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Morphological Properties of *Catharanthus roseus* L. Seedlings Affected by Priming Techniques Under Natural Salinity Stress

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Abstract

Catharanthus roseus is a great medicinal plant used for treating diseases such as cancer, as it contains different biochemicals including terpenoids. Salinity is a major stress, significantly decreasing the growth and yield of medicinal plants, worldwide. However, to our knowledge, there is not any data on the growth improvement in *C. roseus* seedling under salinity stress using the priming techniques tested in the presented research. The objective was to investigate the effects of seed priming including hydro- (distilled water), osmo- (KNO_3 1%), and hormonal priming (salicylic acid, SA 0.5 mM) on the seedling growth of *C. roseus* under natural salinity (salt obtained from Qom Lake, Iran) of 0, 2.95, 5.66, 8.13, and 10.66 dSm^{-1}. The treated seeds were grown in Petri dishes and placed in a germinator (25 ± 1 °C) for germination and the growth of seedlings. The plant seedlings were planted in the greenhouse conditions and treated during the V6–V8 growth stage with the salinity treatments (a factorial design on the basis of a completely randomized block design with three replicates) under hydroponics conditions. Different morphological properties of *C. roseus* including the length and the dry weight of rootlet, plumule, and seedling as well as leaf relative water content (RWC) were measured. The results indicated the alleviating effects of different priming techniques on the growth of *C. roseus* under natural salt stress. Although the effect of priming was not significant on RWC, salt stress and its interaction with priming significantly affected RWC indicating that the alleviating effect of priming on RWC is a function of salinity level. With increasing the level of salinity, rootlet dry weight increased, which indicates the allocation of more carbon to the roots under stress. It is possible to plant *C. roseus* in saline fields using the priming techniques tested in this research work.

Keywords Hydropriming · Osmopriming · Hormonal priming · KNO_3 · Salicylic acid · Saline fields

Abbreviations

SA	Salicylic acid
RL	Root length
RDW	Root dry weight
PL	Plumule length
PDW	Plumule dry weight
SL	Seedling length
SDW	Seedling dry weight
RWC	Relative water content

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Introduction

Catharanthus roseus, from the Apocynaceae family, is among the most important medicinal plants used for treating diseases such as cancer, due to the presence of 120 terpenoids in the plant (Misra and Gupta, 2006). However, similar to the other medicinal plants (Mohammadi and Asadi-Gharneh 2018a), its growth and yield decreases under stresses such as salinity drought, etc. (Omidi et al. 2018). Salt stress decreases seed germination by: (1) decreasing water uptake by the germinating seeds, (2) the toxic effects of Na^+ and Cl^-, (3) disrupting the structure of enzymes and other molecules, (3) negatively affecting cell organelles and plasma membrane, (4) decreasing the respiration of the cells, photosynthesis and the synthesis of proteins (Ibrahim 2016).

The unfavorable effects of salinity on plant growth are due to the osmotic effects of Na^+ and Cl^- as well as their toxic effects and oxidative stress (Miransari and Smith 2019). Under salinity stress, the metabolic activities of plants are negatively affected, which is due to the damage of DNA and peroxidation of membrane lipids as a result of reactive oxygen production (Rahnama et al. 2011; Chang et al. 2014; Gupta and Huang 2014).

Due to the wide distribution of saline fields in different parts of the world, the use of specific indictors, which can clearly specify plant response under stress is of significance. Accordingly, Yan et al. (2013) presented a two-stage practical method, to show plant response under salinity stress. The first stage is related to alteration of plant growth including photosynthesis, plant biomass, and crop yield, and the second stage is a physiological-related stage including the antioxidant activities, regulation of osmosis, and ion homeostasis. They accordingly indicated that the alteration of plant chlorophyll (Sohag et al. 2020), as an indicator of plant photosynthesis under salinity stress, is one of the most useful indexes, which can be used for the illustration of plant response, and subsequent suggestion of alleviating methods, under salinity stress.

Different techniques and methods have been used so far to alleviate the effects of salinity stress on plant growth and yield production such as the use of: (1) tolerant plant species, (2) organic fertilization, and (3) soil microbes (Daei et al. 2009; Miransari 2013; Liang et al. 2018). However, another interesting method, which has been widely used, is the technique of priming including hydro-, osmo-, and hormonal-priming, in which seeds and seedlings are treated with different chemicals and biochemicals (Ibrahim 2016; Tahaei et al. 2016).

The priming techniques enhance seed germination by the following mechanisms: (1) reducing the phases of imbibition and lag, (2) facilitating water absorption, (3) stimulating the metabolic processes required for radicle growth, (4) reducing the physical resistance of endosperm at the time of imbibition, (5) enhancing the growth of damaged membrane, and (6) leaching the barriers of germination (Elouaer and Hannachi 2012; Ibrahim 2016).

The use of osmopriming, for example KNO_3, has been shown to efficiently affect the germination of seeds and the growth of plant seedlings in different crop plants. Potassium (K) and nitrogen (N) are two important macronutrients significantly affecting plant growth and yield production (Mohammadi and Asadi-Gharneh 2018b). For example, K is an essential nutrient for a wide range of functions in plant including the activation of enzymes, the process of photosynthesis, translocation of photosynthates to different parts of the plant, plant water behavior and efficiency, the activity of stomata, and increased plant tolerance under salinity stress (Jha and Subramanian 2016; Mengel 2016; Zamani et al. 2020). Similarly, N also regulates important functioning in plant including the process of photosynthesis, the structure of chlorophyll, plant vegetative growth, the activity of enzymes such as nitrate reductase (Li et al. 2015; Thion et al. 2016).

Salicylic acid as a plant hormone is able to alleviate stresses such as salinity on plant growth by the activation of antioxidant enzymes (Miransari and Smith 2014; Song et al. 2014; Sohag et al. 2020) and accumulation of osmolytes including proline. The exogenous use of SA prevents the reduction of auxin and cytokinin in wheat plants under salinity stress resulting in the increased division of root cells and the subsequent increase of plant growth (Ghassemi-Golezani and Farhangi-Abriz 2018; Bukhat et al. 2019). Exogenous use of SA under salinity stress also increases the accumulation of abscisic acid in the treated seedlings, which contributes to the production of anti-stress proteins and hence maintains plant health under stress (El Tayeb 2005; Hayat et al. 2010). Although not investigated in the presented manuscript, another important aspect of using elicitors such as SA and KNO_3 on the growth and physiology of *C. roseus* under salinity stress, which deserves more investigation, is the enhanced production of alkaloids, which are anti-cancerous in nature (Tonk et al. 2017).

Accordingly, finding methods, which may enhance the production of *C. roseus* in salty lands is of significance, and because to our knowledge, there is not any data on the use of the priming techniques, tested in the presented research, affecting the seedling growth of *C. roseus* under natural salinity stress (Nabaei and Amooaghaie 2019; Sahoo et al. 2019), this research work was conducted. The objective was to determine the effects of hydro- (distilled water), osmo- (KNO_3), and hormonal-priming (SA) on the seedling growth of *C. roseus* seedlings under natural salinity stress.

Materials and Methods

Experimental Method

The seeds obtained from Pakanbazr Company, Isfahan, Iran (www.pkanbazr.com), were sterilized with sodium hypochlorite 10% for 5 min, rinsed with distilled water and resterilized with ethanol 70% for another 1 min, and rinsed with distilled water. The seeds (100 seeds) were examined for the seed-related testings including the rate of germination (Tahaei et al. 2016; Soleymani 2019).

The seeds were then treated with the experimental treatments of different priming techniques including control, hydro- (using distilled water), osmo- (using KNO_3 1%), and hormonal priming (using SA 0.5 mM) for 15 h under the temperature of 15 °C, and were dried for 48 h. The experimental treatments were prepared according to the following: (1) potassium nitrate (1%) by dissolving 1 g KNO_3 in 100 ml distilled water and (2) salicylic acid (0.5 mM) by dissolving 6.90 mg SA in 100 ml distilled water. The treated seeds were grown in Petri dishes containing 15 ml distilled water, covered with parafilm, and were placed in the germinator at 25 ± 1 °C for germination and the growth of the seedlings.

Plastic containers covered with a led, containing three holes of 20 mm diameter in each led, where planted with three seedlings. In each 500-ml container, there was a sponge with the diameter of 30 mm in which the seedlings were planted in the established cracks.

The seedlings were fertigated with a Hoagland's solution (Hoagland and Arnon 1938) adjusted according to the experimental treatments (during the V6–V8 growth stage), and aerated with aquarium pump. Natural salinity levels (salt obtained from Qom lake, Iran) of 0 (S1), 2.95 (S2), 5.66 (S3), 8.13 (S5), and 10.66 (S6) dSm^{-1} using Hoagland's solutions were tested. The experiment was a factorial (completely randomized block design) with three replicates, conducted in the greenhouse. The greenhouse temperature was equal to 22–25 °C being lighted with fluorescent and tungsten lights.

Measured Parameters

Using an oven (70 °C for 24 h), a digital weight (with the precision of 0.0001), and a ruler, the following parameters were measured: rootlet length (RL) and dry weight (RDW), plumule length (PL) and dry weight (PDW), seedling length (SL) and dry weight (SDW) as well as leaf relative water content (RWC) according to the following (Ritchie and Nguyen 1990). The weight of seedlings was determined, and the seedlings were placed in distilled water for 24 h in a cold room (4 °C). Then the saturated weight of the seedlings was measured and the seedlings were dried in an oven for 24 h at 70 °C to determine their dry weight. The leaf relative water content (RWC) was determined according to the following formula:

$$\mathrm{RWC} = \left(\frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}}\right) \times 100,$$

F_w fresh leaf weight right after sampling, D_w leaf dry weight right after drying, S_w leaf saturated weight after placing in distilled water.

Results

According to the analysis of variance, the effects of priming techniques were significant on seedling measured parameters with the exception of RWC. Salinity also significantly affected different measured parameters except SDW. The interaction effect of priming and salinity was also significant on PL, SL, and RWC (Table 1).

The highest RL was resulted by KNO_3 followed by hydropriming. With increasing the level of salinity, RL decreased as the least RL was resulted by the highest level of salinity significantly different from the other levels (Fig. 1). Although with increasing the salinity level PL and SL significantly decreased, the hydropriming and KNO_3 treatments significantly increased PL and SL compared with the other treatments (Fig. 2a, b). The highest ratio of rootlet and plumule length was resulted by the control treatment followed by the SA treatment significantly different from the other treatments (Fig. 3a). With increasing the level of salinity, the ratio of rootlet and plumule length significantly decreased (Fig. 3b).

The KNO_3 treatment resulted in significantly higher RDW than the other treatments followed by the hydropriming and SA treatments not significantly different from each other (Fig. 4a). Increased levels of salinity significantly decreased RDW (Fig. 4b). The highest and significantly different PDW was resulted by the SA treatment, followed by KNO_3 and hydropriming treatments not significantly different from each other (Fig. 5a). Salinity stress significantly decreased PDW (Fig. 5b).

The highest ratio of rootlet weight to plumule weight was also related to the SA treatment significantly different from the other treatments (Fig. 6a). With increasing the salinity level, the ratio of rootlet weight to plumule weight significantly increased as the highest one was related to the highest level of salinity (Fig. 6b). SA treatment resulted in the highest SDW significantly higher than the other treatments (Fig. 7a). Priming techniques did not significantly

Table 1 Analysis of variance indicating the effects of priming techniques and natural salinity on the seedling growth of *Catharanthus roseus*

Trt	d.f	MS						
		RL	PL	SL	RDW	PDW	SDW	RWC
P	3	13.63*	9.27*	45.33*	0.37*	5.65*	7.82*	45.30
S	4	2.67*	8.10*	20.01*	0.41*	1.34*	0.77	167.87*
P x S	12	0.03	0.17*	0.03*	0.009	0.005	0.44	125.73*
Error		0.03	0.03	0.27	0.02	0.04	0.34	32.25
C.V		9.89	10.19	11.49	23.32	7.53	16.95	6.57

*Significant at 0.01 probability level

MS mean of squares, *Trt.* treatment, *d.f.* degree of freedom, *RL* rootlet length, *PL* plumule length, *SL* seedling length, *RDW* root dry weight, *PDW* plumule dry weight, *SDW* Seedling dry weight, *RWC* relative water content, *P* priming, *S* salinity, *C.V.* coefficient of variation

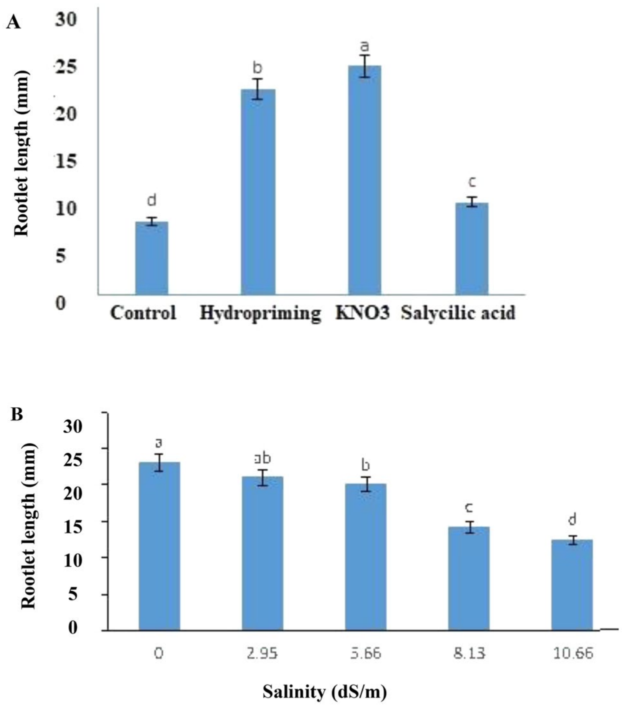

Fig. 1 The effects of priming techniques (**a**) and salinity levels (**b**) on rootlet length

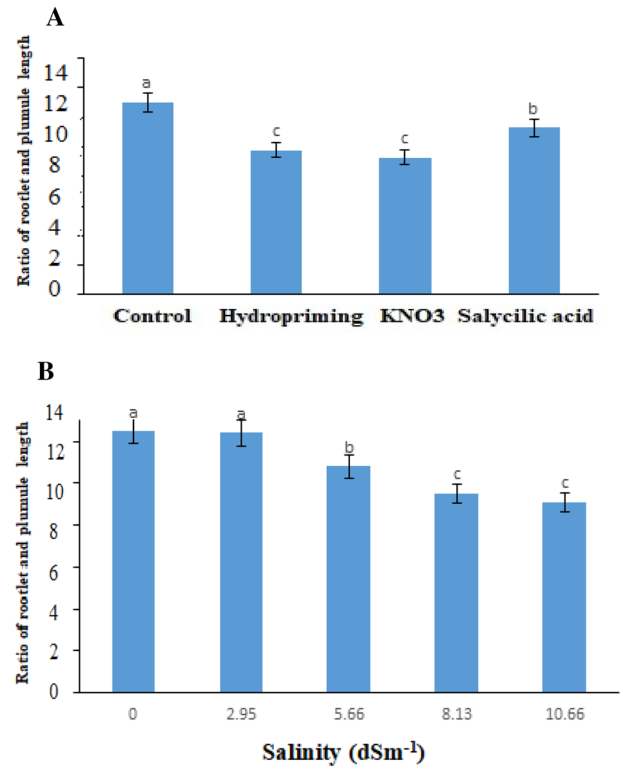

Fig. 3 The effects of priming techniques (**a**) and salinity levels (**b**) on the ratio of rootlet/plumule

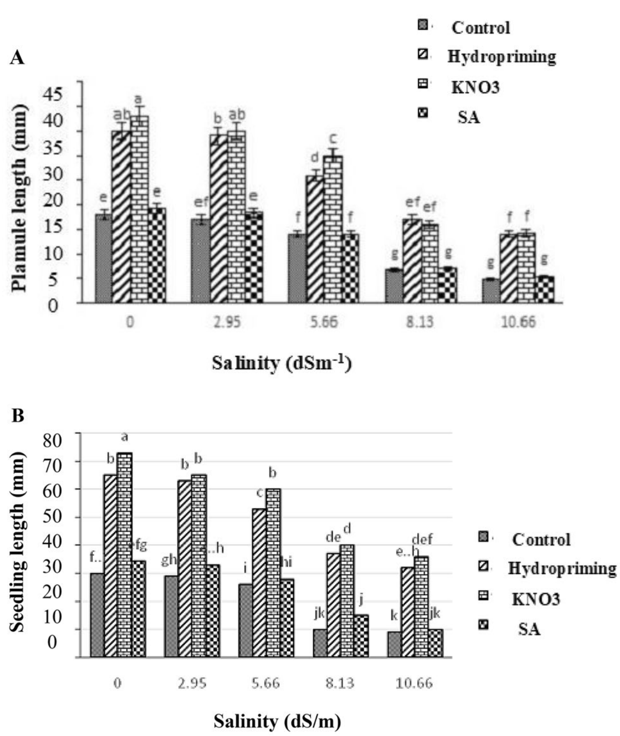

Fig. 2 The effects of priming techniques and salinity levels on (**a**) plumule length and (**b**) seedling length

decrease RWC and there were not clear trends of priming techniques on RWC (Fig. 7b).

Discussion

Priming techniques significantly affected different measured parameters, with the exception of RWC. This indicates that the alleviating effects of the priming techniques tested in this research work are by affecting plant morphology and physiology of *C. roseus* rather than affecting RWC. However, due to the significant effects of salinity and its interaction with priming techniques on RWC, the effectiveness of priming techniques on RWC is a function of salinity level. For example, in the second level of salinity all priming techniques significantly increased RWC related to the control treatment, this was also the case for the highest level of salinity but the difference was not significant (Fig. 7).

Although the response of different plants may differ under salinity stress, there might be some similarities among such responses. For example, Tahjib-UI-Arif et al. (2019) examined the morphology and physiology of sugar beet (*Beta vulgaris* L.) under long-time salinity stress and found that the plant was resistant under mild level of salinity, and its growth and physiology including relative water content was

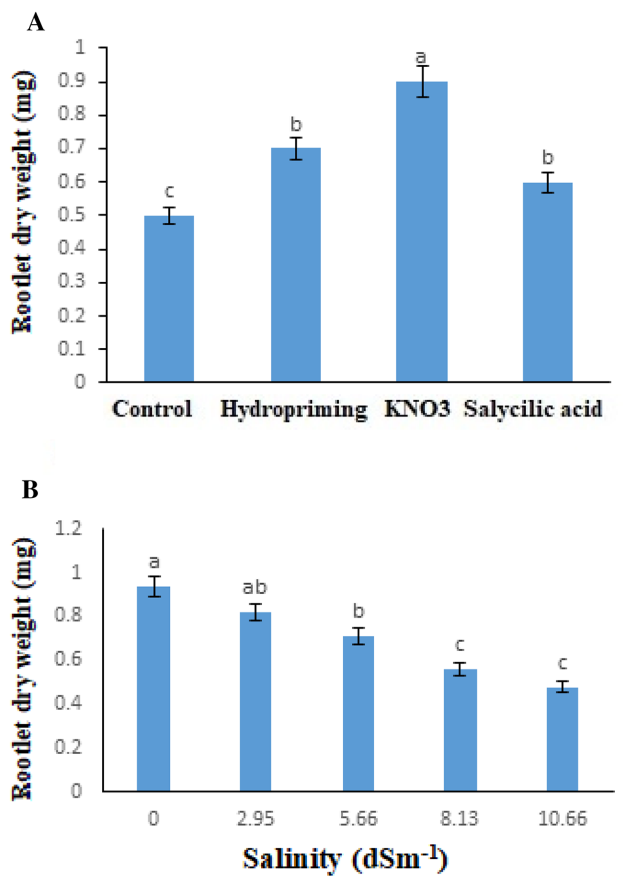

Fig. 4 The effects of priming techniques (**a**) and salinity levels (**b**) rootlet dry weight

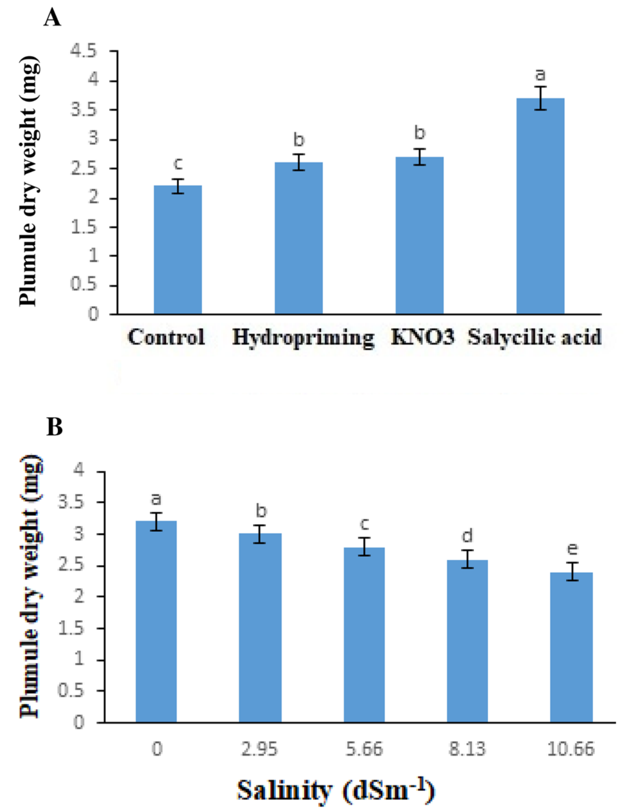

Fig. 5 The effects of priming techniques (**a**) and salinity levels (**b**) on plumule dry weight

enhanced. However, under high level of salinity plant growth significantly decreased.

Although with increasing salinity levels RL, PL, SL, and RDW decreased (Jafarzadeh and Aliasgharzad, 2007), the KNO_3 and the hydropriming treatments were able to significantly increase such parameters compared with the other treatments. This can be due to the positive effects of K and N on the cellular growth of rootlet and plumule. The important role of potassium in plant growth and development is by cellular homeostasis, through ionic balance, adjusting the osmotic potential and catalysis of enzymes. Nitrogen can also significantly affect plant vegetative growth, by affecting the process of photosynthesis, the activity of different enzymes, etc. (Shin et al. 2005).

Çavuşoğlu et al. (2017) investigated the effects of seed priming with KNO_3 on the germination, seedling growth, mitotic activity, and chromosomal abnormalities of *Allium cepa* in control and salt stress conditions. Priming with KNO_3 increased the abnormalities of chromosomes. Salt stress significantly decreased: (1) plant seed germination, (2) seedling growth, (3) the mitotic index in the seed root tip meristems, and (4) increased the number of chromosome abnormalities. However, interestingly the KNO_3 treatment was able to alleviate the negative effects of salt stress on the germination of the seeds, growth of seedlings, mitotic activity, and the abnormalities of chromosomes.

Ibrahim (2016) indicated that KNO_3 priming can ameliorate the negative effects of salinity on seed germination and seedling growth by the following mechanisms: (1) increasing the activity of germination enzymes, (2) altering the mobilization of organic molecules in different parts of the seed, (3) increasing the rate of proteins, free amino acids and soluble sugars, (4) increasing the activity of acid phosphatase and phytase in the seeds and seedlings, and (5) reducing peroxidase activity.

SA was also able to alleviate the stress of salinity on the growth of *C. roseus* by the significant increase of PDW and the ratio of root weight to plumule weight, which are similar to the results by Kang et al. (2012) and Kim et al. (2018). Kang et al. (2012) indicated that the use of 0.5 mM SA enhanced wheat morphological and physiological properties under salinity stress, which was mainly due to the activation of anti-stress proteins. The alleviating effects of SA on the growth of different crop plants under salinity stress have been indicated by different research work (Joseph et al. 2010; Agami 2013; Li et al. 2013). In another interesting

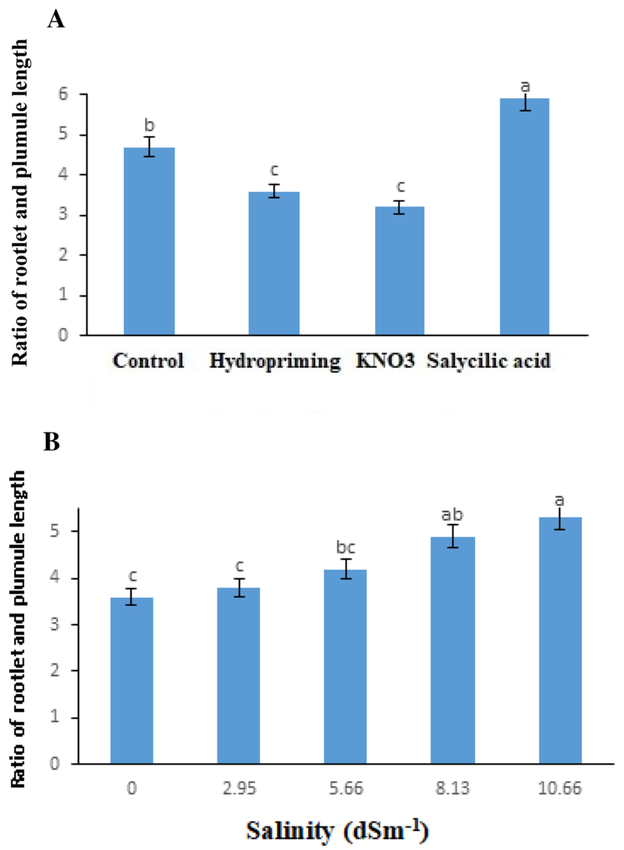

Fig. 6 The effects of priming techniques (**a**) and salinity levels (**b**) on the ratio of rootlet and plumule length

research, Hussein et al. (2007) found that spraying maize plants with SA at 200 mg/l enhanced plant morphological properties under the salinity stress of 4000 mg/l (NaCl). SA acts as an internal signal molecule under stress and induces plant response, which results in the production of different proteins. SA can inhibit the negative effects of Na^+ and Cl^-, and decreased lipid peroxidation (determined by malondialdehyde content and the permeability of membrane), and increase the uptake of different nutrients by plant under salinity stress.

In another research, Bukhat et al. (2019) investigated the effects of SA on the growth improvement in different radish (*Rhaphanus sativus*) genotypes in salt stress conditions by proposing the new roles of SA in the balance of redox potential and the movement of electrons during photosynthesis. The five-day seedlings were sprayed with SA at 0, 2, and 5 mM, which were treated with salt stress of 0, 100, and 200 mM, 48 h later. Although salinity negatively affected seedling growth, chlorophyll concentration and PSII in different genotypes, it enhanced the rate of lipid peroxidation and proline content as well as the production of reactive oxygen species and the activities of antioxidant enzymes. SA alleviated the stress by affecting the movement of photosynthetic electrons and subsequent photochemistry of different genotypes, which increased plant antioxidant activities and decreased lipid peroxidation. The authors accordingly indicated that among the other effects of SA on the alleviation of salt stress, the accumulation of osmolytes can also importantly improve plant growth by enhancing the photosynthesis process, as a result of decreasing oxidative stress, under salinity stress.

Interestingly, our results indicated that with increasing salinity level the ratio of root weight to plumule weight significantly increased. This is consistent with the previous results as plant allocates more carbon to the roots under stress (Miransari et al. 2008; Thalmann et al. 2016).

Conclusion

The effects of different priming techniques including hydro-, osmo- (KNO_3), and hormonal- priming (SA) on the growth and morphology of *C. roseus* under natural salinity stress were investigated. The results indicated the positive effects of the tested priming techniques on *C. roseus* morphology under natural salinity stress, which, to our knowledge, has not been previously investigated. Although the effects of priming techniques were not significant on RWC, salt stress and its interaction with priming techniques significantly affected RWC. This indicates that the alleviating effect of priming techniques on RWC is a function of salinity level. Accordingly, the selection of the proper compound at the certain level of salinity can considerably alleviate the effects of salt stress on *C. roseus* growth and morphology. Under salt stress more carbon was allocated to the plant roots, which is a mechanism usually used by plants to alleviate the effects of stress. The possible physiological mechanisms which may result in such alleviating effects of the tested techniques have been presented. The results elucidate some new insights in the important roles of SA and KNO_3 on the growth improvement in *C. roseus* seedling in natural salt conditions. It is possible to plant *C. roseus* in saline fields using hydro-, osmo- (KNO_3), and hormonal-priming (salicylic acid). However, the question, which may be investigated in the future research is if the combined use of such priming techniques can intensify the alleviating effects of the tested compounds on the growth and morphology of *C. roseus* under natural salt stress.

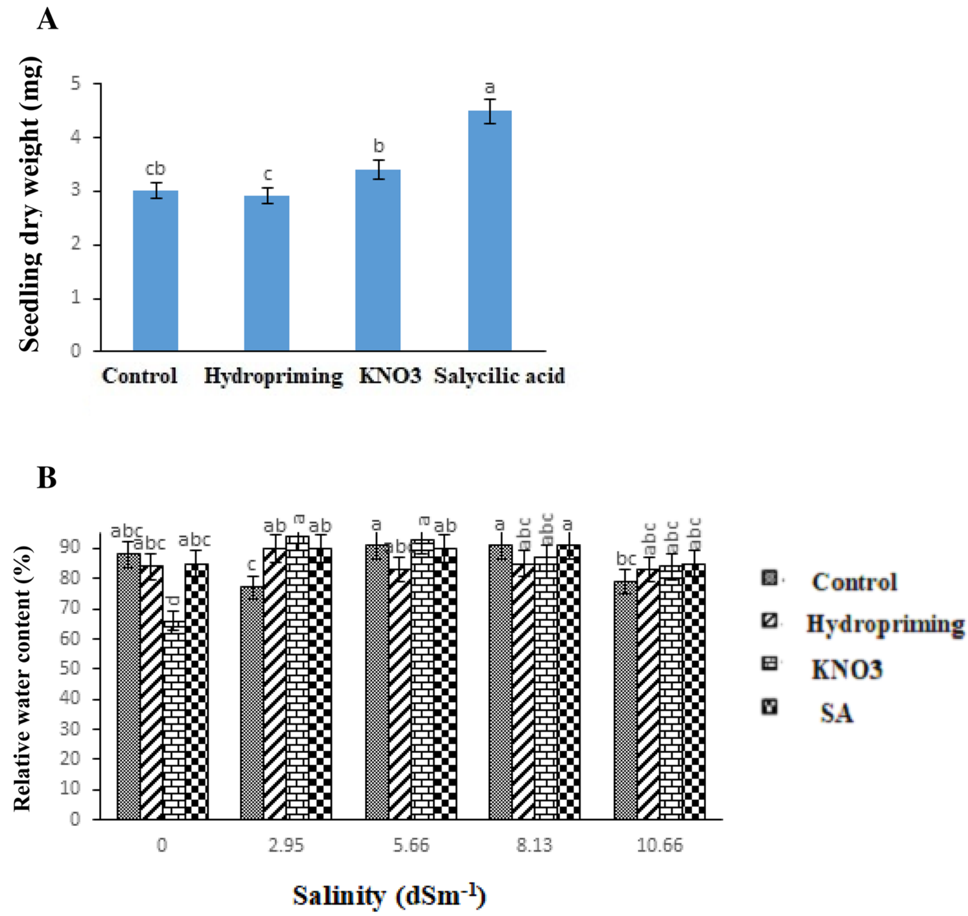

Fig. 7 The effects of priming techniques and salinity levels on (**a**) seedling dry weight and (**b**) leaf relative water content

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Compliance with Ethical Standards

Conflict of interest The authors declare they do not have any conflict of interest.

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