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The effect of natural salinity on seed germination, seedling establishment growth and selected biochemical properties of *Stevia rebaudiana* Bertoni

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Abstract

In dry and semi-arid climates, soil and water salinity reduces plant development and productivity. Due to salty land development and loss of arable land, identifying therapeutic plants resistant to salinity or substances that decrease its effects is crucial. This study examined the seed germination, seedling establishment, and antioxidant potential of a novel *Stevia rebaudiana* (*Stevia* UEM-13) variety to natural salinity. We also examined how salinity affected biochemical and physiological changes in two separate experiments to a CRD with four replications in laboratory and greenhouse. Five degrees of salinity treatments were examined in the natural saltwater Lake of Qom: Control, 4, 8, 12, and 16 ds.m⁻¹. The germination percent, root and shoot seedling dry weight, biochemical properties (medicinal content), and antioxidant enzyme activity were measured. Results indicate considerable impact of salt stress on germination, growth, biochemical, and physiological alterations ($P \leq 0.01$). Increasing salinity stress to 16 ds.m⁻¹ decreased germination (95.08%), root and shoot length (43.93 and 80.88%), seedling dry weight (7.22%), and the best germination and growth index (88.00%) was found in controlled conditions. Shoot osmolytes increased with salinity stress. Example: Maximum protein (31.96%), POD (125%), SOD (64.36%), proline (115%), and soluble sugar (238%), observed at leaves sprout under salt stress (16 ds.m⁻¹). This study shows that salt stress increases secondary metabolites (phenolic chemicals), which modify stevia responsiveness and therapeutic qualities. Salinity stress enhanced antioxidant enzyme activity, carotenoids, anthocyanin, phenol, and malondialdehyde in shoots. The maximum activity was seen at a salinity stress level of 12 ds.m⁻¹. Changing irrigating procedures may modulate stevia's secondary metabolites (medicinal contents).

Keywords: *Stevia*, proline, soluble carbohydrates, protein, antioxidant defense

Introduction

One of the main effects of salinity in the soil or water in arid regions and semi-arid (Koca et al. 2007) is that reduce plant growth and yield (Yilmaz 2007). So because of saline land development and loss of agricultural land desirable for cultivation, identify medicinal plants resistant to salinity or the agents that can reduce the effect of salinity is very important (Shannon et al. 1994). Plant response to salinity stress is very complex and causes physiological changes, the morphological and plant metabolism (Parida and Das 2005). physiological and metabolic changes that may occur in response to salinity stress is increased reactive oxygen species (ROS), including the superoxide (O⁻²) and hydrogen peroxide (H₂O₂) concentration it cause passivity of antioxidant enzymes such as catalase, peroxidase, ascorbate peroxidase, superoxide dismutase and glutathione reductase that these systems could be to remove and inactivate reactive oxygen species (Bailly 2004). Plants under stress conditions created an environment with production and save Osmo regulators to coping with stress. Accumulation proline as a bodyguard in maintaining osmotic balance in rice (Kumar et al. 2007) and sunflower (Mutlu and Bozcuk 2005). Proline one important attribute for measuring the capacity salinity stress in plants. In many plant, proline accumulation response are imposed to biotic and abiotic stresses. Ability of proline is due to osmotic adjustment, stabilize under cell structure, cellular and free radicals, however, increasing levels of proline important role in protecting enzymes involved in antioxidant systems against harmful effects caused by its salinity (Ozturk et al. 2012). In fact, increasing the concentration of proline, the most abundant and most general response is that that as has been observed salinity stress (Suriyan and Chalermopol 2009). It is reported that the accumulation of soluble sugars in cause regulation of osmotic salt stress conditions and reducing water loss cells and preserve cell turgor (Kumar et al. 2007).

Stevia with scientific name *Stevia rebaudiana* Bertoni is known to Honey leaves the plant, the plant sweet and sweet grass. herbaceous plant, perennials, shrubs, herbs and belongs to the sunflower family (Asteraceae) native to northern

South America, Paraguay and Brazil(Sairkar et al. 2009). Given that the sugar obtained from leaves of the plant, which mainly Stevioside is no calories therefore important is this plant as the one medicinal product in terms of food. Stevia in different climatic conditions (Hajar et al. 2014) and poor soils with low nutrient is compatible but the irrigation needs for economical production of this product(Ramesh et al. 2006). Extensive research has been done around the world on this plant but is not well, it seems on the effect of salinity on plant studies of the properties. Accordingly, the objective of this study is to assess the impact of salinity on seed germination and seedling growth, as well as biosynthesis and accumulation of osmolytes and phenolic compounds in seedling of stevia (*Stevia rebaudiana* Bertoni), one of the most economic medicinal plants in the world, under both normal and salt stressed conditions; and to evaluate the potentiality of plants to ameliorate the salt stress on stevia seedlings.

2. Material and methods

2.1. Experimental design and seed preparing

Seed germination experiment (Exp. one)

The experiment was conducted at Seed Biology Laboratory, Department of Environmental Science, Shahed University, Iran, in 2016-2017 in completely randomized design with five treatments, replicated four times. The treatments comprised of natural salinity stress (Control, 4 ds.m⁻¹, 8 ds.m⁻¹, 12 ds.m⁻¹ and 16 ds.m⁻¹) (Table 1).

Plant material and growth condition

The stevia cultivar UEM-13 seeds (*Stevia rebaudiana* Bertoni) were used for our experiment were purchased from PakanBazr Company, Isfahan, Iran. The seeds of *Stevia* variety had initial moisture content (dry weight basis) of 10.5 and the length to width ratio was 2.78 ± 0.04 . Prior to seed germination, seeds rinsed with water and distilled water, then they were surface sterilized in 40% sodium hypochlorite (vol/vol) for 10 min, followed by 70% alcohol (vol/vol) for 10 s, at that moment rinsed thoroughly with sterile deionized water. After that, seeds were taken into a glass beaker and soaked in halo-priming (Bradford, 1987). After one hour(s) the seeds were withdrawn from the solution and dried by keeping in between two layers of filter paper followed by under bright sunlight till it become completely dry i.e. $\pm 10\%$ of initial weight (Basra et al. 2005).

Procure water

For watering plants, natural saline water was used for irrigation which was prepared in Sultan Lake located 40 km from the highway Qom – Tehran. Analysis of water samples showed that various combinations of salt there are in this water and caused high salinity of about 690 (dS. m⁻¹) (Table 1).

Determination of morphological parameters

For each treatment four hundred seeds were kept in between two layers of filter papers in a glass petri plate having 90 mm diameter, Relative humidity (33%) and 16 hours of light and 8 hours of darkness under the photoperiod was placed. The seeds were treated with 10 mL of double distilled water for without saline stress (Control), 20 mL of natural solution base every treatment for all salinity stress. The petri plates were kept inside an incubator at 24 ± 0.5 °C under aphotic condition. Seeds were considered as germinated only when radical length were found to be ≥ 2 mm. Finally, after 14 days' germination percentage (GP), Normal seedling (NS), Abnormal seedling(ANS), Mean germination time (MGT), Root dry weight (RDW), Shoot dry weight (SDW), Seedling total dry weight (TDW) and seedling vigor index (SVI) were recorded to evaluate seed germination performance. Daily germination percentage was recorded; data were subjected to statistical analysis. The root and plumule vigor were calculated as the sum of total radicle length (cm) and plumule length (cm) of all the seedlings divided by number of seedling. Then fresh radicle and plumule were placed in a hot air oven (70 °C for 24 h) to dry (ISTA 2010).

To measure the dry weight of the plant, after drying in an oven at 70 °C, dry weight was measured. To determine the dry weight, the weight of each sample is measured in grams with Sartorius scales BPSIID model with. 0.001 g sensitivity. Each treatment in replications were harvested three bushes and root length and shoot seedling dry weight was measured. For measurement dry weight of the samples for 48 hours at 70 °C were placed in the oven. Radicle and plumule dry weight were measured with electrical balance.

Germination percentage (GP) is calculated using the Eq. (1):

$$GP = \frac{\text{Total seeds germination after day14}}{\text{Total number of planted seeds}} \quad \text{Eq. (1)}$$

To assess the rate of germination, the mean germination time (Nichols and Heydecker) was calculated as follows:

$$MGT = (\sum n(dini)) / (\sum n) \quad \text{Eq. (2)}$$

Coefficient of velocity of germination (CVG) is calculated using the Eq. (3):

$$CVG = (Nt) / (\sum nt) \times 100 \quad \text{Eq. (3)}$$

In this regard, CVG, N , and n are, the speed of germination, total germination at the end of the experiment and germinated seeds at time t , respectively. The seedling vigor index (SVI) was calculated as the product of radicle and plumule length by germination percentage. Seed vigor index was calculated by the Eq. (4) (Omid et al. 2015).

$$SVI = GP \times TSL \times Un \quad \text{Eq. (4)}$$

SVI = Seedling vigor index. GP = germination percent (%), TSL = Total Seedling elongation (cm) and Un = Uniformity

Germination Variance (VG): the following correlation would be used for calculating variance (Omid et al. 2015).

$$VG = (\sum (di - D)^2 / n) / (\sum N) \quad \text{Eq. (5)}$$

$$Uni \text{ of time germination } (U) = 1 / \text{Variance} \quad \text{Eq. (6)}$$

Seedling growth experiment (Exp. Two)

In order to measuring the changes biochemical and physiological tests were implemented in pots. Soil of pots, sand and farm soil with a ratio of three to one was before fill the pots the first sand has been washed and dried and then with farm soil was that using screening, mixing was performed. Within each pot with mouth diameter of 35 cm and 40 cm with a height of 10 Stevia seeds were planted to a depth 1-2 cm. Finally, four seedling were planted in each pot, keeping the pots in the greenhouse under light conditions (16: 8) (light/dark), the intensity of about 190 micromoles of photons per square meter per second, and with 75% humidity and 20 ° C (dark / light). To provide essential minerals plants, pots were irrigated with 1/2 Hoagland solution concentration, pH of approximately 1±7/5 to 3 times a week for 3 weeks. After the plants sufficiently grew (Four- leaf stage), for two weeks every other day, natural salinity stress (Control, 4 ds.m⁻¹, 8 ds.m⁻¹, 12 ds.m⁻¹ and 16 ds.m⁻¹) treatments were applied simultaneously. Salinity levels in 6-leaf stage were applied to create a salt stress to Hoagland solution (Table 2). Also for prevent the accumulation of salts and nutrients within the soil; after each 6 times irrigation with Hoagland, the pots were irrigated with pure water. After sampling of the youngest leaves at the end of flowering, osmolytes and defense system antioxidants, protein content, proline content, soluble sugars content, SOD and peroxidase were recorded. Seedlings leaves collected from all treatments were stored at -80 °C until used for antioxidant assays. After 25 d and 30 d seedlings were washed in distilled water and used for soluble sugars, proline content, Protein and total Catalase activity (CAT), Superoxide dismutase activity (SOD) and peroxidase activity (POD) content.

Leaf relative water content

The relative water content of leaves was measured using the method of Barrs and Weatherley, (1962). The leaves from the top 5 cm were measured. First, the fresh leaves were weighed (FW); then, submerged in distilled water for 12 h, and the swollen leaves were weighed (TW). Finally, the leaves were kept at 70°C for 24 h until a constant weight was reached (DW). The below formula was used to calculate the relative leaf water content.

$$RWC (\%) = \frac{(\text{Fresh Weight} - \text{Dry Weight})}{(\text{Turgur Weight} - \text{Dry Weight})} * 100 \quad \text{Eq. (7)}$$

Proline content

Determination of free Pro content was done according to Bates et al (1973). Gypsophila leaves (0.5 g) were homogenized in 3% sulphosalicylic acid and homogenate was filtered through filter paper. After addition of acid ninhydrin and glacial acetic acid, resulting mixture was heated at 100° C for 1 h in water bath. Reaction was then stopped using ice bath. The mixture was extracted with toluene, and the absorbance of fraction with toluene aspired from liquid phase was read at 520 nm. Pro concentration was determined using calibration curve as mmol proline g⁻¹ FW.

Soluble sugars

Frozen samples (0.1 g) were ground and extracted with 2.5 ml of 80% (v/v) ethanol at 90°C for 60 min, followed by centrifugation at 10000 g at 4°C for 10 minutes. The process was repeated for complete extraction. Total soluble sugar content was determined using anthrone reagent and glucose as standard (Roe 1955). Results were expressed as mg soluble sugar/g DW.

Protein content

Protein determination was made according to the Bradford (Bradford 1976) and using a standard curve prepared with bovine serum albumin. Briefly, Coomassie blue, in response to protein concentration, reacts with basic amino acid residues, especially arginine. When the plants had been treated with salt conditions for 15 and 30 days, 100 mg of the dried aerial portion was placed in a test tube with 2 ml of 50 mM potassium phosphate buffer at pH 7.0. Plant tissues were centrifuged at 7000-12000 rpm. The supernatant was recovered and centrifuged at 3000 rpm for 15 min at 4°C. Samples were diluted 1:100 and read in triplicate in a spectrophotometer at 595 nm.

Superoxide oxide dismutase (SOD, EC 1.15.1.1)

Superoxide dismutase (SOD) activity was estimated according to a modification of Sairam et al. (2005) method. The reaction mixture (3ml) contained 1.5 ml 0.05 M sodium phosphate buffer (pH 7.8), 0.3 ml 130 mM methionine, 0.3 ml 750 µM nitroblue tetrazolium chloride (NBT), 0.3 ml 0.1 mM EDTA-Na₂, 0.3 ml 20 µM riboflavin, 0.01 ml enzyme extract, 0.01 ml 4% (w/v) insoluble polyvinylpyrrolidone (PVPP) and 0.28 ml deionized water. The reaction was started by placing the tubes below two 15 W fluorescent lamps for 10 min and stopped by keeping the tubes in the dark for 10 min. Absorbance was recorded at 560 nm. One unit of SOD enzyme activity was defined as the quantity of SOD enzyme required to produce 50% inhibition of NBT reduction under the experimental conditions, and specific enzyme activity was expressed as units per mg fresh weight of leaf.

Peroxidase activity (POD, EC 1.11.1)

Peroxidase (POD) activity was assayed according to the method Plewa *et al* (1991) with some modifications. Their action was started by adding 0.15 mL of 100 mmol/L H₂O₂ and the optical density at 470 nm was recorded in a spectrophotometer Shimadzu against an identical mixture to which no H₂O₂ was added, using the extinction coefficient of 26.6 mmol.L⁻¹.Cm⁻¹.

Determination of Photosynthetic pigments

Measurements of photosynthetic pigments, including chlorophyll a, b, total chlorophyll and carotenoids (carotenoids and xanthophyll) with the method introduced by Lichtenthaler (1987). 5.0 grams of frozen leaves from the end of each plant with 15 ml of 80% acetone was added and after filtration, the absorption read with a spectrophotometer with wavelengths of 646.8, 663.20 and 470 nm.

$$\text{Chlorophyll } a = 12.7(A_{663}) - 2.69(A_{645}) \times \frac{V}{100W}$$

$$\text{Chlorophyll } b = 20.13(A_{645}) - 5.03(A_{663}) \times \frac{V}{100W}$$

$$\text{Total Chlorophyll} = 20.2(A_{645}) + 8.02(A_{663}) \times \frac{V}{100W}$$

$$\text{Carotenoids} = (A_{470} - (17.1 \times (Chl a + Chl b) - 9.479 \times \text{anthocyanin})) / 119.26$$

V; volume of upper phase of solution and W; weight of plant fresh sample

Anthocyanins

To measure the anthocyanins, 5.0 g of completely sandblasted shoot in a porcelain mortar with 10 ml of methanol acid (hydrochloric acid pure methanol volume ratio of 1: 99) and juice was poured in test tubes, and placed in a dark environment, at 25 ° C for 24 hours used by Wagner (1979). Then, centrifuged for 10 minutes at 4000g and determine the solution absorbance at 550 nm. In order to calculate the concentration using the extinction coefficient M-1cm-1 at 33000 and presented in micromoles per gram of fresh weight.

$$\text{Anthocyanin} = 0.08173 A_{537} - 0.00697 A_{647} - 0.002228 A_{663} \quad \text{Eq. (9)}$$

Phenolic Compounds

For this purpose, we used the method by Sonald and Laima, 5.0 g sample of the shoots and roots were boiled up in 10 ml of 80% ethanol. The extracts were centrifuged for 10 min at 4000 rpm and saturated then sodium carbonate was added and diluted and centrifuged again. Finally, measured by a spectrophotometer, at a wavelength of 640 nm. Standard curves were plotted using catechol. The calculations were based on the number of phenolic compounds in mg per gram of fresh weight.

PAL and Malondialdehyde (MDA)

To prepare the enzyme extract 500 mg of leaf tissue with 5.6 ml of buffer Tris-HCl (8 / 8pH, 50 Mill molar) containing Betamarkato ethanol (15 Mill molar) were worn in the cold mortar. The extract obtained was centrifuged for 30 minutes with g50000 away. The supernatant was used to measure enzyme activity. One unit of PAL activity equivalent to 1 mol of cinnamic acid in one minute is the and measured in terms of MDA content by using thiobarbituric acid reacting substances (TBARS). In order to determine the concentration of malondialdehyde in the leaf, first half a gram of fresh leaves were completely powdered in a solution of 20% TCA (containing 5% Thiobutyric acid 4) and then mixed for 25 minutes in The temperature of 95 ° C was heated in a bin Mary bath and then the mixture was cooled in an ice bath and, according to the Valentiniotik et al. Method (30), the concentration of malondialdehyde was measured at 532 nm wavelength.

Sodium (Na⁺) and Potassium (K⁺) contents

For Na⁺ and K⁺ quantification, plant material, dried in oven at 80 1^C to a constant. The contents of Na⁺ and K⁺ in the mineral extract were then determined using a flame photometer (Al-Khayri 2002). Chloride was determined by titration after samples of oven-dried and ground material were extracted in demineralized water for 30 min (Chen et al. 2001).

Data, statistical methods analysis

All analyses were performed based as a CRD experiment in completely randomized design. Each data point was the mean of four replicates (n = 4) and the Duncan's Multiple Range Test (Duncan) option of Statistical Analysis System software (Version 9.4; SAS Institute; USA). The data recorded on seed germination, germination rate, radicle elongation, plumule elongation, dry weight seedling, vigor index and Radicle length, Length seedling, Mean germination time, germination rate, were pooled to get a mean value and analyzed statistically. The F-test was considered significant at p ≤0.05 between treatments for halo-priming.

Results

Morphological and physiological traits under salinity

Germination, normal and Abnormal seedling

Germination, seedling number and abnormal seedling of *Stevia rebaudiana* Bertoni UEM-13 was significantly affected (P ≤0.01) by salinity; however, under 16 ds/m, germination decreased by 95% in comparison to the control. With an increase in salinity, normal seedling and abnormal seedling of stevia tends to be reduced (Table 4; Fig IA and B). At 16 ds/m salinity, the normal seedling was less than other salinity levels.

Root, Shoot and Seedling dry weight

Analysis of variance significant effect on the level of salinity on seed germination, root and shoot length and seedling dry weight were significantly (P ≤0.01) (Table 3). The results showed that salinity reduced seedling (7.22 %), root (12 %) and shoot (16 %) dry weight than control, respectively. Was observed highest seedling dry weight (88%) under controlled conditions and seedling dry weight and the lowest salinity level 16 (dS. m⁻¹) (Table 4).

Root, Shoot and Seedling length

It was determined that with increasing salinity, seedling length, root length and shoot length decreased significantly (P ≤0.01) (Table 3). On the other hand, salinity -stress significantly inhibited the elongation of shoot and root as well as their fresh and dry masses (Table 4), in a concentration. Similarly, Puniran-Hartley *et al.* (2014) declared that NaCl treatment has inhibited the growth of wheat and barley.

Physiological parameter

Increasing the salt concentration of soluble carbohydrates in the shoots, roots and seedlings increased (P ≤0.01) (Table 3). Results greatest amount of soluble carbohydrates shoots and seedlings in salinity levels of 16 (dS. m⁻¹) and soluble carbohydrates in the roots salinity level 8 (dS. m⁻¹) indicated (Table 4). The protein content of the aerial parts, root, and seedling increased salinity levels. The results showed that the shoot protein in salinity level 4 (dS. m⁻¹) and the protein content of seedling and root system in salinity levels 8 and 12 (dS. m⁻¹) was observed (Table 4).

Photosynthetic pigments

Based on the results of variance analysis effects salinity on the photosynthetic pigments such as chlorophyll a, chlorophyll b, total chlorophyll and anthocyanin content at was significantly (P ≤0.01) (Table 3). The highest amounts of chlorophyll a (19.85 %) and b (11.05 %) content were obtained in control and 12 (dS. m⁻¹) salinity and the lowest pigments after the 16 (dS. m⁻¹) salinity, content of chlorophyll a (6.29 %) and chlorophyll b (7.59 %) content was recorded in 12 (dS. m⁻¹) and control salinity, respectively. Changes of others photosynthetic pigments as cartenoied and antocyanin shown in Table 4.

Metabolite profile under salinity

Analysis of variance showed that the effect of salinity on osmolytes and antioxidant enzymes shoots in the seedling stage were statistically significant (P ≤0.01) (Table 3). Mean comparison showed that the average salinity levels lead to increased activity of POD antioxidant enzymes at shoots in salinity 8 (dS. m⁻¹) and at the salinity 4 (dS. m⁻¹) the lowest in terms of in the control and 16 (dS. m⁻¹) condition (Table 4). The SOD enzyme activity in aerial shoots

increased with increasing salinity levels so that the enzyme at shoots enzyme activity SOD in salinity levels of 8 (dS.m⁻¹) increased and the least amount of enzyme was observed in the control condition (Table 4). Increasing salinity levels, increase peroxidase activity in shoot showed. So that POD activity in the shoots at the level of salinity 8 (dS. m⁻¹) and seedling salinity level 12 (dS. m⁻¹) increased (Table 4).

Shoot potassium (K⁺) and sodium (Na⁺) content

Results showed that the effect of salinity on shoot potassium (K⁺) and sodium (Na⁺) content in the seedling stage were statistically significant ($P \leq 0.01$) (Table 3). Mean comparison showed that the salinity levels lead to increased sodium (Na⁺) content and decreasing potassium (K⁺) at shoots in salinity 12 (dS. m⁻¹); and at the control salinity the lowest sodium and potassium content recorded. In the 16 (dS. m⁻¹) condition in terms of shoot potassium (K⁺) and sodium (Na⁺) content no seedling growth (Fig. III A, B and C).

Discussion

Tolerance to salinity during germination is critical for the establishment of plants growing in arid saline soil regions. Seeds require adequate water for vital activities and germination. If the water absorption is disturbed, the activity inside the seed is slowly applied and the time required for root exit from the seed increases, that is, the rate of germination decreases. It seems that in the germination under stress and drought due to the loss of osmotic potential, the absorption of water is impaired, and then the activity of the enzyme alpha-amylase is inhibited.

Several studies have shown levels of salinity stress reduced germination of mung bean, lentil (Kazerouni et al. 2005). Sorghum and Millet (Khalesro and Aghaali khani 2006). Mainly salinity stress by low osmosis potential and ionic poisonous can reduce germination and for this reason, it can influence on germination percent more than drought (Alebrahim et al. 2008). The causes of growth retardation at different levels of salinity, maybe decreased photosynthesis, increased sodium and chlorine in the plant and not producing some of the proteins and enzymes concentration in solution. the root and stem length were the most important parameters in the germination stage under tension conditions, because the root is in direct contact with the soil and absorbs water from the soil, and the stem transfers water and soluble materials from root to other points and the salinity is high The cause of water absorption decreases root and shoot lengthening

Proline one of the primary responses of plant proportion to salinity (Fedina et al. 2002). In this study, salinity led to the accumulation of the amino acid proline content in the flowering shoots, proline and proline root seedling that results were agree with the results (Esra et al. 2010), (Suriyan and Chalernpol 2009) and (Ozturk et al. 2012). Proline in osmotic adjustment of an adaptive response to stress that involves an increase in the cell soluble substances turgor to maintain the cells and keep stomata open, then process of photosynthesis and plant growth during the stress period (Oncel et al. 2000). Proline is a protective to maintain osmotic balance in rice (Kumar et al. 2007) and sunflower (Mutlu and Bozcuk 2005).

The results showed that the relative water content (RWC) of leaves was reduced, fresh and dry shoot weight decreased significantly, the contents of malondialdehyde (MDA), proline (Pro) and soluble sugar (SS) increased, and the activities of superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and increased during mild and moderate salinity but were inhibited in severe salinity.

In the salinity, increasing levels of proline as a compatible osmolytes, protective and cleansing oxygen macromolecules has been active (Desingh and Kanagaraj 2007). Reported that the free amino acids soybeans increased with increasing salinity (Ramana et al. 2012). Increased amino acids in plant tissues under salt stress on the protein (Hussein et al. 2007). Conditions accumulated stress protein and acts as a regulator of osmotic (Fariduddin et al. 2003). increasing salinity, soluble carbohydrates shoots, and seedlings increased. The effect of salinity on the sugar accumulation in many plant species, such as sugar beets, tomatoes, rice, and berries have been reported (Wanichananan et al. 2003). It is reported that the plant is resistant to salinity, increasing its internal sugar more (Kerepesi and Galiba 2000).

Increasing the concentration of soluble carbohydrate is starch hydrolysis enzyme thus increasing the hydraulic needs. Sugar is the main molecules in various physiological responses, the photosynthetic gene regulation, metabolism, and defense responses is influenced (Crowe et al. 1990). Carbohydrate accumulation in plant tissues under conditions of osmotic adjustment due to environmental stresses (Dhanapackiam and Ilyas M.H 2010).

This research was done on antioxidant system enzymes activity increased with increasing salinity. Induction of antioxidant enzymes general adaptation strategy that plants use to overcome oxidative stress (Foyer and Noctor, 2003). Previous studies showed that the level of antioxidative enzymes such as CAT, SOD, and POD increases when plants are exposed to oxidative stress including salinity (Smirnov 1995). It is reported that the activity of these enzymes increased under various environmental stresses (Hernández et al. 2000). Suggests that activity of antioxidant enzymes play an important role in plant resistance to stress. It is reported that hydrogen peroxide is toxic for cells so rapidly antioxidant defense system has been cleared and converted to water and oxygen (Gao et al. 2008). Increase the activity of peroxidase (POD) in plants exposed to salinity Phaseolus mungo (Dash and Panda 2001) and rice (Satoh et al.

2010) have been observed. Increase SOD in salt concentration in tomato plants in response to salt stress have been reported (Tari et al. 2015). Study of salinity increased peroxidase activity (POD) and catalase (CAT) in barley (Xu et al. 2008), corn (de Azevedo Neto et al. 2006) and sesame (Koca et al. 2007).

In this study, salinity stress had a more negative effect on Na⁺, K⁺ and ratio of Na⁺/K⁺ which was in contrast with the results reported by (Al-Khayri 2002). Previous researchers have shown that reduction in Na⁺ and K⁺ was mainly due to ionic effect especially the proportional increase in Na⁺ concentration. Moreover, the presence of Na⁺ and Cl⁻ ions in the cells may induce changes in protein activity because ions affect the structure of the hydration water which surrounds the protein molecule (Singh and Sharma 2016; Shahverdi et al. 2017).

The concentration of Na⁺ in leaves of Stevia increased with increasing salinity (Table 4), and in all treatments, the shoot sodium concentration was higher than others that treatments. That is, an increase in salinity from Control to 16 increased leaf sodium concentrations by 100% and 250%, respectively. Salt tolerance in glycophytes is associated with the ability to limit uptake and/or transport of saline ions (mostly Na⁺ and Cl⁻) from the root zone to aerial parts.

Conclusion

In conclusion, the data presented here provide new information on the key mechanisms that underlie stevia in salinity. The trends of metabolite, mineral and morpho-physiological changes in the stevia exposed to increasing salinity reveal that, first the main reactions to salinity take place in the shoot consistently second, metabolic changes under salinity occur mainly in the shoots and reveal that the different responses to salinity. Also, data indicate that Stevia is more tolerant to salt stress during germination than the seedling stage. The germination data further suggests that Stevia UEM-13) is a salt-tolerant glycophyte rather than a halophyte. The significant increment of proline in the roots suggests a positive role of this amino acid as a compatible solute in balancing the accumulation of Na⁺ and Cl⁻ as a result of salinity.

Disclosure statement

The authors declare that they have no conflict of interest.

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Author contributions

Heshmat Omid developed the original idea and the protocol, abstracted and analyzed data, wrote the manuscript, and is guarantor **Mohammad Hosein Bijeh Keshavarzi** contributed to the development of the protocol, abstracted data, and prepared the manuscript.

References

- Al-Khayri JM (2002) Growth, proline accumulation and ion content in sodium chloride-stressed callus of date palm. *In Vitro Cellular & Developmental Biology - Plant* 38(1): 79-82 <https://doi.org/10.1079/IVP2001258>
- Alebrahim M, Janmohammadi M, Sharifzade F Tokasi S (2008) Evaluation of salinity and drought stress effects on germination and early growth of maize inbred lines (*Zea mays* L.). *Electronic J. Crop Prod* 1: 35-43
- Bailly C (2004) Active oxygen species and antioxidants in seed biology. *Seed Science Research* 17: 93-107
- Basra SMA, Farooq M Khaliq A (2005) Comparative study of pre-sowing seed enhancement treatments in fine rice (*Oryza sativa* L.). *Pakistan Journal of Life and Social Sciences* 1: 5-9
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72(1): 248-254 [https://doi.org/https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/https://doi.org/10.1016/0003-2697(76)90527-3)
- Chen S, Li J, Wang S, Hüttermann A Altman A (2001) Salt, nutrient uptake and transport, and ABA of *Populus euphratica*; a hybrid in response to increasing soil NaCl. *Trees* 15(3): 186-194 <https://doi.org/10.1007/s004680100091>
- Crowe JH, Carpenter JF, Crowe LM Anchordoguy TJ (1990) Are freezing and dehydration similar stress vectors? A comparison of modes of interaction of stabilizing solutes with biomolecules. *Cryobiology* 27(3): 219-231 [https://doi.org/https://doi.org/10.1016/0011-2240\(90\)90023-W](https://doi.org/https://doi.org/10.1016/0011-2240(90)90023-W)
- Dash M Panda SK (2001) Salt Stress Induced Changes in Growth and Enzyme Activities in Germinating Phaseolus Mungo Seeds. *Biologia Plantarum* 44(4): 587-589 <https://doi.org/10.1023/A:1013750905746>
- de Azevedo Neto AD, Prisco JT, Enéas-Filho J, Abreu CEBd Gomes-Filho E (2006) Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany* 56(1): 87-94 <https://doi.org/https://doi.org/10.1016/j.envexpbot.2005.01.008>
- Desingh R Kanagaraj G (2007) Influence of salinity stress on photosynthesis and antioxidative systems in two cotton varieties. *Appl. plant physiology* 33: 3-4
- Dhanapackiam S Ilyas M.H M (2010) Effect of salinity on chlorophyll and carbohydrate contents of *Sesbania grandiflora* seedlings. *Indian Journal of Science and Technology* 3(1) <https://doi.org/10.17485/ijst/2010/v3i1/29647>

Esra K, İşlek C ÜstÜN A (2010) Effect of Cold on Protein, Proline, Phenolic Compounds and Chlorophyll Content of Two Pepper (*Capsicum annum* L.) Varieties. *Gazi University Journal of Science* 23: 1-6

Fariduddin Q, Hayat S Ahmad A (2003) Salicylic Acid Influences Net Photosynthetic Rate, Carboxylation Efficiency, Nitrate Reductase Activity, and Seed Yield in *Brassica juncea*. *Photosynthetica* 41(2): 281-284
<https://doi.org/10.1023/B:PHOT.0000011962.05991.6c>

Fedina IS, Georgieva K Grigorova I (2002) Light-Dark Changes in Proline Content of Barley Leaves under Salt Stress. *Biologia Plantarum* 45(1): 59-63
<https://doi.org/10.1023/A:1015175802847>

Gao S, Ouyang C, Wang S, Xu Y, Tang L Chen F (2008) Effects of salt stress on growth, antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedlings. *Plant, Soil and Environment* 54: 374-381
<https://doi.org/10.17221/410-PSE>

Hajar EWI, Sulaiman AZB Sakinah AMM (2014) Assessment of Heavy Metals Tolerance in Leaves, Stems and Flowers of *Stevia Rebaudiana* Plant. *Procedia Environmental Sciences* 20: 386-393
<https://doi.org/https://doi.org/10.1016/j.proenv.2014.03.049>

Hernández JA, Jiménez A, Mullineaux P Sevilla F (2000) Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defences. *Plant, Cell & Environment* 23(8): 853-862
<https://doi.org/https://doi.org/10.1046/j.1365-3040.2000.00602.x>

Hussein M, Balbaa L Gaballah M (2007) Salicylic Acid and Salinity Effects on Growth of Maize Plants. *Res. J. Agric. Biol. Sci.* 3

ISTA (2010) International rules for seed testing, International seed testing association (ISTA).

Kazerouni E, Akramian M, Tokassi S Eghbali S (2005) Physiological effects of salt and drought stresses on germination and seedling growth of lentil (*Lens culinaris*) and mungbean (*Vigna radiata* L.). *Horticultural journal*: 445-448

Kerepesi I Galiba G (2000) Osmotic and Salt Stress-Induced Alteration in Soluble Carbohydrate Content in Wheat Seedlings. *Crop Science - CROP SCI* 40
<https://doi.org/10.2135/cropsci2000.402482x>

Khalesro S Aghaalkhani M (2006) Effect of salinity and drought stress on seed germination in forage sorghum (*Sorghum bicolor* L.) and Pearl millet (*Pennisetum americanum* L.). *Horticultural Journal* 77: 153-163

Koca H, Bor M, Özdemir F Türkan İ (2007) The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environmental and Experimental Botany* 60(3): 344-351
<https://doi.org/https://doi.org/10.1016/j.envexpbot.2006.12.005>

Kumar V, Shriram V, Jawali N Shitole MG (2007) Differential response of indica rice genotypes to NaCl stress in relation to physiological and biochemical parameters. *Archives of Agronomy and Soil Science* 53(5): 581-592
<https://doi.org/10.1080/03650340701576800>

Lichtenthaler HK (1987). [34] Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology*, Academic Press. 148: 350-382.

Mutlu F Bozcuk S (2005) Effects of salinity on the contents of polyamines and some other compounds in sunflower plants differing in salt tolerance. *Russian Journal of Plant Physiology* 52(1): 29-34
<https://doi.org/10.1007/s11183-005-0005-x>

Nichols MA Heydecker W Two Approaches to the Study of Germination Data.

Omid H, NaghdiBadi HA Jafarzadeh L (2015) *Seeds of medicinal plants and crops*, Shahed University Press.

Oncel I, Keleş Y Ustün A (2000) Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. *Environmental pollution (Barking, Essex : 1987)* 107: 315-320
[https://doi.org/10.1016/S0269-7491\(99\)00177-3](https://doi.org/10.1016/S0269-7491(99)00177-3)

Ozturk L, Demir Y, Ünlükara A, Karataş İ, Kurunc A Duzdemir O (2012) Effects of long-term salt stress on antioxidant system, chlorophyll and proline contents in pea leaves. *Romanian Biotechnological Letters* 17

Parida AK Das AB (2005) Salt tolerance and salinity effects on plants: a review. *Ecotoxicol Environ Saf* 60(3): 324-349
<https://doi.org/10.1016/j.ecoenv.2004.06.010>

Ramana GV, Padhy SP Chaitanya KV (2012) Differential responses of four soybean (*Glycine max.* L) cultivars to salinity stress. *Legume Research* 35(3): 185 - 193

Ramesh K, Singh V Megeji NW (2006). *Cultivation of Stevia [Stevia rebaudiana (Bert.) Bertoni]: A Comprehensive Review*. *Advances in Agronomy*, Academic Press. 89: 137-177.

Roe JH (1955) The determination of sugar in blood and spinal fluid with anthrone reagent. *J Biol Chem* 212(1): 335-343

Sairam RK, Srivastava GC, Agarwal S Meena RC (2005) Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. *Biologia Plantarum* 49(1): 85-91
<https://doi.org/10.1007/s10535-005-5091-2>

Sairkar P, Chandravanshi M, Shukla N Mehrotra N (2009) Mass production of an economically important medicinal plant *Stevia rebaudiana* using in vitro propagation techniques. *J Med Plants Res* 3

Satoh K, Kondoh H, Sasaya T, Shimizu T, Choi IR, Omura T Kikuchi S (2010) Selective modification of rice (*Oryza sativa*) gene expression by rice stripe virus infection. *J Gen Virol* 91(Pt 1): 294-305
<https://doi.org/10.1099/vir.0.015990-0>

Shahverdi MA, Omid H Tabatabaei SJ (2017) Effect of nutri-priming on germination indices and physiological characteristics of stevia seedling under salinity stress. *Journal of Seed Science* 39

Shannon MC, Grieve CM Francois LE (1994) Whole plant response to salinity. New York, USA, Marcel Dekker.

Singh J Sharma P (2016) Comparative effects of soil and water salinity on oil quality parameters of *Brassica juncea*. *Journal of Oilseed Brassica* 7: 29-37

Smirnoff N (1995) Antioxidant systems and plant response to the environment, Bios Scientific Publishers, Oxford.

Suriyan C-u Chalermopol K (2009) Proline Accumulation, Photosynthetic Abilities and Growth Characters of Sugarcane (*Saccharum officinarum* L.) Plantlets in Response to Iso-Osmotic Salt and Water-Deficit Stress. *Agricultural Sciences in China* 8(1): 51-58
[https://doi.org/https://doi.org/10.1016/S1671-2927\(09\)60008-0](https://doi.org/https://doi.org/10.1016/S1671-2927(09)60008-0)

Tari I, Horváth E, Poór P, Takács Z Szepesi A (2015) The Alleviation of the Adverse Effects of Salt Stress in the Tomato Plant by Salicylic Acid Shows A Time- and Organ-Specific Antioxidant Response. *Acta Biologica Cracoviensia s. Botanica* 57
<https://doi.org/10.1515/abcsb-2015-0008>

Wanichananan P, Kirdmanee C Vutiyano C (2003) Effect of salinity on biochemical and physiological characteristics in correlation to selection of salt-tolerance in aromatic rice (*Oryza sativa* L.). *ScienceAsia* 29: 333-339
<https://doi.org/10.2306/scienceasia1513-1874.2003.29.333>

Xu Q, Xu X, Zhao Y, Jiao K, Herbert SJ Hao L (2008) Salicylic acid, hydrogen peroxide and calcium-induced saline tolerance associated with endogenous hydrogen peroxide homeostasis in naked oat seedlings. *Plant Growth Regulation* 54(3): 249-259
<https://doi.org/10.1007/s10725-007-9247-2>

Yilmaz DD (2007) Effects of salinity on growth and nickel accumulation capacity of *Lemna gibba* (Lemnaceae). *J Hazard Mater* 147(1-2): 74-77
<https://doi.org/10.1016/j.jhazmat.2006.12.047>

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Table 1. Chemical analysis of water samples pool Howz-e Soltan

EC (dS/m)	Cl ⁻ gr l ⁻¹	SO ₄ ⁻² gr l ⁻¹	Ca ⁺² gr l ⁻¹	M g ⁺² gr l ⁻¹	Na ⁺ gr l ⁻¹	K ⁺ gr l ⁻¹
690	218.7	48.8	0.086	19.5	128	1.23

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Table 2. The Hoagland solution for the growth and development of seedling *Stevia rebaudiana* plants

Component	Stock Solution	mL Stock Solution/1L
Macronutrients		
2M KNO ₃	202 g/L	2.5
1M Ca(NO ₃) ₂ •4H ₂ O	236 g/0.5L	2.5
Iron (Sprint 138 iron chelate)	15 g/L	1.5
2M MgSO ₄ •7H ₂ O	493 g/L	1
1M NH ₄ NO ₃	80 g/L	1
Micronutrients		
H ₃ BO ₃	2.86 g/L	1
MnCl ₂ •4H ₂ O	1.81 g/L	1
ZnSO ₄ •7H ₂ O	0.22 g/L	1
CuSO ₄ •5H ₂ O	0.051 g/L	1
H ₂ MoO ₄ •H ₂ O or	0.09 g/L	1
Na ₂ MoO ₄ •2H ₂ O	0.12 g/L	1
Phosphate		
1M KH ₂ PO ₄ (pH to 6.0)	136 g/L	0.5

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Table 3. A One-way ANOVA (Mean of Squares) for the effect of salinity treatment on the different measured parameters in germination and growth parameters of *Stevia rebaudiana* seedlings (numbers represent F values;

Parameter	Salinity	Error	Coefficients of Variation (%)
Germination percentage	4325.11**	15.12	14.22
Normal seedling	207.425**	1.300	13.90
Abnormal seedling	39.9250**	0.8333	19.42
Mean germination time	3.4833**	0.1625	22.29
Root dry weight	1.6917**	0.0418	20.35
Shoot dry weight	1.658**	0.0396	18.96
Seedling dry weight	6.6342**	0.1018	15.52
Root length	219.45**	21.42	7.85
Shoot length	7.452**	0.064	7.52
Seedling length	706.639**	2.926	7.61
Seedling vigor	7.083 **	0.4614	13.85
Chlorophyll a content	240.6454**	6.2034	13.38
Chlorophyll b content	2.5971 **	69.785	12.70
Chl a/Chl b content	69.7859**	2.5971	3.44
Chlorophyll total content	451.3977**	7.935	15.87
Protein content	7501.254**	112	14.25
Proline content	45321.215**	283.215	5.36
Soluble sugar content	45214.123**	612.254	8.25
Superoxide dismutase (SOD) activity	22312.1232**	814.325	9.45
Peroxidase (POD) activity	14321.1245**	215.321	5.75
Shoot potassium (K ⁺) content	81818.805**	4861.28	18.52
Shoot Sodium (Na ⁺) content	3.1871**	0.0271	11.16
Shoot K ⁺ /Na ⁺ content	0.57741**	0.00786	15.28
Cartenoied content	3444499.72**	12437.04	11.52
Antocyanin content	0.0013**	0.00012	17.47
phenol content	0.000764**	0.0025	11.83
Malondialdehyde content	5.400**	0.1773	12.32
Relative water content (RWC)	3889.42**	4.486	3.82

(ns; non-significant; *P < 0.05; **P < 0.01).

Table 4. Effect of natural salinity stress on the content of seed germination and some growth seedling parameter in the shoot of stevia seedlings. Values are mean $\pm$ standard error of four independent replicates.

Parameter\ Salinity (dS/m)	Control dS/m	4 dS/m	8 dS/m	12 dS/m	16 dS/m
Germination percentage (%)	88.00 $\pm$ 0.98 ^a	60.33 $\pm$ 0.75 ^b	43.00 $\pm$ 0.35 ^c	14.33 $\pm$ 0.38 ^d	4.33 $\pm$ 0.28 ^e
Root length (cm)	5.94 $\pm$ 0.08 ^a	5.07 $\pm$ 0.06 ^b	4.36 $\pm$ 0.05 ^c	3.92 $\pm$ 0.03 ^c	3.33 $\pm$ 0.08 ^d
Shoot length (cm)	4.50 $\pm$ 0.02 ^a	2.46 $\pm$ 0.20 ^b	1.96 $\pm$ 0.09 ^c	1.66 $\pm$ 0.12 ^c	0.86 $\pm$ 0.22 ^d
Seedling length (Cm)	33.39 $\pm$ 0.04 ^a	31.24 $\pm$ 0.05 ^a	25.69 $\pm$ 0.03 ^b	21.87 $\pm$ 0.04 ^c	0.12 $\pm$ 0.02 ^d
Shoot dry weight (gr)	1.60 $\pm$ 0.03 ^a	1.47 $\pm$ 0.04 ^{ab}	1.27 $\pm$ 0.05 ^b	0.90 $\pm$ 0.03 ^c	0.00 ^d
Root dry weight (gr)	1.42 $\pm$ 0.04 ^{ab}	1.60 $\pm$ 0.03 ^a	1.27 $\pm$ 0.05 ^b	0.72 $\pm$ 0.05 ^c	0.00 ^d
Seedling dry weight (gr)	8.63 $\pm$ 0.35 ^a	5.93 $\pm$ 0.26 ^c	6.90 $\pm$ 0.76 ^b	5.53 $\pm$ 0.41 ^c	2.40 $\pm$ 0.25 ^d
Seedling vigur	0.24 $\pm$ 0.05 ^c	0.44 $\pm$ 0.06 ^b	0.58 $\pm$ 0.09 ^b	0.88 $\pm$ 0.04 ^a	0.00 $\pm$ 0.00 ^d
Protein content (μ g /gFW)	391.33 $\pm$ 4.28 ^c	551 $\pm$ 2.46 ^a	516.56 $\pm$ 11.45 ^a	458.07 $\pm$ 6.71 ^b	369.42 $\pm$ 5.54 ^c
Proline content(μ g /gFW)	302.12 $\pm$ 9.48 ^d	475.16 $\pm$ 8.45 ^c	432.12 $\pm$ 7.44 ^c	545.21 $\pm$ 5.41 ^b	650 $\pm$ 14.58 ^a
Soluble sugar content (μ g /gFW)	252.85 $\pm$ 10.48 ^e	352.68 $\pm$ 7.44 ^d	653.85 $\pm$ 5.41 ^c	725.35 $\pm$ 9.42 ^b	852.35 $\pm$ 8.41 ^a
Peroxidase (POD) activity (u/min/mg pro)	82.25 $\pm$ 3.46 ^d	185.26 $\pm$ 4.42 ^a	175.32 $\pm$ 5.44 ^a	153.5 $\pm$ 6.41 ^b	98.32 $\pm$ 3.43 ^c
Superoxide dismutase (SOD) activity (u/min/mg pro)	275.32 $\pm$ 12.48 ^d	412.25 $\pm$ 11.75 ^{ab}	452.54 $\pm$ 9.42 ^a	332.25 $\pm$ 8.48 ^b	295.23 $\pm$ 7.48 ^c
Chlorophyll a (μ g/ gFW)	19.85 $\pm$ 1.08 ^a	15.39 $\pm$ 0.9 ^b	11.71 $\pm$ 0.58 ^b	6.29 $\pm$ 0.88 ^c	0 ^d
Chlorophyll b (μ g/ gFW)	7.59 $\pm$ 1.08 ^b	8.42 $\pm$ 1.11 ^b	8.41 $\pm$ 1.05 ^b	11.05 $\pm$ 1.05 ^a	0 ^c
Chlorophyll a / chlorophyll b	2.61 $\pm$ 0.25 ^a	2.02 $\pm$ 0.15 ^a	1.40 $\pm$ 0.13 ^b	0.56 $\pm$ 0.08 ^c	0 ^d
Chlorophyll total (μ g/ gFW)	27.44 $\pm$ 2.28 ^a	23.80 $\pm$ 3.72 ^{ab}	20.13 $\pm$ 3.67 ^{bc}	17.34 $\pm$ 3.54 ^c	0 ^d
Cartenoied (μ g/ml)	2122.22 $\pm$ 0.08 ^a	2024.20 $\pm$ 0.08 ^a	2146.80 $\pm$ 0.08 ^a	1985.07 $\pm$ 0.08 ^a	0 ^b
Cartenoied / chlorophyll a	108.456 $\pm$ 8.68 ^b	137.69 $\pm$ 9.14 ^b	169.49 $\pm$ 5.31 ^b	378.57 $\pm$ 6.22 ^a	0 ^c
Antocyanin (μ g/ml)	0.015 $\pm$ 0.05 ^{cd}	0.035 $\pm$ 0.08 ^{ab}	0.048 $\pm$ 0.06 ^a	0.223 $\pm$ 0.07 ^b	0 ^d
Phenolic compounds (μ g/mL)	0.035 $\pm$ 0.04 ^d	0.041 $\pm$ 0.08 ^c	0.058 $\pm$ 0.01 ^b	0.066 $\pm$ 0.05 ^a	0 ^b
MDA(mg/gFW)	1.58 $\pm$ 0.01 ^b	2.32 $\pm$ 0.02 ^a	2.71 $\pm$ 0.01 ^a	2.82 $\pm$ 0.02 ^a	0 ^c
RWC (%)	70.65 $\pm$ 2.08 ^a	70.04 $\pm$ 2.17 ^b	68.29 $\pm$ 2.05 ^b	63.68 $\pm$ 2.14 ^c	0 ^d

Same letters indicate no significant difference ($P \leq 0.05$) as analyzed by Duncan test

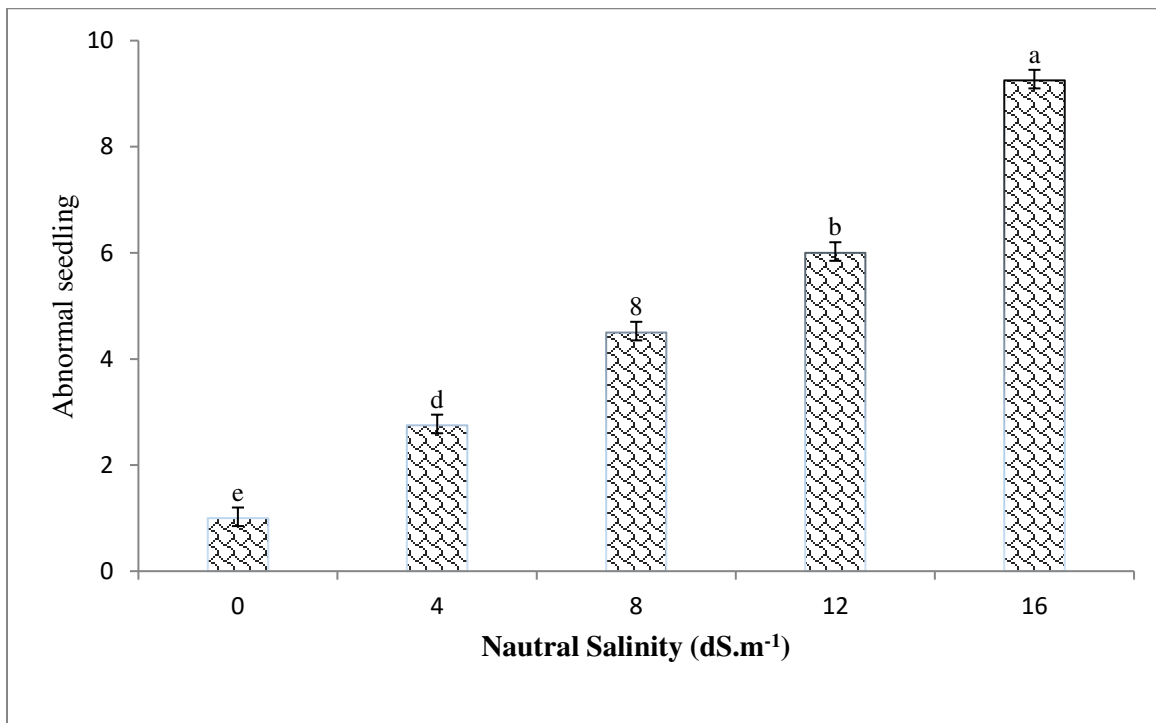
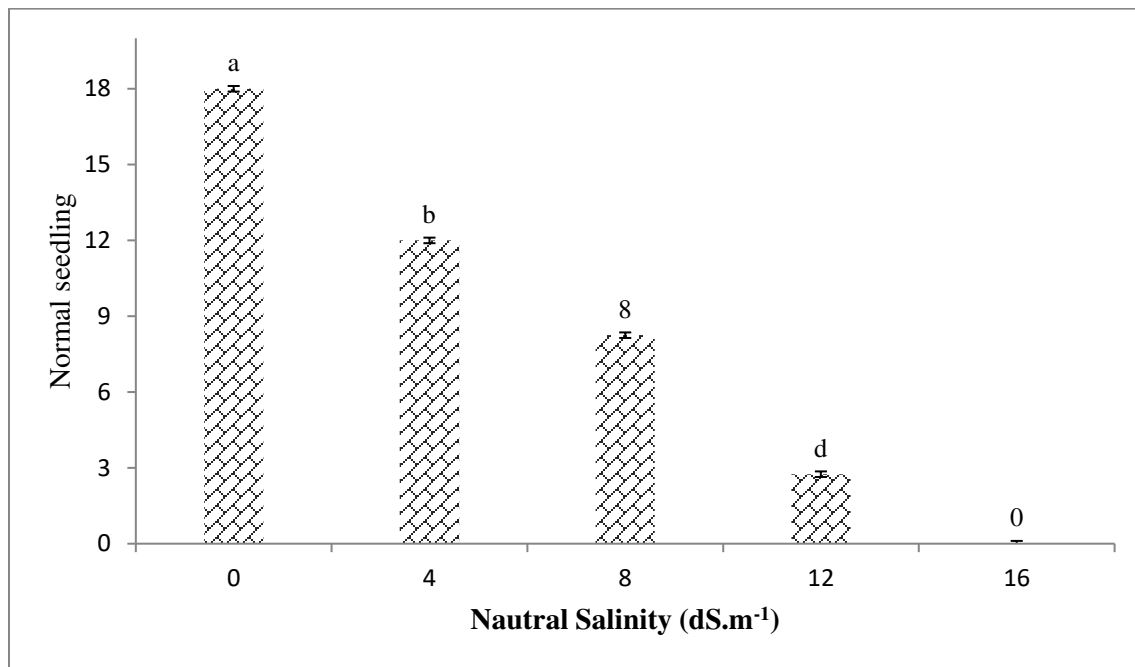


Fig. 1. Effect of salinity concentration on *Stevia rebaudiana* UEM-13 normal seedling number (A) and abnormal seedling number (B). Different letters indicate significant differences by Duncan's test at ($P \leq 0.05$). Vertical bars represent $\pm$ S.D.

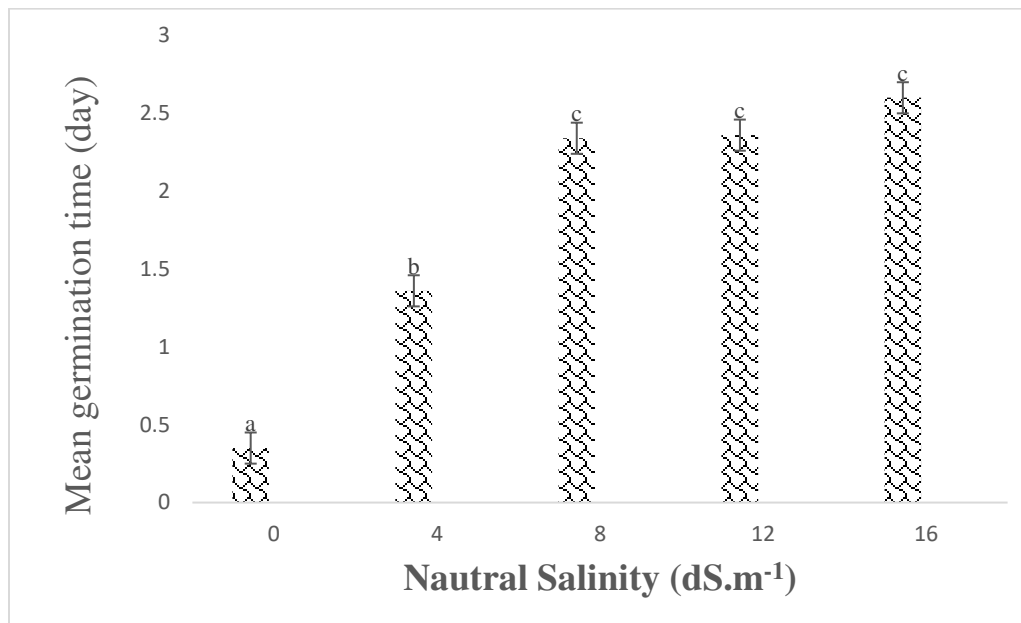


Fig. 2. Effect of salinity concentration on *Stevia rebaudiana* UEM-13 mean germination time. Different letters indicate significant differences by Duncan's test at ($P \leq 0.05$). Vertical bars represent $\pm$ S.D.

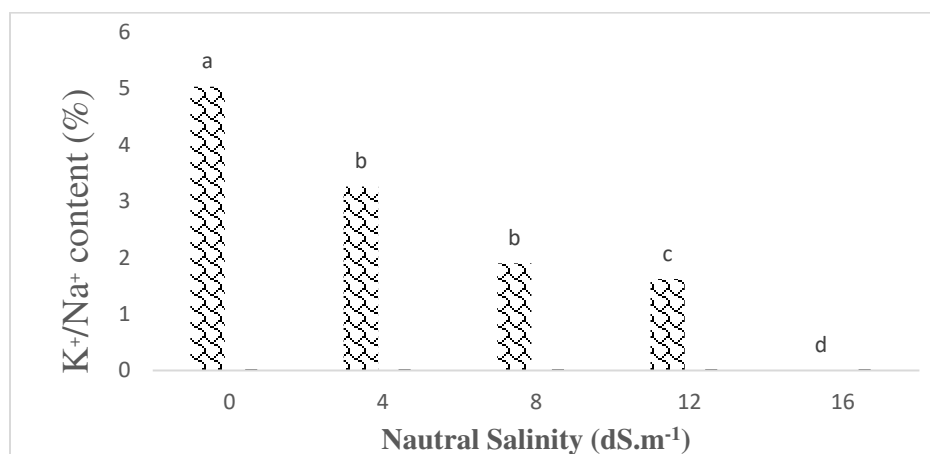
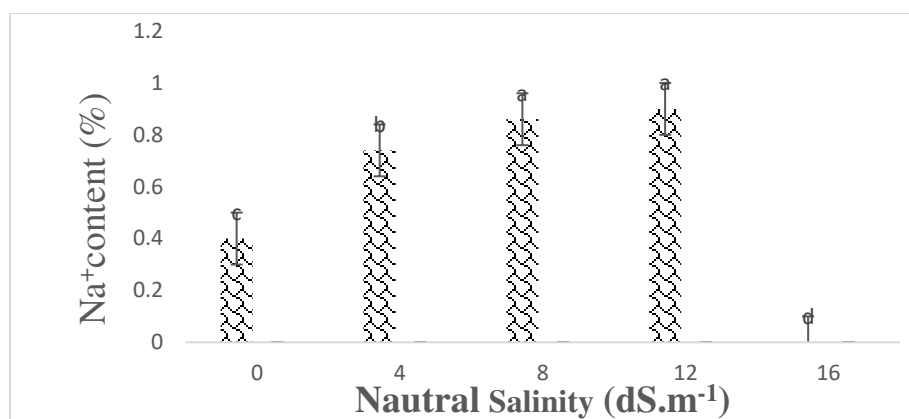
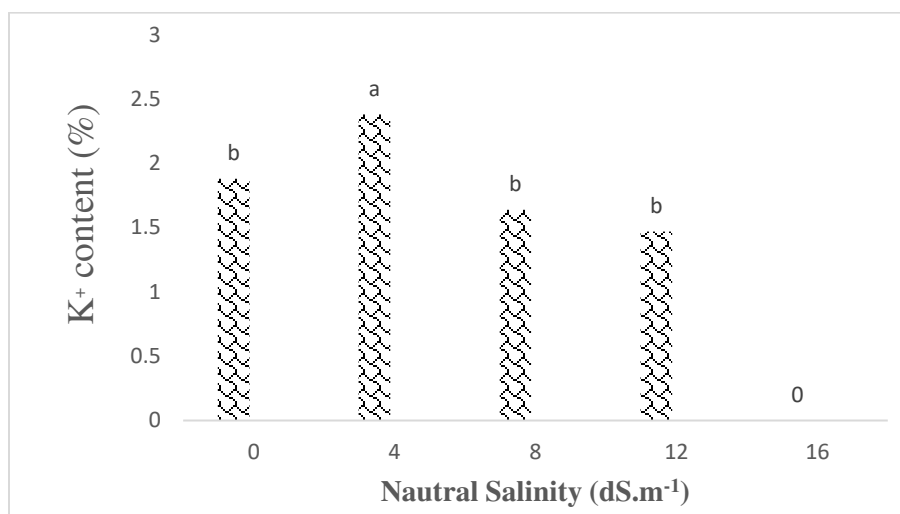


Fig. 3. (A, B and C). Effect of salinity concentration on *Stevia rebaudiana* UEM-13 K⁺/Na⁺ content, Na⁺ content and K⁺/Na⁺ content. Different letters indicate significant differences by Duncan's test at 5%. Vertical bars represent $\pm$ S.D.

Means within a column followed by the same letter are not significantly different ($P \leq 0.05$).