doi:10.14720/aas.2025.121.2.13627 Original research article / izvirni znanstveni članek

# Evaluation of salinity tolerance in seedlings of *Hyppeastrum reticulatum* (L'Hér.) Herb.

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Received May 23, 2023, accepted April 16, 2025
Delo je prispelo 23 maj 2023, sprejeto 16. april 2025

**Evaluation of salinity tolerance in seedlings of *Hyppeastrum reticulatum* (L'Hér.) Herb.**

**Abstract:** Amaryllis (*Hyppeastrum* Herb.) is one of the bulbous ornamental plants that is distributed around the world. Regarding the cultivation of ornamental plants in landscaping, it is essential to use salinity-resistant ornamental species. Less research has been done on the impact of salt irrigation on the growth and development of bulbous ornamental plants like this plant. So, in order to investigate salinity tolerance in amaryllis, the experiment was done with five salinity concentrations [control (distilled water) with EC = 0dSm$^{-1}$, and electrical conductivity (EC) at 2, 4, 6 and 8 dSm$^{-1}$] with four replication on leaf freshness, leaf length and width, proline, nitrogen (N), potassium (K), phosphorous (P) content, and peroxidase enzyme activity. Results showed that increasing salinity led to decreased leaf nutrients and growth parameters like plant height, shoot mass, leaf length, width, mass, and corm mass, and increased proline and peroxidase activity.

**Key words:** leaf, nitrogen, phosphorous, potassium, proline, peroxidase

**Ovrednotenje sejancev križancev amarilisa (*Hippeastrum reticulatum* (L'Hér.) Herb. na slanost**

Izvleček: Amarilis (*Hippeastrum* Herb.) je ena izmed čebulastih okrasnih rastlin, ki je razširjena po vsem svetu.. Pri uporabi okrasnih rastlin v ozelenjevanju je pomembno, da se uporabljajo na slanost odporne okrasne rastline, pri čemer je bilo opravljenih le malo raziskav o vplivu zalivanja s slano vodo na rast in razvoj čebulastih okrasnih rastlin. Z namenom preučiti toleranco amarilisa na slanost je bil izveden poskus s petimi slanostmi in štirimi ponovitvami. Obravnavanja s slanostmi so bila: kontrola (distilirana voda) z električno prevodnostjo EC = 0dSm$^{-1}$ in obravnavanja z električno prevodnostjo (EC) 2, 4, 6 in 8 dSm$^{-1}$.V rastlinah so bili ocenjeni/izmerjeni naslednji parametri: svežost listov, dolžina in širina listov, vsebnost prolina, dušika (N), kalija(K), fosforja in aktivnost peroksidaze. Rezultati so pokazali, da se je s povečevanjem slanosti zmanjševala vsebnost hranil v listih, zmanjševali so se tudi rastni parametri kot so višina rastlin, masa poganjkov, dolžina, širina in masa listov, masa celotnih rastlin, povečali sta ste vsebnost prolina in aktivnost peroksidaze.

**Ključne besede:** nadzemni del rastline, list, dušik, fosfor, kalij, prolin, peroksidaza

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## 1 INTRODUCTION

Amaryllis (*Amaryllis* Herb.) is one of the bulbousornamental plants that is distributed around the world. This plant belongs to the Amaryllidaceae family and *Hippeastrum* genus. Amaryllis (*Hippeastrum x hybridum* Hort.) are used as flowering plants, pot plants, and cut-flowers or limitedly in landscape designing. In Iran, they are mostly grown in the northern regions (the provinces of Mazandaran, Gilan, and Golestan). In Persian, amaryllis is called *Nasrin* (Azimi, 2024)

Salinity is an abiotic stress that usually occurs in semi-arid and arid areas, influencing plant growth and agricultural productivity (Porcel et al., 2012). Ionic toxicity is caused by an accumulation of Na+ and Cl- ions at high salt concentrations, which harms plant growth and development and interferes with the uptake of potassium, phosphorus, calcium, and nitrogen ions, leaving the plant with insufficient quantities of those components (Ulczycka-Walorska et al., 2020) and causing physiological changes (Fatma et al., 2016). As a result, these physiological changes reduce cell division, expansion, or promotion of cell death and induce a decrease in growth rate and yield. They also destroy chlorophyll in leaves, which leads to leaf senescence (Rahemi et al., 2017).

Additionally, it was mentioned that osmotic stress is induced by an increase in sodium and chlorine ions. Furthermore, it was mentioned that oxidative stress (secondary stress) is brought on by an increase in reactive oxygen species (ROS), such as superoxide, hydroxyl radicals, and peroxide, which are ROS that have a negative impact on normal cell growth and metabolism (Aroca et al., 2013).

It is essential to use ornamental species that are tolerant to increased salinity or to develop a resistance trait through plant breeding and physiological techniques when growing attractive plants for landscaping (Bayat et al., 2013). This researcher also reported that the flower number and diameter of *Gerbera aurantiaca* Sch. Bip. exposed to salinity decreased compared to control plants.

The references state that compared to other horticultural products, less research has been done on how salt irrigation affects the growth and development of ornamental plants, particularly bulbous plants. Therefore, due to the salinity problem, which is considered a limiting factor for landscape development, the physiological and morphological study of *Hippeastrum* is important.

## 2 MATERIALS AND METHODS

The seeds of amaryllis (*Hyppeastrum reticulatum* (L'Hér.) Herb.) were obtained from the "Ornamental Plants Research Center (OPRC) of Mahallat, Iran". The seeds were cultivated in a cultivation tray and kept in a greenhouse with 70 ± 5 % relative humidity and 25 ± 5 °C conditions. The seedlings were transplanted at the three-leaf stage into the pots. Then, the uniform seedling genotypes were selected and transplanted into the pots filled with loamy soil, rotten animal manure, and compost (1:1:1); then transferred to open space. The experiment consisted of five salinity concentrations [control (distilled water) with EC = 0 $dSm^{-1}$, and electrical conductivity (EC) at 2, 4, 6, and 8 $dSm^{-1}$] with four replicas. In order to make experimental solutions, 1.28, 2.56, 3.84, and 5.12 $g.l^{-1}$ of NaCl were used for EC = 2, 4, 6, and 8 $dsm^{-1}$.

For two months (July-August), salinity treatments were used twice a week. The volume of applied saline water was 300 ml for each treatment. To prevent salt accumulation, the pots were leached twice a week. Leaf freshness, leaf length and width, fresh and dry mass shoots, crown diameter, bulb mass, proline, nitrogen (N), potassium (K), and phosphorous (P) content, and peroxidase enzyme activity were measured.

### 2.1 MEASUREMENTS OF GROWTH

Digital callipers and rulers were used to measure leaf length and width, plant height, bulb diameter, and crown diameter. Fresh and dry shoots and crowns were assayed by digital balance.

Nitrogen, phosphorous, and potassium were measured using the Khejeldal device, a spectrophotometer and a falme photometer, respectively (Tekaya et al., 2014).

### 2.2 PEROXIDASE ACTIVITY

The Guaiacol technique was used to measure the peroxidase (POD) activity (Oraee et al., 2020). For three minutes, the variations in 470 nm absorbance were used to track how well guaiacol was being oxidised. 50 ml 100 mM PBS (pH 6.0), 19µl 30 % $H_2O_2$, 28µl guaiacol comprised the reaction mixture solution. The enzyme extract was added to the solution of the reaction mixture to begin the reaction.

The following equation was used to calculate POD activity:

*POD activity* ($\Delta A470/min{\cdot}g\ FM) = \Delta A470 \times VT / M \times VS \times t$. *$\Delta A470$: the changes of absorption; were VT: total volume of the extracted solution; VS: volume of enzyme solution for testing; M: the mass of samples*".

### 2.3 PROLINE CONTENT

The method developed by Oraee et al. (2020) was used to measure the proline content in the leaves. In 10 ml of 30 ml $l^{-1}$ sulfosalicylic acid, fresh leaves (1.0 g) from each of the four replications were homogenized and the extract was used to spectrophotometrically measure proline.

### 2.4 STATISTICAL ANALYSIS

Eight different seedling genotypes were planted in each of the three replicates of the experiment's factorial, complete randomised block design.

Using the SAS statistical programme, data were examined by variance mean comparison and the Duncan multiple range test.

## 3 RESULTS AND DISCUSSION

### 3.1 GROWTH CHARACTERISTICS

The highest and the lowest plant heights were related to control (36.97 cm) and EC = 8 dsm-$^{1}$ (22.33 cm) (Figure 1). By increasing salinity stress, plant height was reduced by 9.8, 18.87, 26.68, and 39.6 %, respectively. This trend was the same for other vegetative traits such as leaf number, width, and length (Figure 1). The highest decrease was obtained with EC = 8 dsm$^{-1}$ at 50, 44 and 25 % for leaf number, width, and length as compared to control, respectively. Plants treated with EC = 0 dsm$^{-1}$(control) to EC =8 dsm$^{-1}$ showed a decreasing trend in fresh and dry shoot mass, corm mass, and crown length in comparison with control (Figures 1 and 2). The highest and the lowest values were attributed to EC = 8 dsm$^{-1}$ and control in all the traits.

Reduced growth traits are one of the earliest impacts of salt stress on plants. According to Sarvandi et al. (2020), plants' reduced ability to absorb water as a result of osmotic stress brought on by salt is the reason why their leaf surface area is decreasing (Sarvandi et al., 2020). Additionally, it was claimed that the synthesis and transportation of hormones between roots and shoots are impacted by the absorption of chloride and sodium ions, which reduces leaf area and plant dry biomass and lowers specific leaf area (SLA). In addition to lowering leaf area (LA), salinity inhibits the growth of the root system, delays the production of apical buds, and induces chlorosis with subsequent necrosis on the leaf edge (Oliveira et al., 2017). Dry matter reduction under stress conditions has also been reported due to decreased leaf area index, photosynthesis rate, growth of aerial organs, and the relative growth rate of the plant (Soheili-Movahed et al., 2017). In response to elevated salt concentrations in *Poa pratensis* L., fresh and dry mass of roots and shoots decreased (Esmaeili and Salehi, 2016).

Vegetative growth, including leaf width and length, number of leaves, and number of shoots, decreased as the concentration of sodium chloride increased (Naseri Moghadam et al., 2020) and salinity stress has more detrimental effects than drought stress on the development, aesthetic, and physiological aspects of *N. tazetta* L. flowers (NaseriMoghadam et al., 2020). Regarding salinity's impacts on leaf area, salinity inhibits the root system, causes a large increase in Na+ content across all plant tissues with growth, delays in the development of apical buds, and raises the concentration of NaCl in the nutritional solution (Dlamini et al., 2019). A restriction in leaf expansion followed by a reduction in leaf area is one of the first signs of plants exposed to excessive salinity. It can be explained by alterations in the cells and a decline in leaf turgor. Reduced cell elongation and cell division cause leaves to appear more slowly and to grow to a smaller size in the end. Leaves become smaller and thicker as a result of a shift in cell size that reduces area more than thickness (Go´mez-Bellot et al., 2013).

According to our results, the growth characteristics decreased with the increased salinity. These findings were similar to above finding. This means that in ornamental plants, salinity stress reduces growth, flower size, flower turnover, and visual quality (Toscano et al. 2020). It is well known that salinity reduces photosynthesis and carbohydrate levels, which are useful for flower production and development. The consequence of this is a reduction in biomass accumulation, as observed in plants and flowers. These results were also observed and confirmed in amaryllis in our research (Trivellini et al., 2023). In fact, stunted growth is an adaptive mechanism for survival, which allows plants to combat salt stress. Salt stress might reduce the expression of key regulatory genes involved in cell cycle progression (e.g., cyclin and cyclin-dependent kinase), leading to decreased cell numbers in the meristem and a growth inhibition which impacts the plant's ability to absorb nutrients and water efficiently and to a lesser extent, cell division, is affected, resulting in a lower root and leaf growth rate. After the occurrence of salinity stress, the lateral shoot enlargement is affected, leading to apparent differences in overall growth. This response is due to changes in the cell–water relation resulting from osmotic changes outside the root. The osmotic effect leads to a reduction in the capability of plants to absorb water (Balasubramaniam et al., 2023).

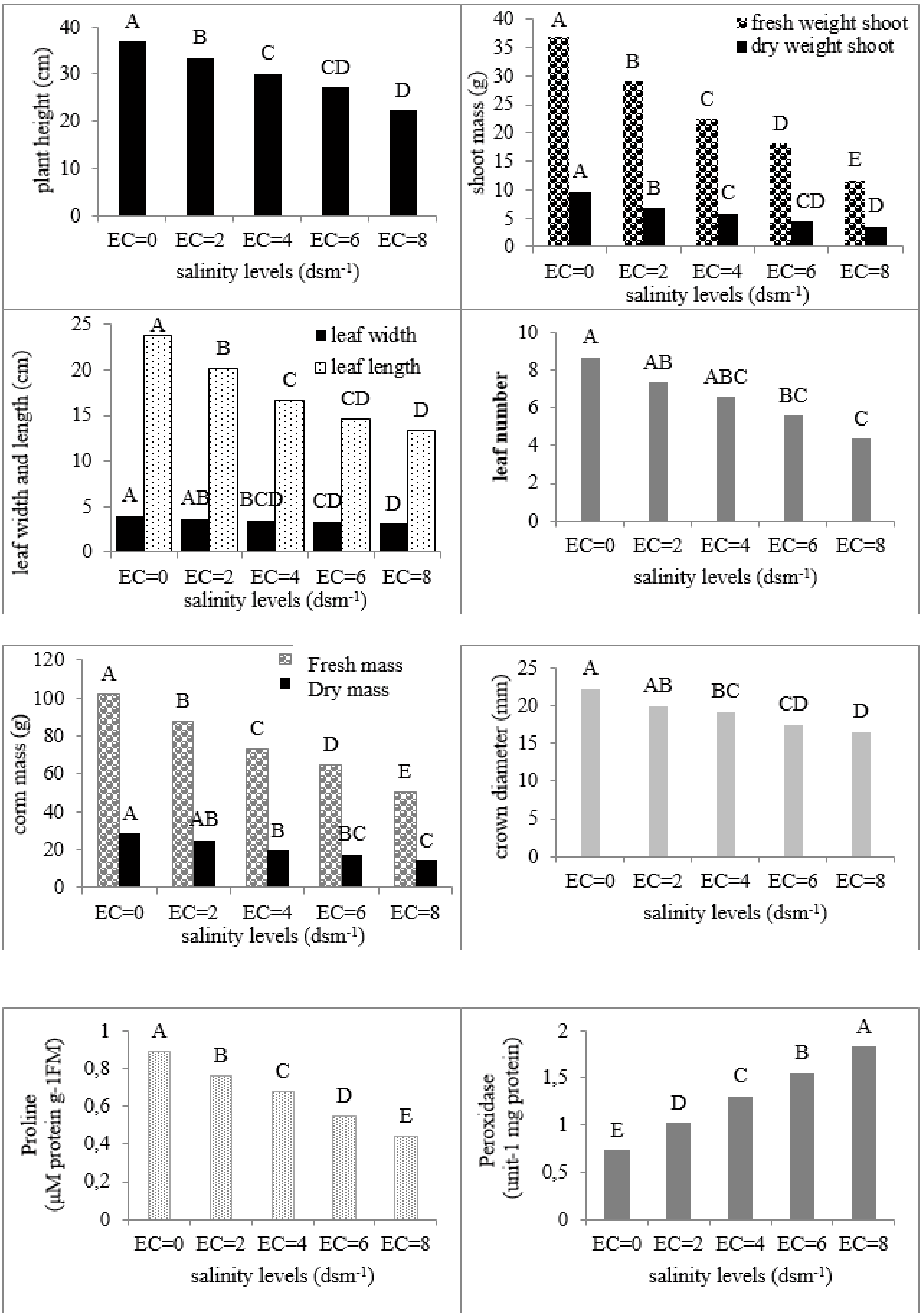


**Figure 1:** Effects of salt stress on morpho-physiological and biochemical traits

### 3.2 PROLINE CONTENT AND PEROXIDASE ACTIVITY

Considering proline and peroxidase contents in leaves, they increased by 102 and 151 % for EC = 8 $dsm^{-1}$ as compared to control, respectively (Fig 1).

Proline content increases when the water potential of the leaf decreases, which leads to the maintenance of cellular turgor and reduces the damage to the membrane in the plant; therefore, with osmotic adjustment, tolerance to water stress increases (Rahdari and Hosseini, 2012). It also serves as an enzyme and membrane protector, as well as a reservoir of energy and nitrogen for utilisation (García-Caparrósand Lao, 2018). Proline accumulation is a well-known adaptive mechanism in plants against salt stress. Additionally, because the rise in proline content may be positively linked with the degree of tolerance, proline accumulation has been proposed as a selection criterion for salt tolerance (García-Caparrósand Lao, 2018). The rate of proline synthesis depends on the development of stress, the age of the plant organ, and genetic variation (Bajji et al., 2001). The proline concentration changes at different levels of salinity showed that with increasing salinity, the proline content of genotypes increased (García-Caparrós et al., 2016).

### 3.3 PEROXIDASE ACTIVITY

The maximum peroxidase activity was present in plants tend to counteract the reactive oxygen species produced by stress (Kaya et al., 2013). Plants subjected to salt stress exhibited up-regulation of the antioxidant defense system (Hussain et al., 2016). According to these studies, salinity increased the activity of peroxidase enzymes in salt-sensitive cultivars. One of the typical responses of plants to saline circumstances is the acceleration of the production of reactive oxygen species (ROS), which include the lethal superoxide radical ($O_2$), singlet oxygen ($1O_2$), hydroxyl radical (OH-), and hydrogen peroxide ($H_2O_2$). Peroxisomes, chloroplasts, and mitochondria are the key cell components that generate ROS. These reactive oxygen species are involved in a variety of activities, including protein oxidation, lipid peroxidation, and DNA damage (Shams and Khadivi, 2023).

In order to overcome the negative effects of ROS at the cellular level, plants show a mechanism of scavenging of these species through the antioxidative machinery composed by enzymatic and non-enzymatic components such as superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POX) and catalase (CAT) (García-Caparrós and Lao, 2018).

Regarding the status of macro elements in leaves, nitrogen, phosphorous and potassium decreased with increase in salinity level by 56.7, 44 and 58 % as compared to control (Fig 2). These results were in agreement with Ulczycka-Walorska et al. (2020) who stated that high salt concentration in plants disturbs the absorption of potassium, phosphorus, calcium and nitrogen ions leading to insufficient levels of those elements in the plant. The results of our study were in agreement with previous studies that excess salt, restricting plants' ability to absorb water and minerals such as $K_+$ and $Ca^{2+}$ (Mircea et al., 2025).

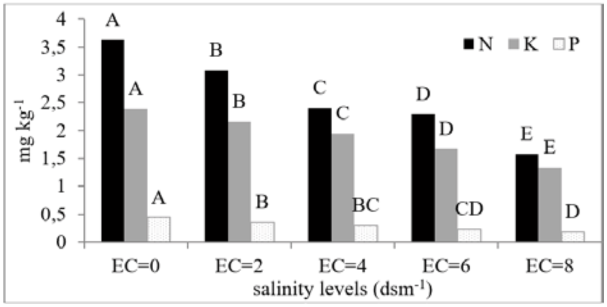


**Figure 2:** Effects of salt stress on leaf nitrogen, potassium and phosphorous

### 3.4 MULTIVARIATE ANALYSIS

In order to group the salinity levels based on increasing dissimilarity, a hierarchical agglomerative cluster assessment was performed (Fig 3). The first group

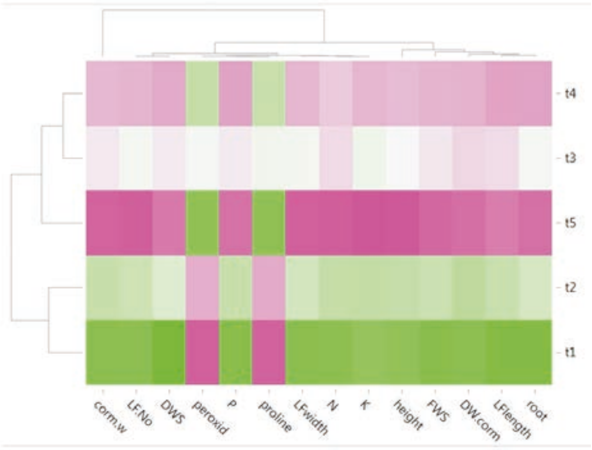


**Figure 3:** Cluster analysis of salinity stress on amaryllis based on physical and chemical properties of leaf (gradient from low (pink), white (medium) to high (green)).Abbreviations: corm.w: corm mass, LFNo: leaf number, DMcorm: Corm dry mass, FMS: leaf fresh mass, LFwidth: leaf width, LF: leaf length and t1 to t5: EC = 0,2,4,6 and 8 ds/m, respectively.

(Cluster I, Figure 5), which included t4 and t3, t4 had the highest levels of peroxidase and proline. The second cluster (Cluster II, Figure 5), which included t5, showed low values of leaf nutrients (N, P and K), corm, root, corm dry mass, leaf length, width, and number, and high values for proline and peroxidase. The third group (Cluster III) included t1 and t2, within this cluster, t1 had the highest vegetative traits and nutrient contents and the lowest values for proline and peroxidase

## 4 CONCLUSION

Amaryllis is described as a plant with low water requirements, with water surpluses being detrimental to the development of the crop. The level of tolerance to salinity in *Hippeastrum* hybrids showed that this ornamental plant was susceptible to EC. Increasing salinity led to decreased leaf nutrients and growth parameters like plant height, shoot mass, leaf length, width, mass, and corm mass, and increased proline and peroxidase activity.

**Acknowledgement:** The financial support of the Ornamental Plants Research Center is gratefully acknowledged.
**Statements & Declarations**
**Funding:** There is no funding with this work.
**Compliance with ethical standards (Conflict interest):**The authors declare that they have no conflict of interest.
**Author contributions:** Conceived and designed the experiments: Performed the experiments: Azimi, M.H, Khalaj, MA, Analyzed and wrote the paper: Sayyad-Amin P, Revised and edited the paper: Hosseinpour, N

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