RUDN Journal of Agronomy and Animal Industries
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО
2019; 14(4):319—328
http://agrojournal.rudn.ru

DOI: 10.22363/2312-797X-2019-14-4-319-328

Research article

# Influence of salinity on vegetative growth and photosynthetic pigments of bitter almond rootstock

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**Abstract.** Bitter almond rootstock is considered one of the most vital rootstocks for stone fruit species but it is classified as a plant sensitive to salinity. This experiment was carried out to study the effect of salt stress on vegetative growth and photosynthetic pigments of bitter almond rootstock as an attempt to sustain growth and increase its tolerance to high salt concentrations. However, the seeds were soaked in salt solution of NaCl as 1, 3, and 5 $dsm^{-1}$ for 48 hours before stratification. After that, nuts were sown in perlite and treated with different saline solutions subsequently stratified at 6 °C for eight weeks. Sprouted seeds were cultivated in pots with a mixture of peat and perlite and treated only with the highest salt concentration 5 $dsm^{-1}$. The treatments were arranged in a complete randomized block design with three replications. Vegetative traits and photosynthetic pigments content were estimated. The results revealed that soaking and pre-treating seed of bitter almond rootstock by means of high salt concentration 5$dsm^{-1}$ during the germination period and subsequently after planting produced stronger transplants that had hardening, adaptation and could avoid the hyperosmotic shock of salt stress after planting. It is obvious throughout; increment of stem diameter, plant height, total number of leaves\plant, fresh and dry weight of leaves, photosynthetic pigments and total carbohydrate content of such transplants. While other coming seedlings from low salt concentrations were exposed to hyperosmotic shock and salt injury therefore inhibit growth rate of such plants, increased falling of leaves and finally reduced photosynthetic pigments content in the resulting seedlings.

**Key words:** ion toxicity, salinity, NaCl, stratification, adaptation

**Article history:**
Received: 17 May 2018. Accepted: 2 September 2019

**For citation:**
Badran AM, Savin IY. Influence of salinity on vegetative growth and photosynthetic pigments of bitter almond rootstock. *RUDN Journal of Agronomy and Animal Industries*, 2019; 14(4): 319—328. doi: 10.22363/2312-797X-2019-14-4-319-328

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## Introduction

Salinity is one of the oldest environmental problems affecting approximately 30% of irrigation land. Salinity affects almost all stages of plant growth, involving emergence, vegetative growth and reproductive development [1]. NaCl is the most of the soluble salts in saline soil. Na ions are among the predominant exchangeable bases [2]. However, soil salinity causes ion toxicity, osmotic stress, nutrient (nitrogen, calcium, potassium, phosphorus, ferrous, and zinc) reduction and oxidative stress on plants. Moreover, the ions $Na^+$ and $Cl^-$ penetrate into cells of plants and may be accumulated in the vacuole for the tolerant plants or in the cytoplasm which in turn are known to be very toxic to the plant cell by injuring the cytoplasmic enzymes in sensitive cultivars [3]. Thus, salinity is considered to be one of major factors limiting sustainable agriculture.

The possibility of employing saline water in irrigation, as well as underground water, well water, or diluted sea water are taken into consideration as limiting factors for sustainability of agriculture in newly reclaimed lands. Regarding bitter almond (*Prunus amygdalus*) rootstock, Family: *Rosaceae* is counted as one of the most vital rootstock species for stone fruits. Whereas, it is mentioned to be drought resistant, the tolerance of almond rootstock to water stress is probably attributed to adaptive mechanisms that founded in their leaves or roots. These mechanisms involve osmotic adjustment, stomatal conductance reduce of transpiration water loss, leaf shedding acceleration, and root depth and density increase [4]. Added to that, almonds may be employed in semi-arid lands to control soil erosion and desertification because of their high adaptation to undesirable environmental conditions [5]. Although it is a vigorous and long-lived plant, almond trees are considered as a sensitive plant to salinity. Today, the preparation of fruit trees to tolerate salt stress is required to sustain agricultural production. This study aimed to elevate resistance and tolerance of bitter almond rootstock to salt stress in the soil and irrigation water.

## Material and Methods

The present investigation was carried out in 2016—2017 to study the effect of salt stress on vegetative growth and photosynthetic pigments content of bitter almond rootstock. Therefore, almond nuts require a cold stratification under humid conditions to emergence from dormancy and enhance germination. Hence, temperatures between 2 and 7 °C are markedly the most efficient [6].

Germination under saline conditions may be a rabid test to explore salt tolerant plants [7]. So, the seeds were collected from a vitality genotype at North Sinai Governorate, Egypt. Uniformly sized and vitality nuts were soaked in salt solutions as follows: 1, 3 and 5 $dSm^{-1}$ in two days, where ppm = Ec (ds/m) × 640, for Ec between 0.1 and 5 $ds/m^{-1}$ [8]. Salt solutions 1, 3 and 5 $dSm^{-1}$ were chosen in the consideration of general specifics of plant resistance for salinity [9].

Nuts were then cultivated in perlite and treated with different saline concentrations subsequently stratified at 6 °C for two months (15 October — 13 December, 2016). After cold treatment, non-germinated seeds were treated by 22 °C for 3 weeks (13 December, 2016 — 5 January, 2017) to enhance emergence. Three seedlings/replicate were obtained and cultivated in pots (15 × 20 cm) which had a mixture of peat and perlite, then treated with the highest salt concentration 5 $dSm^{-1}$ once/week at the end of February up to the end of august.

Measurements:

1. Stem diameter increment / plant (mm) was estimated using venire caliper as a following: The increment in stem diameter = stem diameter (at the end of August) – stem diameter (at the end of February).
2. The increment in plant height (cm) was measured as follows: The increment in plant height = plant height (at the end of August) – plant height (at the end of February).
3. Number of leaves / plants was counted.
4. The fresh and dry weight of leaves / plant (g) was measured.
5. Chlorophyll a, band carotenoids ($mg \cdot g^{-1}$) concentrations were determined as described by [10].
6. Total carbohydrate ($mg \cdot g^{-1}$ DW) was determined as described in [11].

The experiment was designed in a randomized complete block design with three replicates, and three plants for each. The obtained results were submitted to analysis of variance (ANOVA)-MSTAT software.

## Results and Discussion

Results obtained respecting the effect of nuts soaked and irrigated by different salinity concentrations on vegetative content and pigments content of bitter almond rootstock were presented in figures 1—6.

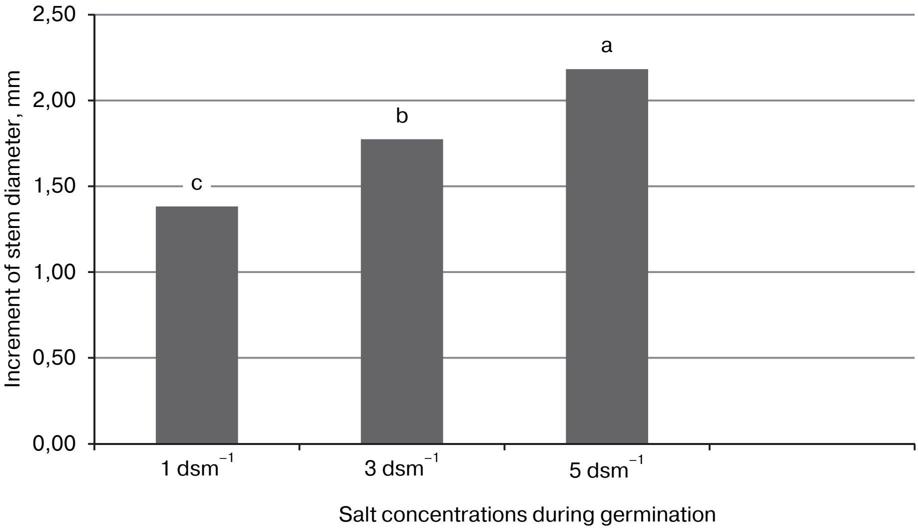


**Fig. 1.** Effect of salt stress on increment of stem diameter (mm) of bitter almond rootstock

Concerning the seedlings that germinated under low salt concentrations 1 and 3 $dsm^{-1}$ (Figure 1), it was found that irrigated such seedlings with height salt concentration 5 $dsm^{-1}$ after germination period caused a subsequent decrease in growth rate of stem diameter compared to the seedlings that already have been germinated under salt stress 5 $dsm^{-1}$ which gained the adaptation case in germination period and its ability to tolerate salt stress after planting was improved. The gained data in this study in the line with these mentioned early [12]. The data reported that using of NaCl in germination period would be applied as an adaptation method to promote salt tolerance of germinated plants. However, salinity inhibit the cell cycle transiently by reducing the expression and activity of cyclins and cyclin-dependent kinases, thus produce a fewer cells in the meristem, so limiting growth [13]. Additionally the previous research [14] demonstrated, that the growth reduction was attributed to sever changes in the ion relationships of plants as a result of the contrary effect of Na and Cl ions on metabolism or from deranged water relations.

Also, soaking and treating seeds of bitter almond by low salt concentration 1 and 3 $dsm^{-1}$ before planting positively increased the stem length of bitter almond seedlings compared to the highest salt concentration 5 $dsm^{-1}$. Nevertheless, these seedlings when irrigated with high saline solution 5 $dsm^{-1}$ during the growth period, the growth rate was aggressively decreased as a result of the adverse effects of salt stress 5 $dsm^{-1}$ (Figure 2). In this respect, the highest growth and the best length of plant was obtained when the seeds were irrigated with 5 $dsm^{-1}$ during the germination period and later after planting. Generally, the reduction in stem length and diameter means a depression in plant growth. This depression could be attributed to inhibit cell division and cell elongation under conditions of increasing salinity at high salt concentration. This explanation agrees with that reported in [15] on peach seedlings. Salinity promoted abscisic acid which may inhibit cell cycle regulation [16]. The studies demonstrated, that increasing salt stress caused reduction in shoot length of bitter almond [17].

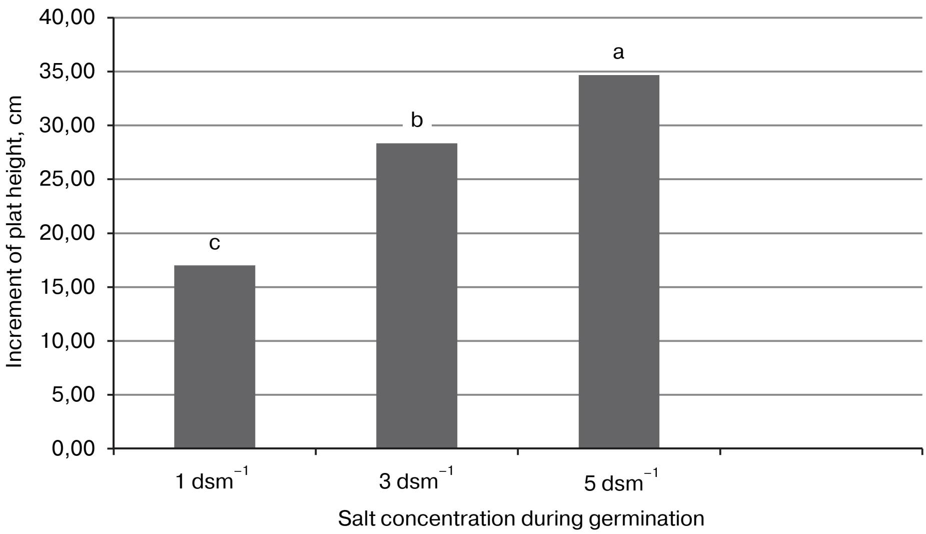


**Fig. 2.** Effect of salt stress on (the) increment of plat height (cm) of bitter almond rootstock

Regarding the seedlings that germinated under low salt concentrations 1 and 3 $dsm^{-1}$, it was found that irrigation of those seedlings in high salt solution 5 $dsm^{-1}$ during the growth period caused the increase in leaf falling as a result of the hyper osmotic shock of salt stress which in turn marked the decrease in the total number of leaves/plant of bitter almond rootstock (Figure 3). In this respect, the highest number of leaves/plant was obtained when the seeds were irrigated with 5 $dsm^{-1}$ during the germination period and subsequently irrigated with the same concentration during the growth period. It can be concluded from the previous results, that soaking seeds of bitter almond rootstock in high saline solutions before planting lead to adaptation the coming seedlings to salt stress in irrigation water and having more number of leaves compared to those plants which did not/or received low concentrations of salinity. Additionally, salinity accelerates plant senescence and fallen of leaves as a result of the excessive toxic levels of $Na^+$ and/or $Cl^-$ ions. This can be an adaptive way to translocate accumulated amount of $Na^+$ and/or $Cl^-$ out of juvenile to the older leaves, reserving them away from the physiologically active tissue which in turn resulted in fallen of older leaves [18].

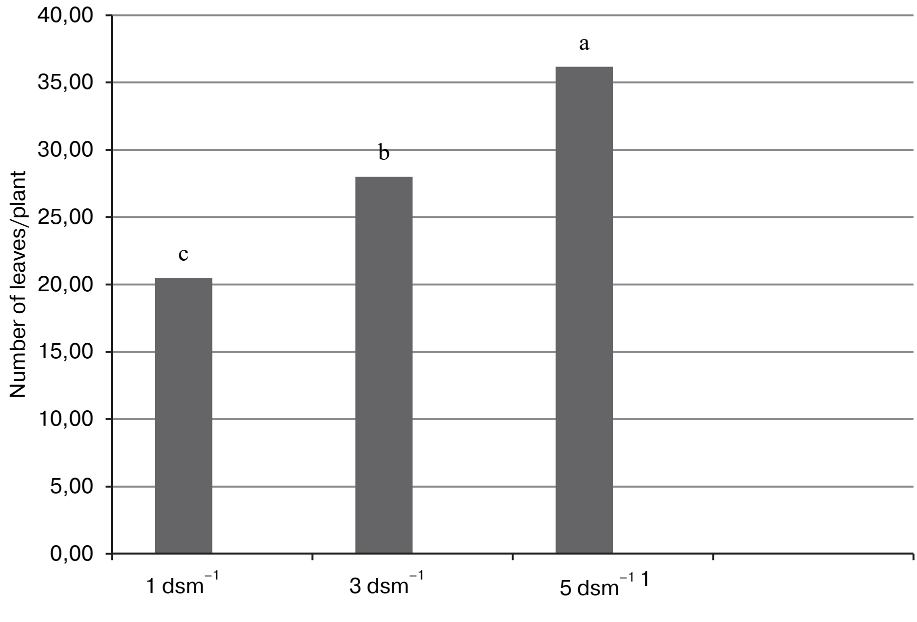


**Fig. 3.** Effect of salt stress on number of leaves/plant of bitter almond rootstock

The data in Figures 4 and 5 demonstrated that, soaking and treating the seeds of bitter almond with salt concentrations reduced both fresh and dry weight of leaves/plant due to changing salt concentration from 1 and 3 $dsm^{-1}$ in germination period to only 5 $dsm^{-1}$ after germination. This reduction was proportional to the salt level. Additionally, irrigating by 5 $dsm^{-1}$ in germination period and later during the vegetative growth period produced the highest increment in both of fresh and dry weight of leaves/plant. In this regard, the previous researches [19] found that the deleterious consequences of high salt concentrations caused in hyper osmotic shock and ionic imbalance effect in plant cells.

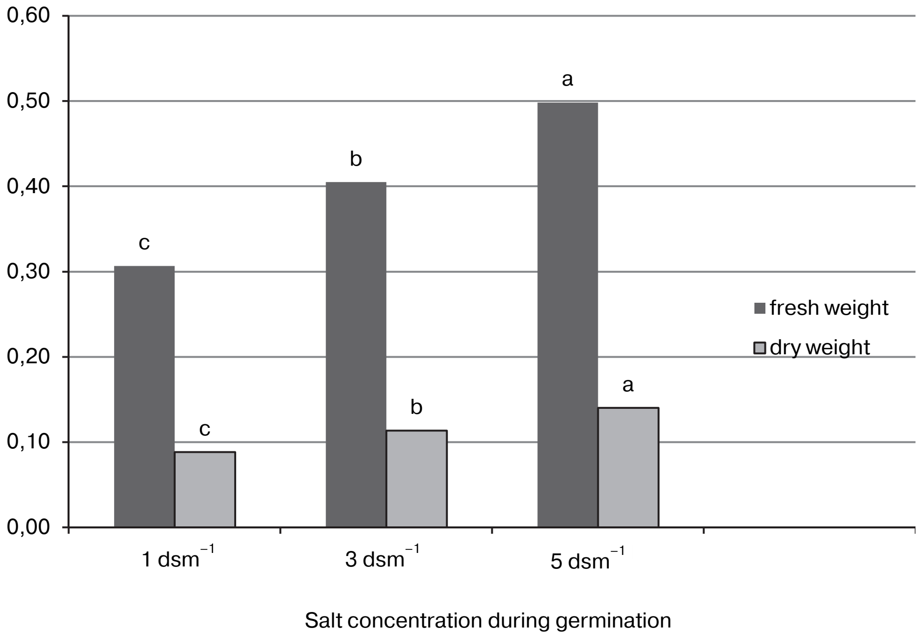


**Fig. 4.** Effect of salt stress on fresh and dry weight of leaves/plant (%) of bitter almond rootstock

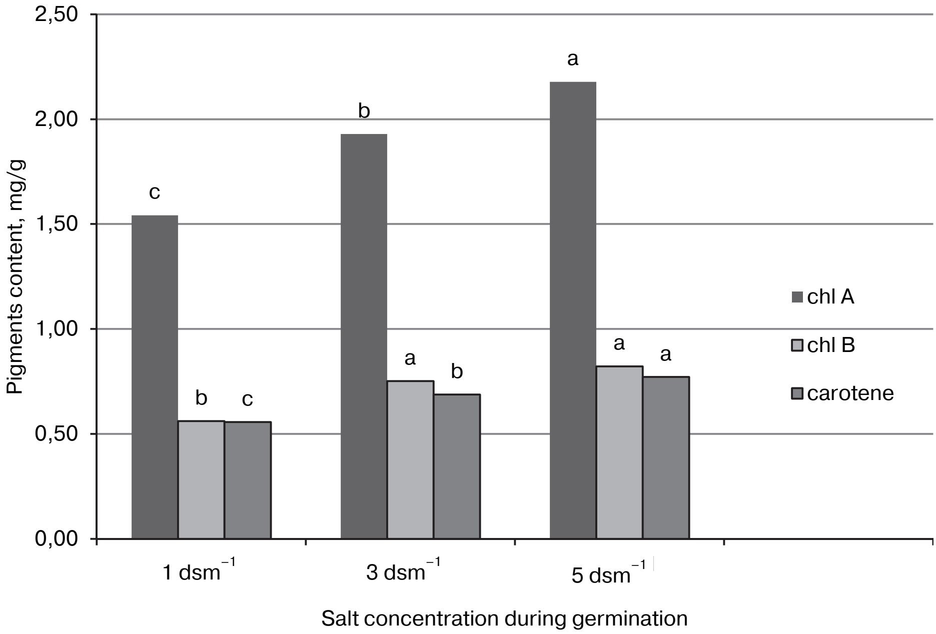


**Fig. 5.** Effect of salt stress on photosynthetic pigments content (mg/g) of bitter almond rootstock

The results presented in Fig. 5 show the effect of pre-treating seed by means of various salt concentrations during the germination period subsequently by 5 dsm$^{-1}$ after planting on photosynthetic pigments content of bitter almond rootstock. It was found that, the resulting seedlings from high salt concentration 5 $dsm^{-1}$ had hardening and adaptation and could avoid the hyper osmotic shock of salt stress and thus positively reflected on vegetative growth and chlorophyll a, band carotenoids content in the leaves of plants. While other coming seedlings from low salt concentrations were exposed to hyper osmotic shock and salt injury which in turn inhibited growth rate of plants, increased falling of leaves and finally reduced photosynthetic pigments content in the plants. Researchers noted that excess ions of $Cl^-$ or $Na^+$ in the plant leaves influenced stomatal closure, causing excessive water evaporate and leaf injury symptoms as happened in drought damage [20]. In this regard, salinity promotes the synthesis of abscisic acid which in turn closes stomata when translocated to guard cells. As a result of stomatal closure, photosynthesis decline and oxidative stress conducted. Additionally, the reduction of photosynthetic pigments in stressed transplants can be attributed to the decrease in the uptake of elements required for chlorophyll biosynthesis (i.e., iron and magnesium) [21], or throughout inhibition of chlorophyll synthesis [22].

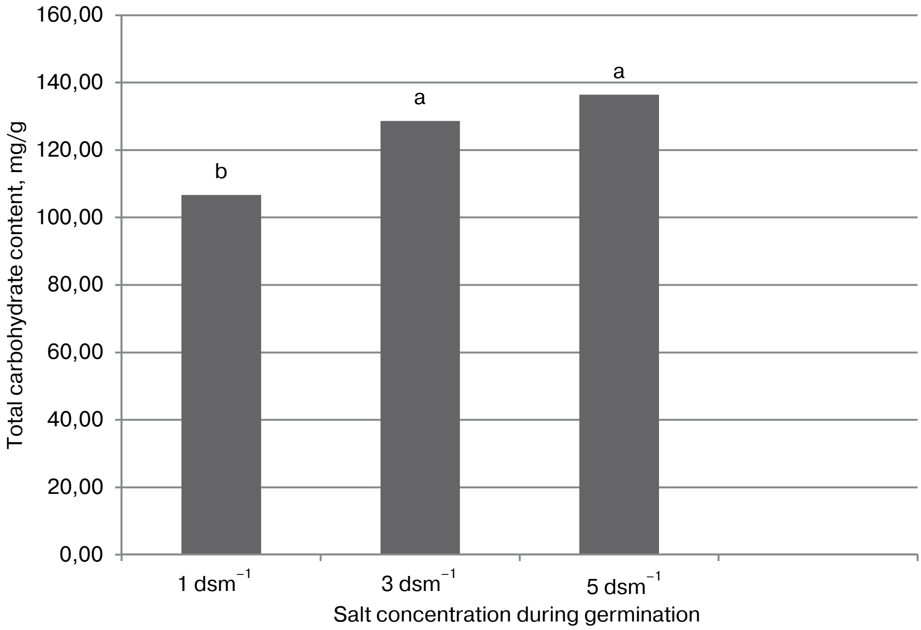


**Fig. 6.** Effect of salt stress on total carbohydrate content (mg/g) of bitter almond rootstock

Concerning the effect of nuts soaking in saline solutions at the stratification period and before planting, it increased the total carbohydrate content of bitter almond rootstock. It is therefore clear that the germinated seeds under high concentration of salinity 3 and 5 $dsm^{-1}$ produced seedlings which had adaptated to salinity in irrigation water 5 $dsm^{-1}$ after planting. It resulted in enhanced vegetative growth, increased number of leaves, leaf area and total chlorophyll content which in turn increased the rate of photosynthesis process, subsequently accumulation of total carbohydrate in the plants. While, the other seeds that germinated under control treatment showed less resistance and significantly gave the lowest value of total carbohydrate content in the plants.

## Conclusion

Increasing the salt concentrations from 1 and 3 $dsm^{-1}$ in soaking and stratification periods up to 5 $dsm^{-1}$ after planting resulted in decrease of vegetative growth and photosynthetic pigments due to hyper osmotic shock and salt injury of salt stress. On the other hand, pre-treating and irrigation of bitter almond transplants with high saline solution 5 $dsm^{-1}$ produced adapted and stronger seedling and thus positively reflected on vegetative traits and photosynthetic pigments. For growers and nurseries, this investigation can recommend to cultivate bitter almond seedlings under saline conditions or in new reclaimed soil; that's soils which face desertification dangers or that will be irrigated with diluted sea water or underground wells should be subjected to soaking and irrigation with saline water during germination period and early stages of growth in nursery.

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DOI: 10.22363/2312-797X-2019-14-4-319-328

Научная статья

# Влияние засоления на вегетативный рост и фотосинтетические пигменты горького миндаля

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**Аннотация.** Горький миндаль является одним из наиболее используемых в качестве подвоя сортов косточковых плодовых культур, однако отличается чувствительностью к солевому стрессу. Проведены опыты по изучению воздействия различных уровней засоленных поливных вод на вегетативный рост и фотосинтетические пигменты горького миндаля с задачей поиска метода увеличения его устойчивости к засолению. Орехи до стратификации замачивали в растворе NaCl различной степени: 1 $dsm^{-1}$, 3 $dsm^{-1}$ и 5 $dsm^{-1}$ за 48 ч. Затем семена посеяли в перлит и в течение 8 недель при температуре 6 °C поливали раствором NaCl различной степени. Проросшие семена высаживали в горшки со смесью торфа и перлита и поливали водным раствором с самой высокой концентрацией соли 5 $dsm^{-1}$. Проведены полные блочные рандомизированные исследования с троекратным повторением. Результаты показали, что замачивание и орошение семян горького миндаля водой с высоким концентратом соли 5 $dsm^{-1}$ в период прорастания, а затем после посадки приводило к большей силе рассады с наилучшей адаптацией к солевому стрессу после посадки, что четко выявляется по таким параметрам, как прирост диаметра стебля, высота растения, общее количество листьев/растений, свежая и сухая масса листьев, количество фотосинтетических пигментов и общее содержание углеводов. При меньшей концентрации соли саженцы подвергались гиперосмотическому шоку, поэтому солевые травмы препятствовали росту таких растений, происходило увеличение падения листьев и снижение содержания фотосинтетических пигментов в полученных саженцах.

**Ключевые слова:** NaCl, ионная токсичность, засоленность, стратификация, адаптация

**История статьи:**

Поступила в редакцию: 17 мая 2018 г. Принята к публикации: 2 сентября 2019 г.

**Для цитирования:**

Badran A.M., Savin I.Y. Influence of salinity on vegetative growth and photosynthetic pigments of bitter almond rootstock // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2019. Т. 14. № 4. С. 319—328. doi: 10.22363/2312-797X-2019-14-4-319-328

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