traits (B) of dragon fruit

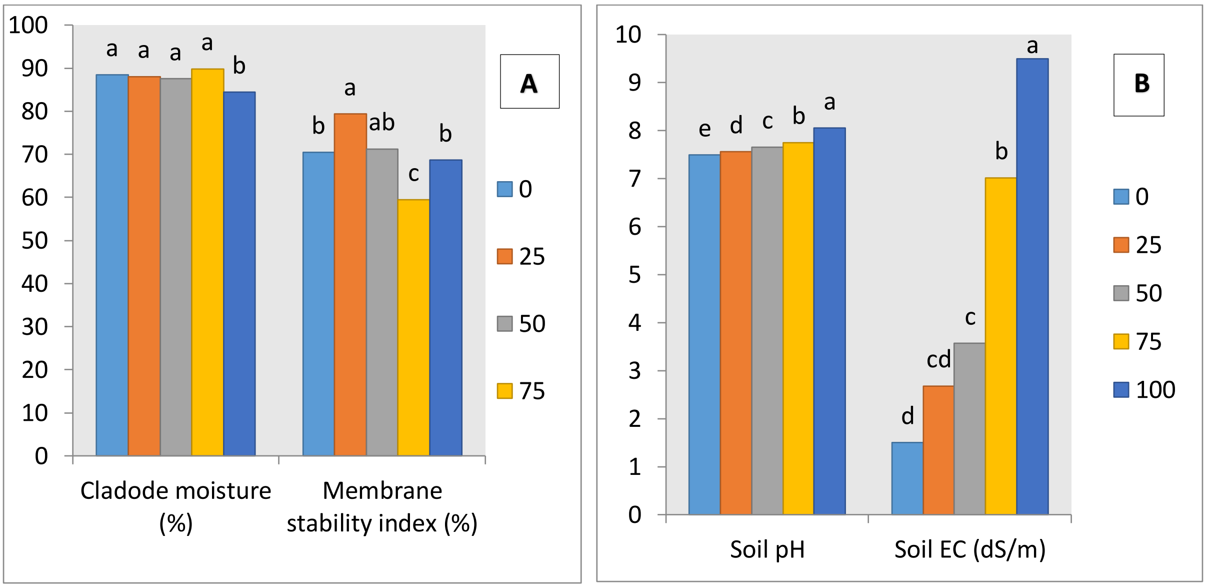


Fig. 3: Effect of different saline water levels on cladode moisture and membrane stability index (A), and soil pH and EC (B) of dragon fruit