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MORPHOLOGICAL RESPONSE OF BITTER ALMOND UNDER SALINE CONDITIONS[1]

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Bitter almond (*Prunus amygdalus*) is considered one of the most important rootstocks for stone fruit species but classified as a plant sensitive to salinity. This experiment was conducted to investigate the effect of saline water irrigation on seed germination and the first stages of bitter almond growth as an attempt to raise its resistance to high salt concentrations. In this regard, nuts were soaked in salt solutions of NaCl as (1, 3, and 5dsm^{-1}) for 48 hours prior to stratification. Subsequently, the seeds were sown in perlite and irrigated with different saline solutions then stratified at 6 °C for eight weeks. Germinated seeds were sown in pots with a mixture of peat and perlite. The treatments were arranged in a randomized complete block design with three replications and 25 seeds for each replicate. Germination measurements and first stages of growth parameters were estimated. The results revealed that salinity treatments significantly affected seed germination and the first stages of bitter almond growth. Soaking and irrigation of bitter almond seeds with high salt concentrations reduced germination percentage, germination rate, stem length and diameter by 1.1, 1.5, 1.7, and 1.3 times respectively comparing to the lowest salt concentration. However, elongation of secondary and primary root / plant and number of secondary roots/plant markedly increased under high salt concentrations by 1.3, 1.7 and 1.8 times more than its values in the lowest concentration. Finally, the general transplants vigor index was significantly improved under 5dsm^{-1} of salt concentration; therefore, such transplants adapted and gained an ability to tolerate high salt concentrations.

Keywords: ion toxicity; salinity; seed dormancy; stratification; vigor index

МОРФОЛОГИЧЕСКИЙ ОТКЛИК ГОРЬКОГО МИНДАЛЯ НА ЗАСОЛЕНИЕ

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Горький миндаль (*Prunus amygdalus*) является одним из наиболее важных видов подвоя для косточковых плодовых культур, но классифицируется как чувствительное растение к засолению. Проведены эксперименты по изучению влияния в различной степени засоленных

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поливных вод на прорастание семян и первые фазы роста горького миндаля с целью поиска метода повышения его устойчивости к высоким концентрациям солей. Семена перед стратификацией замачивались в растворе NaCl различной концентрации: 1 dsm^{-1}, 3 dsm^{-1} и 5 dsm^{-1} в течение 48 ч. После этого они были высажены в перлит и в течение 8 недель при температуре 6 °C поливались раствором NaCl различной концентрации. Пророщенные семена высаживались в горшки со смесью торфа и перлита. Опыт был проведен в рандомизированном полном блочном дизайне в трехкратной повторности с 25 семенами в каждой повторности. Результаты показали, что концентрация соли значительно влияет на прорастание семян и первые стадии роста горького миндаля. Замачивание и орошение семян горького миндаля водой с высоким содержанием соли уменьшает процент прорастания, скорость прорастания, длину и диаметр стебля в 1,1; 1,5; 1,7 и 1,3 раза соответственно по сравнению использованием воды с низкой концентрацией соли. Однако удлинение вторичного и первичного корня и количество вторичных корней растений заметно увеличилась при высоких концентрациях соли: в 1,3; 1,7 и 1,8 раза больше, чем их значения при самой низкой концентрации. Кроме того, общий показатель сильной рассады был значительно улучшен при концентрации соли 5 dsm^{-1}. Следовательно, эти сеянцы имели наибольшую адаптацию к солевому стрессу, и их способность выдерживать высокую концентрацию соли была выше.

Ключевые слова: ионная токсичность, засоленость, семенной покой, стратификация, индекс силы

Introduction

Salinity is one of the most serious and oldest environmental problems affecting approximately one-third of irrigation land. Thus, soil salinity is a major factor limiting sustainable agriculture. The USDA salinity laboratory defines saline soil as having electrical conductivity of the saturated paste extract (ECe) of $4dSm^{-1}$. High concentrations of soluble salts such as chlorides of sodium, calcium and magnesium contribute to the high electrical conductivity of saline soils. NaCl contributes to most of the soluble salts in saline soil. Sodium ions are among the predominant exchangeable bases [1]. Soil salinity in agriculture soils refers to the presence of a high concentration of soluble salts in the soil moisture of the root zone. These concentrations of soluble salts through their high osmotic pressures affect plant growth by restricting the uptake of water by the roots. Salinity can also affect plant growth because the high concentration of salts in the soil solution interferes with balanced absorption of essential nutritional ions by plants [2].

Nutrient disturbances under salinity reduce plant growth by affecting the availability, transport, and partitioning of nutrients. However, soil salinity imposes ion toxicity, osmotic stress, nutrient (nitrogen, calcium, potassium, phosphorus, ferrous, and zinc) deficiency and oxidative stress on plants. Salinity also indirectly limits plant productivity through its adverse effects on the growth of beneficial and symbiotic microbes. High salt concentrations in soil impose osmotic stress and thus limit water uptake from soil. Sodium accumulation in cell walls can rapidly lead to osmotic stress and cell death [3]. Moreover, ion toxicity is the result of replacement of K+ by Na+ in biochemical reactions, and Na+ and Cl– induced conformational changes in proteins. For several enzymes, K+ acts as cofactor and cannot be substituted by Na+. In this regard, high K+ concentration is also required for binding transfer ribonucleic acid (tRNA) to ribosomes and thus protein synthesis [2]. Ion toxicity and osmotic stress cause metabolic imbalance, which in turn leads to oxidative stress [4]. Additionally, the ions Na+ and Cl– penetrate into plant cells and can be accumulated in the vacuole for the tolerant plants or in the cytoplasm which are known to be very toxic to the plant cell by injuring the cytoplasmic enzymes for sensitive cultivars [5]. Environmental stresses such as drought and salinity increase superoxide dismutase and peroxidase activities, which are implicated in cell membrane damage in sensitive species [6].

Salinity affects almost all aspects of plant development, including germination, vegetative growth and reproductive development. General symptoms of damage by salt stress are growth inhibition, accelerated development, senescence and death during prolonged exposure. In addition, salt stress induces the synthesis of abscisic acid which closes stomata when transported to guard cells. As a result of stomatal closure, photosynthesis declines and photoinhibition and oxidative stress occur. Moreover, the decline of photosynthetic pigments in salt stressed transplants maybe due to the decrease in the absorption of minerals needed for chlorophyll biosynthesis (i.e., iron and magnesium) [7], or due to inhibition of chlorophyll synthesis [8]. Salinity affects photosynthesis mainly through a reduction in leaf area, chlorophyll content, and stomatal conductance; and to a lesser extent through a decrease in photosystem II efficiency [9]. Salinity imposed osmotic stress leads to cell turgor loss and cell volume change. An immediate effect of osmotic stress on plant growth is its inhibition of cell expansion either directly or indirectly through abscisic acid.

The possibility of using saline water for irrigation, especially underground water, well water, or diluted sea water are considered as limiting factors. In this respect, bitter almond (*Prunus amygdalus*), Family: *Rosaceae* is considered one of the most important rootstock species for stone fruits. As it is known to be drought resistant, the tolerance of almond trees to water stress is presumably related to adaptive mechanisms present in their leaves or roots. Such mechanisms include osmotic adjustment, stomatal conductance decrease of transpiration water loss, leaf shedding acceleration, and root density and depth increase [10]. Additionally, almonds can be used in semi arid areas to control soil erosion, afforestation and desertification because of their high adaptation to unfavorable environmental conditions [11]. Although it is a hardy and long-lived plant, almonds are classified as a sensitive plant to salinity. The development of salinity tolerant crops is required to sustain agricultural production. The present investigation aimed to raise resistance and adaptation of bitter almond to salt concentration in the soil and irrigation water.

Material and Methods

This study was conducted in 2016–2017 to investigate the effect of salinity on seed germination and first stages of bitter almond growth. In this regard, almond seeds (*Prunus amygdalus*) require a cold treatment under humid conditions to overcome dormancy and promote germination. Seed dormancy is an adaptive mechanism that protects many plant species from freezing damage during the winter. This phenomenon affects seed germination and later seedling growth [12].

The treatments utilized in breaking seed dormancy can be divided. First, the removal of seed coat when dormancy is induced by the seed coat. In almond nuts, the endocarp has a mechanical effect, affecting gas exchange and preventing seed imbibition. In this regard, washing out tegument hormones caused by modification of the stratification period required for germination [13]. In addition, tegument removal has been described as a mechanism to accelerate seed germination [14]. In this way, previous studies [15; 16] characterized seed dormancy in peach as two independent mechanisms: tegument dormancy (external), with a hormonal nature, and manifested in the inhibition of germination, and embryo dormancy (internal) with a genetic nature and expressed mainly in the later plant growth. Secondly, stratification at temperatures near freezing is usually the most effective for breaking dormancy. The low temperature can depress the growth-inhibitor content of the dormant seeds [17]. In this regard, temperatures between 2 °C and 7 °C are generally the most efficient [12]. Germination under salt stress could be a quick test to look for salt tolerant plants [18].

Bitter almond seeds were collected from a vigor genotype at Rafah, North Sinai Governorate, Egypt. Uniformly sized and healthy nuts were soaked in salt solutions in the following manner: $1 dSm^{-1} = 640$ ppm, $3 dSm^{-1} = 1920$ ppm and $5 dSm^{-1} = 3200$ ppm for 48 hours, where ppm = Ec (ds/m) × 640, for Ec between 0.1 and $5 dSm^{-1}$ [19]. Water was replaced on a daily basis to remove growth inhibitors that are leached from the seeds. The heavier nuts settled to the bottom while the light nuts, pulp, damaged and unusually small nuts were eliminated [20].

Nuts were then sown in perlite at depths of 10mm and irrigated with different saline solutions then stratified at 6 °C for eight weeks (15 October – 13 December, 2016). To prevent water loss during stratification, the upper surface of pots was covered by a sack. After cold stratification, non-germinated nuts were exposed to 22 °C for three weeks (13 December – 5 January, 2017) to promote germination. Germination period is the time (in days) between the first time of sprouts and the end of germination because of the seeds which placed in the optimum conditions do not germinate immediately after sowing [18]. Seed germination was defined by the emergence of a radicle at least 2mm in length. Three plants per replicate from every treatment were obtained and sown in black polyethylene bags (15 cm × 20 cm) containing a mixture of peat and perlite, and subsequently irrigated with salt concentrations once a week through the end of February. Salt solutions ($1dSm^{-1}$ = 640 ppm, $3dSm^{-1}$ = 1920 ppm and 5dSm–1 = 3200 ppm) were selected considering general specifics of plant tolerance for salinity [21]. The accumulated salts were removed every three irrigation cycles from the bags by irrigation with tap water, and then followed by re-irrigation with the same salt solution in the next irrigation cycle to avoid salt buildup. The plants were grown at a temperature of 25 ± 2 °C; a photoperiod of 16 hours light, eight hours dark; and alight intensity (PAR) of 500–700 μM m–2 s–1.

Estimated measurements:

1. The seed moisture content (g) was determined before study and after complete germination.

2. The germination percentage was determined weekly until the end of germination period. The germination percentage (GP) was calculated using the following formula:

$$GP = \Sigma G/N \cdot 100.$$

Where GP is the germination percentage, G is the numbers of germinated nuts and N is the numbers of all nuts [22].

3. The germination index was calculated via the following formula:

$$GI = \Sigma Gt \, / \, Dt.$$

Where Gt is the germination percentage after t days and Dt represents germination days according to previous research [23].

4. Germination rate (GR) was calculated using following equation:

$$Gr = \Sigma n/\Sigma \, (Dn) \cdot 100.$$

Where n is the number of nuts that germinated on days and D is the number of days counted from the beginning of the test [24].

5. The stem length (cm) was measured from the soil surface to the end of the growing point.

6. The stem diameter (mm) of each plant was measured using venire caliper.

7. The mean length of primary root / plant (cm) was measured.

8. The mean number and length of secondary roots/plant (cm) were measured.

9. The seedling vigor index (V) was calculated by the following formula [25]:

$$Vigor\ index = germination\% \ x \ mean\ of\ seedling\ length\ (root + shoot).$$

The treatments were arranged in a randomized complete block design with three replicates, and 25 seeds for each replicate. The obtained data were tabulated and subjected to analysis of variance (ANOVA) with the MSTAT software package, and means were compared using last significant differences (LSD) range at the 5 % level.

Results and Discussion

Data obtained regarding the effect of seed soaked and irrigated with salt solution on germination and seedling growth of bitter almond were tabulated in figures 1–9.

From the obtained data, it was found that seed moisture content was significantly decreased by increasing the salt concentration (Fig. 1). The highest seed moisture content was recorded with $1dsm^{-1}$ treatment, followed by $3dsm^{-1}$ and that received $5dsm^{-1}$ respectively. This means that the seed absorbed minimal water from high osmotic pressure and salinity of soil solution compared to low salinity and control treatments. This played a vital and direct role in nuts germination parameters of bitter almond. In this regard, previous research [26] proposed delay in citrus germination as attributable to the rate of imbibition. They found that fresh seeds at approximately 50 % moisture content are close to the water content required for the process of germination to begin and suggest that extra time is required for dry seeds to imbibe water and initiate the germination phase. Additionally, decrease in water potential in germination medium because of drought or salinity prevent water absorption needed for germination process to start [27].

There was a marked decrease in germination percentage parallel to increasing salt concentration up to 5dsm^{-1} (Fig. 2). In this respect, the lowest germination percentage (73.78 %) was achieved when the nuts were treated with 5dsm^{-1} and the highest germination percentage (80.44 and 76 %) was recorded under control treatment and low salt concentration respectively. Germination percentage under 5dsm^{-1} was deceased by 1.1 times compared to the lowest salt concentration. It can be attributed to high water content of nuts at low salt concentration caused in increasing biological activity and induced the embryo to germinate [28]. In Arabidopsis, 200 mmole (mM) NaCl stress caused in 90 % ovule abortion that takes place as a result of the adverse effects of salinity on reproductive development. This enhances cell death in some tissue types caused in ovule abortion and senescence of fertilized embryos [29]. The results obtained in this work agree with these obtained by previous studies [30; 31]. They found that increasing salinity caused a significant reduction in germination percentage.

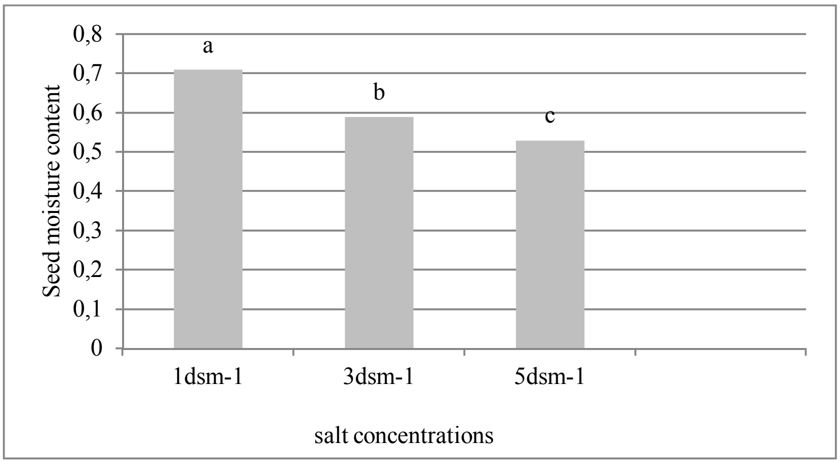

Fig. 1. Effect of salt concentrations on seed moisture content of bitter almond seeds

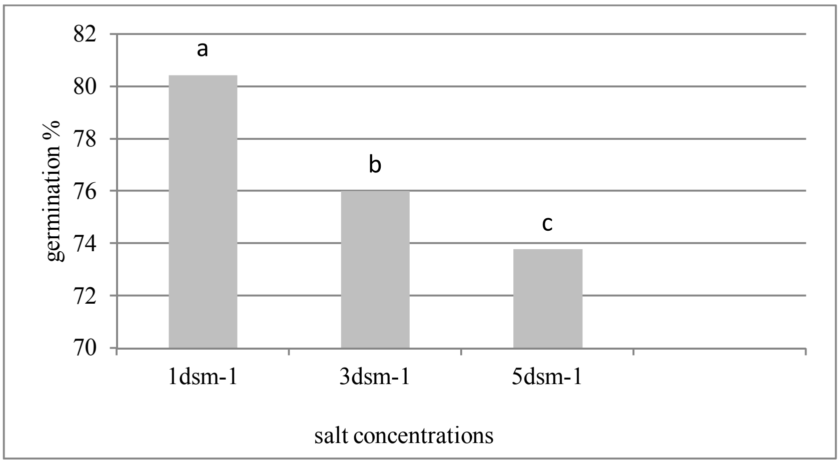

Fig. 2. Effect of salt concentrations on germination percentage of bitter almond seeds

It is evident from Figure 3 that increasing salt concentrations from 1dsm^{-1} to 5dsm^{-1} significantly decreased average germination index of bitter almond seeds by 1.4 times. Subsequently, the highest pronounced reduction effect was obtained due to the high dose of salinity treatment (5dsm^{-1}). In the other hand, the highest index of germination was detected on nuts that were treated with 1dsm^{-1} and 3dsm^{-1} respectively. These results are in line with previous research [32] that reported salinity stress limits the productivity of agricultural crops, with adverse effects on germination, plant vigor and crop yield. The reduction in growth parameters of different plant organs maybe attributed to osmotic pressure of the substrate which may restrict the uptake of water [33]. In addition, studies [34] suggested that the reduction in growth was due to drastic changes in the ion relationships of plants as a result of the adverse effect of Na+ and Cl– ions on metabolism or from disturbed water relations. Moreover, salinity arrests the cell cycle transiently by reducing the expression and activity of cyclins and cyclin-dependent kinases (enzymes that plays an important role in regulating the cell cycle or cell division) resulting in fewer cells in the meristem, thus limiting growth [35].

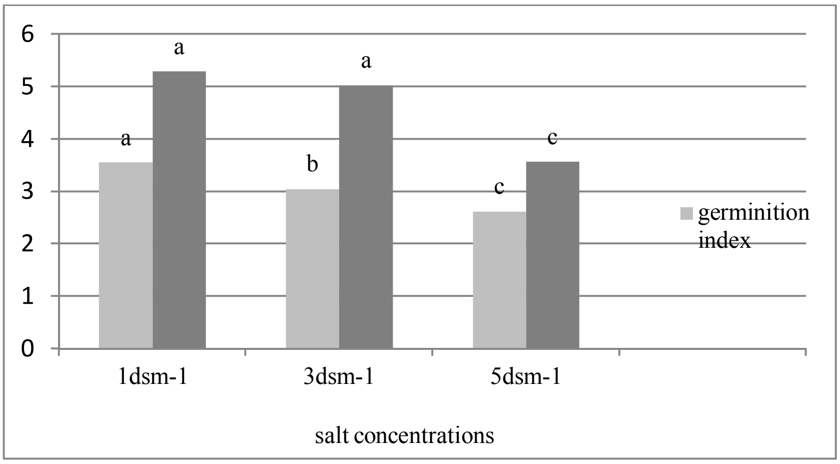

Fig. 3. Effect of salt concentrations on germination index and rate of bitter almond seeds

Salinity treatments significantly affected the germination rate of bitter almond seeds (Fig. 3). It was evident that the highest salt concentration substantially decreased the rate of germination by 1.5 times relative to low salt concentration and control treatment which rapidly increased the rate of germination of bitter almond seed. The differences between 1dsm^{-1} and 3dsm^{-1} were insignificant. These results are in harmony with those previously [25] suggested that the decreased rate of germination and the subsequent steps of germination at lower temperatures in cultivated citrus seeds was a result of a reduced rate of initial water uptake although a long germination period was employed (eight weeks). Furthermore, seeds supplied with insufficient moisture will show unsynchronized seedling emergence which is an important problem for nurserymen [36]. Moreover, salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment. Seed germination, seedling emergence and early survival are particularly sensitive to substrate salinity [37]. Studies showed that the germinated seed number and the germination percentage had an inverse relation with salinity of substrate [38].

The obtained results in Figure 4 & 5 showed that, soaking and irrigating bitter almond nuts with saline water at different salinity concentrations reduced both stem length and diameter by 1.7 and 1.3 times due to changing salt concentration from 1 to 5dsm^{-1}. This reduction was proportional to all salinity concentrations. In this respect, treatment of 5dsm^{-1} (3200 ppm) produced the greatest reduction in both values of stem length and diameter, followed by 3dsm^{-1} (1920 ppm) and 1dsm^{-1} (640 ppm). These treatments decreased the stem length and diameter without significant differences between salinity treatments in terms of stem diameter. The control and 3dsm^{-1} gave the best growth and had the greatest length and diameter, while the treatment of 5dsm^{-1} showed the lowest growth. The previous results agree with previous research [39] that concluded that tomato shoot height and stem diameter reduced under salinity stress caused by photosynthesis reduction, tissue expansion reduction, and cell division inhibition. However, tomato plant height reduced significantly after transplant under 4dsm^{-1} and 3dsm^{-1}, respectively [40]. With bitter almond, researchers noticed that increasing salinity resulted in reduction in shoot length [41]. Salt stress-induced abscisic acid (ABA) may inhibit cell cycle regulation [42]. In this regard, salinity induced early plant senescence as a result of the accumulation of toxic levels of some ions (Na+ and / or Cl–). This may be an adaptive mechanism to translocate excess amount of Na+ and / or Cl– out of younger leaves to the older leaves, keeping them away from the physiologically active tissue [43]. The control plants (non-stressed plants) did not show such decline in plant organs, likely due to the balanced ions composition in their tissue. They were able to remain physiologically active to the relatively longer period than the saline one.

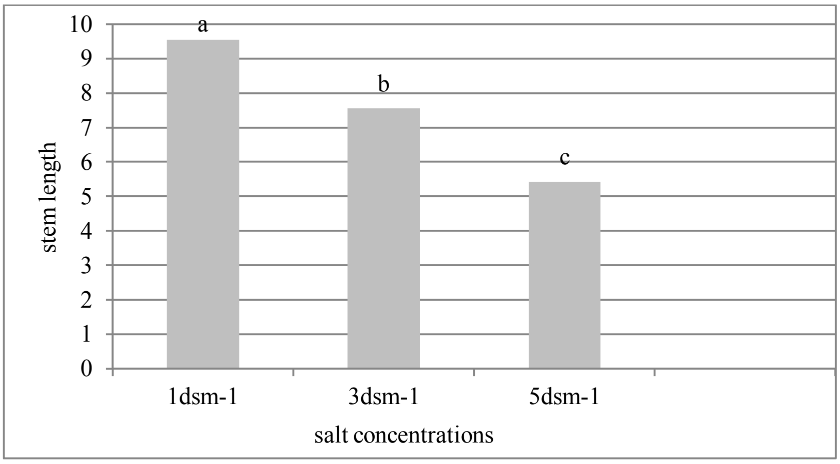

Fig. 4. Effect of salt concentrations on stem length of bitter almond seedlings

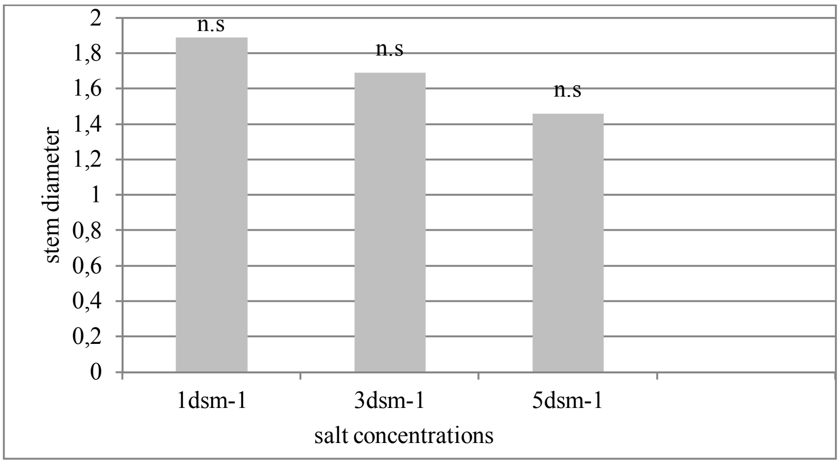

Fig. 5. Effect of salt concentrations on stem diameter of bitter almond seedlings

Regarding the number of secondary roots / plant of bitter almond seedlings affected by different salinity concentrations, data represented in Figure 6 revealed clearly that the two concentrations of saline solutions (3 and 5dsm^{-1}) exhibited an obvious increase in root quantity. Such an increase was significant compared to transplants irrigated with 1dsm^{-1}. Data show that the saline solution at 5dsm^{-1} concentration developed more secondary roots / plant than 3dsm^{-1}, and that the effect was highly significant. Previous studies mentioned that accumulation of Cl– or Na+ ions in the plant leaves affected stomatal closure, causing excessive water loss and leaf injury symptoms similar to those of drought damage [11]. Other researchers described similar conclusions such as root system increases in mango tree under water stress [44].

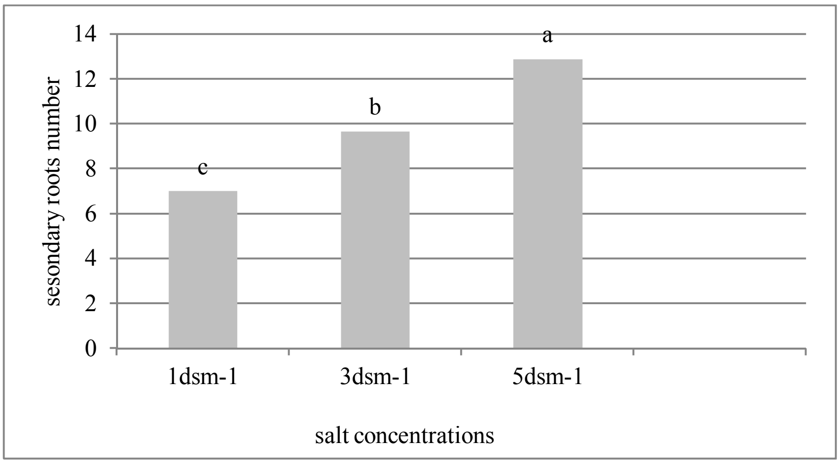

Fig. 6. Effect of salt concentrations on number of secondary roots of bitter almond seedlings

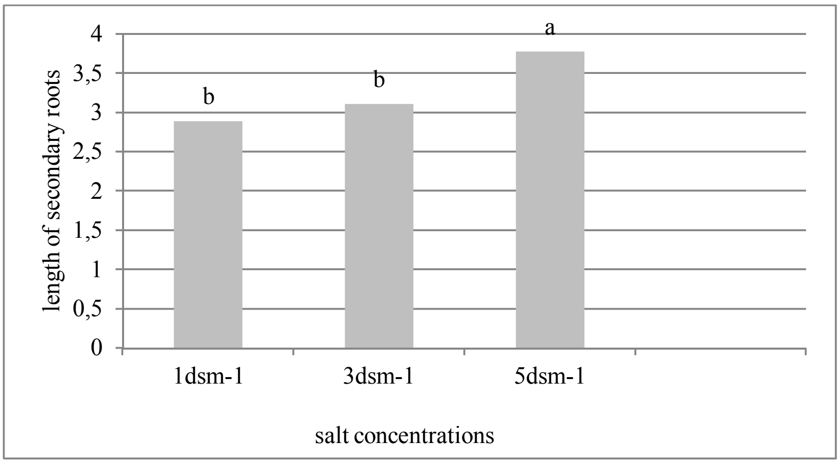

Fig. 7. Effect of salt concentrations on length of secondary roots of bitter almond seedlings

The length of secondary roots / plant during the early stages of bitter almond seedlings growth was significantly affected by salinity treatments as shown in Figure 7. The greatest increment of secondary roots length / plant was gained by those seedlings supplied by 5dsm^{-1} salt concentration with significant differences between 5dsm^{-1} and the other treatments. On the other side, bitter almond transplants that received 1 and 3dsm^{-1} of saline water irrigation produced the lowest length of secondary roots/plant without any significant differences between them. In contrast, when induced with salt stress by NaCl at 8 g/l, Tijib date palm cultivar showed the longest epicotyl length, principal root and more lateral roots compared to Nakhlahamra cultivar [6].

Relative to the effect of seed pre-treating and irrigation with different concentrations of salt solution, it is clear that the germinated nuts under high concentrations of salinity 5dsm^{-1} (3200 ppm) created strong seedlings which had a tallest length of primary root / plant, and also gave the highest number and length of secondary root / plant. Seedlings showed adaptation to salt stress and an ability to tolerate high salt concentration was increased, while other nuts which germinated under low salinity concentrations 3dsm^{-1} (1920 ppm) and 1dsm^{-1} (640 ppm) showed less resistance and gave short length and lower root values. Moreover, elongation of secondary and primary root / plant and number of secondary roots / plant markedly increased due to 5dsm^{-1} by 1.3, 1.7 and 1.8 times more than values in the lowest concentration. The results obtained in this work agree with these reported previously [45]. The results showed that applying of NaCl salt in germination stage could be used as an adaptation method to improve salt tolerance of seeds. There is also reasonably adequate information on the effects of salinity on germination and emergence [46].

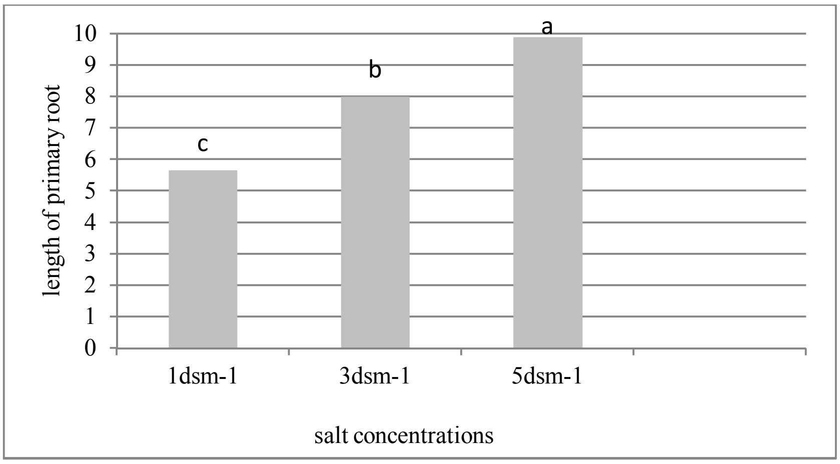

Fig. 8. Effect of salt concentrations on length of primary root of bitter almond seedlings

Concerning the vigor index of seedlings it was found that, on average, salinity treatments significantly increased the general vigor index of bitter almond transplants. Significantly, the highest vigor index was attributed to the 5dsm^{-1} treatment. Conversely, the transplants vigor index was decreased under low salt concentration and control treatments. This increase in the general vigor index under height salt concentrations may be attributed to the increase of primary roots. Under these conditions, the water hardly absorbed by the roots due to height osmotic stress in the root zone so that the primary root penetrated the layers of soil looking for water and nutrient elements. These results are in line with reported data showing that osmotic stress depressed the hypocotyls growth more than the radicle in seed germination stage of Four Wild Almond Species and root systems were more droughts tolerant [47]. In contrast, other researchers [48] found that all water saline treatments significantly decreased the vegetative growth parameters and total chlorophyll content.

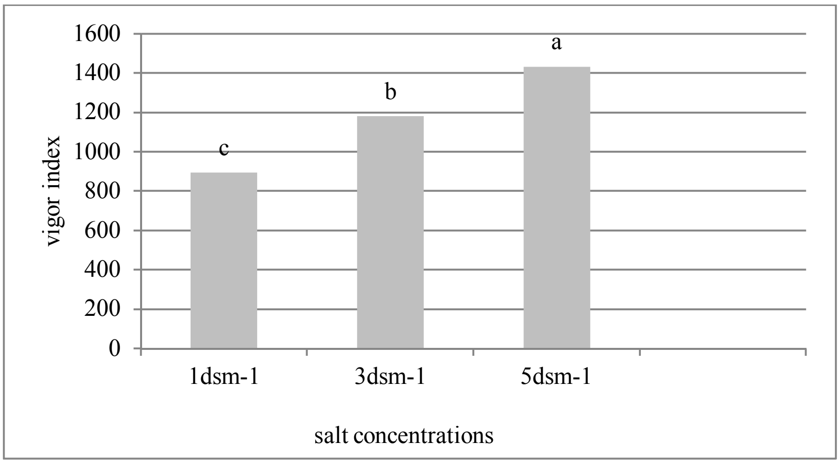

Fig. 9. Effect of salt concentrations on vigor index of bitter almond seedlings

Conclusion

From the previous results, soaking and irrigation of nuts and bitter almond transplants in early stages of growth with different concentrations of salt solution resulted in a decrease of germination parameters, stem length and diameter; due to osmotic stress of soil solution, nutrient deficiency, ion toxicity, tissue expansion reduction and cell division inhibition. Additionally, high salt concentration gave stronger seedlings which showed adaptation, and can resist high salinity concentrations throughout its tallest and deepest underground parts. For nurseries and growers, this study can conclude that seedlings of bitter almond will cultivate in saline soil and new reclaimed soil; that soils which face desertification dangers or that will be irrigated with diluted sea water or underground wells must be subjected to pre-treating and irrigation with saline water during germination and early stages of growth in nursery.

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АНАЛИЗ ВИДОВОГО СОСТАВА СЕМЕЙСТВА МАРЕВЫХ (*CHENOPODIACEAE* Vent.) ФЛОРЫ КАЛМЫКИИ

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Семейство *Chenopodiaceae* во флоре Калмыкии занимает по числу видов третье место после злаков и сложноцветных и пятое место по числу родов. Ведущими родами в семействе являются: *Atriplex* L., *Salsola* L., *Chenopodium* L., *Suaeda* Forssk., *Petrosimonia* Bunge. Приводится анализ видового состава семейства маревых (*Chenopodiaceae*) по типам ареалов, по отношению к засолению субстрата, по экобиоморфологическим особенностям. В пределах семейства отмечена различная экологическая пластичность видов и высокая специализация адаптаций к условиям опустынивания Калмыкии: микрофилия, галофильность, афильность, способность к перегреву и высокой солнечной инсоляции, летний покой и позднелетне-осенняя или же реже ранневесенняя вегетация.

Ключевые слова: семейство, род, доминанты, засоление, галофиты, жизненные формы, космополиты, ареал, коэффициент сходства

ANALYSIS OF THE SPECIES COMPOSITION OF THE CHENOPODIACEAE Vent. FAMILY FLORA OF KALMYKIA

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The family *Chenopodiaceae* in the flora of Kalmykia occupies the third place in terms of the number of species after *Poaceae* and *Asteraceae* and fifth in the number of genera. The leading genera in the family are: *Atriplex* L., *Salsola* L., *Chenopodium* L., *Suaeda* Forssk., *Petrosimonia* Bunge. The article provides an analysis of the species composition of the *Chenopodiaceae* family according to the types of areals, in relation to salinization of the substrate, according to ecobiomorphological features. Within the family, different ecological plasticity of species and a high specialization of adaptations to the conditions of desertification of Kalmykia are noted: microphilia, halophylicity, affinity, ability to overheat and high solar insolation, summer rest and late summer-autumn or less early spring vegetation.

Keywords: family, genus, dominants, salinization, halophytes, life-forms, cosmopolitans, area, similarity coefficient