Plant Responses to Individual and Combined Effects of Abiotic Stress: *Lycium depressum* L. Vegetative Parameters under Salinity and Drought

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Abstract. *Lycium depressum* L. is the only native tree-like life-form species inhabited in saline and alkaline regions of Turkmen Sahra located at Golestan province in Northern Iran. During past years, efforts have been made to increase vegetation cover of the area by cultivation of *L. depressum* L. to reduce water and wind erosions and dust storm challenges; however, the cultivation of this species has not been quite successful. Regarding the importance role of *L. depressum* in the ecosystem, a greenhouse experiment was conducted in January 2018 with different levels of salinity and drought stresses. Salinity stress (control (4 dS/m), 14, 24, 34 and 44 dS/m) applying NaCl solution and drought stress (control (0), 0.25, 0.50, 0.75 and 1 MP) applying PEG 8000 as well as their combinations were conducted on plant cuttings (clones) using factorial experiment and Response Surface Method (RSM). Data were collected for leaf number (LN), root length (RL) and plant height (PH). Through the findings, it was concluded that drought stress had higher negative effect on plant function than salinity stress which dramatically reduced LN, RL and PH parameters (P<0.05). It was also inferred that combined treatments had higher negative effects on plant function than the individual treatments. Additionally, the result showed a significant difference between aerial organs and underground organs with regard to the severity of being affected by salinity and drought stresses so that LN and PH were highly affected comparing with RL. Generally, we observed that higher levels of salinity (higher than 30 dS/m) and drought (higher than 0.25 MP) will adversely affect the growth of plant organs leading to reduction of plant yield; even in some cases, it causes the plant total death. Finally, it was concluded that *L. depressum* is highly affected by drought rather than salinity.

Key words: *Lycium depressum* L., Salinity, Drought, RSM Analysis, Abiotic Stress
Introduction

Plants live in two different conditions including soil and atmosphere which continuously change through the time. Under biotic stresses consisting of pathogen infection and herbivores attacks and abiotic stresses such as drought, salinity, heat, chilling, oxidative stresses, nutrient deficiency (Awasthi et al., 2014; Carmo-Silva et al., 2012), and flooding, toxic elements included aluminum, arsenate and cadmium (Mahajan and Tuteja, 2005) condition for plant growth and development is unfavorable and stressful. As the most important and prevalent environmental factors, salinity and drought stress affect the distribution of plants in the nature and restrict plant yield and function as a result, it threatens the ecosystem sustainability (Khan et al., 2017; Fedoroff et al., 2010; Zandalinas et al., 2018). The influence of these factors is even higher in arid and semi-arid regions of the world. Regarding the fact that a vast area of Mediterranean region is under the influence of arid and semi-arid climate (Barlow et al., 2016; Saadi et al., 2015), salinity and drought stress are typical phenomenon of this region which dramatically decrease plant production (Munns and Tester, 2008; McNutt, 2014), leading to a wide range of responses through plant species including morphological, physiological, biochemical and molecular alteration (Cvikrová et al., 2013; Danquah et al., 2014; Gilroy et al., 2014).

According to recent global climate change model, the probability that plants encounter new and even extremer abiotic stresses in the future is higher than what had been predicted previously (Rizhsky et al., 2004; Suzuki et al., 2014). On one hand, it was shown through a huge body of research that interaction effects of stresses such as drought-heat, salinity-heat, drought-salinity, ozone-salinity, ozone-heat, nutrient-drought, nutrient-salinity, UV-heat, UV-drought and high light intensity-heat are higher than their individual effects on plants (Mittler and Blumwald, 2010; Suzuki et al., 2014). On the other hand, many studies have shown beneficial effects of interaction influences of different stresses which were simultaneously imposed (Suzuki et al., 2014). The stress matrix generated by Mittler (2006) and updated by Mittler and Blumwald (2010) and Suzuki et al. (2014) is shown in Fig. 1.

Fig. 1. Environmental stress interactions and their effects on plant (Mittler and Blumwald, 2010; Suzuki et al., 2014)
In a study conducted on cultivated barely as an example which showed either salinity or drought have reduced plant growth, chlorophyll content, photosynthesis rate, water potential and osmotic potential while application of both stresses simultaneously had higher negative influence on aforementioned parameters than individual state (Ahmed et al., 2013). In contrast, while imposing stresses individually has negative effect on plants, the combination effects of some stresses act in the favor of them. For instance, the combination of ozone and drought stresses on plant, in which reduction of stomatal conductance caused by drought stress occurred, could decrease ozone uptake through stomatal (Iyer et al., 2013).

Assessing the effects of abiotic stresses on plants function and production has a significant role particularly regarding plantation of plants in natural lands in which the success of plant cultivation operation is highly dependent on understanding the water, soil and plant relations and the interactions within stresses and plant responses (Ahmad et al., 2011).

Turkmen Sahra, located at northern part of Golestan Province in Northern Iran and southern part of common border between Iran and Turkmenistan, is the region for one of the most saline and alkaline plain in Iran. The region contains a salinity ranges between 0 dS/m from eastern part of the region increasing toward 80 dS/m or even higher (Akhani, 2004) at western part of the province adjacent to Caspian Sea. Salinity stress goes hand-in-hand with drought stress in the region contributed to the restriction of growth and distribution of plants. Since part of Turkmen Sahra has altered into desert condition in previous years (FRWMO\(^1\), 2017), the occurrence of water and wind erosion and dust storm phenomenon have led land managers, experts and scientists of natural resources to be worried about the situation and coming up with strategies and solutions to reduce the rate of erosion and increase soil stability. Rehabilitation of region with plantation of native species tolerant to salinity and drought stress is the most practical solution to combat with the aforementioned challenges. Moreover, it has been cited through many studies that pastoralists inhabited in this region encounter many obstacles due to lack of vegetation cover and poor ecosystem services (Sharifiyan Bahraman et al., 2014a, 2014b; Niknahad-Gharmakher and Sharifiyan Bahraman, 2017). Although the region has the area of 13353 km\(^2\), the fact that only 130 plants mostly annual species exist in the region has made Turkmen Sahra a poor region with regard to plant biodiversity and vegetation cover. In these circumstances, selection of a compatible and native species for plantation is even harder.

*Lycium depressum* L. from *Lycium* genus and Sloanaceae family is the only native perennial tree-like species in the region which is salt tolerant and it has been reported as a drought tolerant plant as well. Recently, this species had been planted to promote vegetation cover by Golestan Natural Resources and Watershed Management office in Iran. Although *L.depressum* L. has some habitats in Turkmen Sahra and its reproduction occurred naturally, plantation and establishment of the species have faced many problems and conducted project in 2017 with plantation of 20000 plant cuttings had faced failure.

Regarding the role of this species in conservation of water and soil and preventing erosion in Turkmen Sahra, this study was aimed to assess combined and individual effects of salinity and drought stresses on vegetative parameters of *L. depressum* L.

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1- Forest, Rangeland and Watershed Management Organization of Iran
Material and Methods

Study area
In order to assess the effects of salinity and drought stress on *Lycium depressum* L., 300 homogenous and healthy clones were selected from the species habitat in Gonbad Kavus county rangelands located in 37° 37' 51" N and 54° 49' 43" E at Golestan Province, Iran in January 2018. Clones were transported into a greenhouse at Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. The geographical location of Turkmen Sahra in Asiatic Continent and Iran country is showed in Fig. 2.

![Fig. 2. Turkmen Sahra geographical location in Asia and Iran](image)

Plant Material and Growth Conditions
Prior to cultivation, whole the clones were soaked into Indole-3-butyric acid 3000 ppm for 5 seconds. Then, clones were grown in pots (size: 20-30 cm) containing a mixture of sand and garden soil (2:3 and 1:3 respectively). Clones were irrigated each six days for 60 days for root growth period. Mean temperature during the experiment ranged from 21°C in January to 27°C in May. Afterward, among 300 rooted clones, 150 were selected for uniformity from the stuck for the experiment and were planted in plastic pots size 12 cm. Prior to transporting clones into plastic pots, primary leaf number (PLN), primary root length (PRL), primary plant height (PPH) and primary branch number (PBN) were recorded as vegetative parameters to assess their responses to salinity and drought stresses (Álvarez and Sánchez-Blanco, 2014; Cirillo et al., 2016; Álvarez and Sánchez-Blanco, 2105; Salachna and Piechocki, 2016).

Five levels of salinity as (control (4dS), 14, 24, 34 and 44 dS/m) using NaCl solution and five levels of drought stress as (control (tap water), 0.25, 0.50, 0.75 and 1 MP) using PEG 8000 were assessed using a factorial experiment based on a completely randomized design with six replications. Therefore, we had 25 treatments with six replications, generally
giving us 150 pots. The treatments were continued for 75 days. By the end of experiment period (end of May 2018), vegetative parameters were measured again. Finally, the differences between secondary and primary leaf number (LN), secondary and primary root length (RL) and secondary and primary plant height (PH) were calculated as final indices to assess plant morphological responses to salinity and drought stress (Álvarez and Sánchez-Blanco, 2015; Salachna and Piechocki, 2016). Due to the lack of difference between primary and secondary branch numbers, this parameter was eliminated.

**Statistical Analysis**

Data analysis was done using Two-way Analysis of Variance (ANOVA), to compare the main and interactions effects (which were 25 treatments). Then, response surface analysis method (RSM) was applied to determine the most optimized level of salinity and drought stress for species to have higher LN, RL and PH. Using this method, individual treatments of salinity and drought stress were compared with each other predicting interaction effects of combined salinity and drought on plant. Data were analyzed using SPSS version 25, rsm package in RStudio software and Excel version 2019. Pictures regarding *Lycium depressum* L. morphology, its flower and fruits are shown in Fig. 3.

**Result**

**Leaf number (LN)**

Regarding *L. depressum* L., results showed that LN was dramatically reduced by increasing salinity and drought levels. As it is shown in Fig. 4, LN was negatively affected by drought stress rather than salinity stress. Comparing the amount of LN in treatments which salinity level is constantly 4 dS/m and drought stress increases from 0 to 1 MP, it can be noticed that LN is highly reduced while in treatments where drought stress is constantly 0 MP and salinity stress
increases from 4dS/m toward 44 dS/m, LN did not have a noticeable reduction. Nonetheless, there were significant differences between salinity treatments of 4dS/m, 14dS/m, 24dS/m and 34dS/m and 44dS/m without drought stress (p<0.05). The higher value of LN was obtained in 4dS/m during the experiment. Therefore, it cannot be expected that *L. depressum* L. has high tolerance to drought owing to the fact that it can tolerate high amount of salinity. In our experiment, it was also showed that the combined treatments of salinity and drought had significantly higher negative effects than their individual effects on LN (Fig. 4).

![Leaf number (LN)](image_url)

**Fig. 4.** Leaf number of *Lycium depressum* L. with different levels of salinity and drought stress.

A 3D-graph of the way at which salinity and drought affect LN is shown in Fig. 5. We only used the data from treatments contained drought and salinity stress and we did not enter the data of combined effects. So, we could predict the combined effect of salinity and drought through RSM analysis. Based on the result of this graph, it is predicted that the most optimized level of salinity and drought (red cells) which will lead to the highest amount of LN would be 20dS/m without drought stress. This graph also shows that LN is more sensitive under drought stress than salinity stress. Purple, blue, green, yellow and orange colors show the decline in leaf number, respectively. This spectrum is right for root growth and plant height RSM graphs as well.
**Root Length**

Assessing of RL indices showed that root length reduced with increasing of salinity and drought levels. Considering the result of Fig. 6, root length is affected by drought stress higher than salinity stress. It was also depicted that a combination of salinity and drought stress has higher negative effect on RL than their individual effects. Comparing the mean of different groups of treatments, it can be noticed that RL would not decrease highly if drought stress stayed constantly 0 MP even by increasing salinity stress up to -0.5MP.

![Root length graph](image)

**Fig. 6.** Root length of *Lycium depressum* L. with different levels of salinity and drought stress

RSM result also showed that RL dramatically decreased by increasing salinity and drought stress. The root production of plant was stopped in 44dS/m coupled with -0.75 MP and 44dS/m-1.00MP treatments and some cases led to
death of the plant (Fig. 6). Comparing the result of Fig. 6 and RSM analysis (Fig. 7), it can be noticed that RSM is successful in predicting the combined effects of salinity and drought stress.

![Graph showing interaction effects of salinity and drought stress on root growth](image)

**Plant Height (PH)**
Comparison of different treatment groups on plant height (PH) showed that similar to LN and RL indices, plant height was also decreased significantly with increasing salinity and drought stress. In other words, PH had not increased in treatments with high salinity and drought levels and it had not been increased in proportion to primary plant height.

As it is illustrated in Fig. 8, it can be noticed that PH is influenced by drought stress more than salinity stress. According to the result, with drought stress increasing from 0 MP toward 1MP at all salinity levels (4, 14, 24, 34 and 44 dS/m), PH had significantly decreased, except for treatments 34dS and 44dS which drought stress did not impose; PH did not decrease noticeably but a significant difference was observed in 44dS/m compare to other treatments.
Fig. 8. Plant’s height of *Lycium depressum* L. with different levels of salinity and drought stress

3D-graph of interaction between salinity and drought stress and PH response is shown in Fig. 9. Considering the result of RSM analysis, it can be predicted that the most optimized level of salinity and drought stress for plant to have higher amount of PH is 10-20dS/m-0 MP.

Fig. 9. Interaction effects of the salinity and drought stress on plant height

**Discussion**

**Individual effects of salinity and drought stress**

It was shown through the findings of this research that vegetative parameters were affected by drought stress significantly but they were not affected by salinity as much as they did under drought stress. Previous studies have concluded that species with high salinity tolerance are not necessarily tolerant to drought as well and vice versa (Bhatnagar-Mathur *et al.*, 2007; Kefu *et
Initial responses of plant to salinity and drought stresses were fundamentally as same as each other (Munns, 2002). Based on the result, the higher the amount of salinity and drought were, the lower the rate of LN, RL and PH were. It can be inferred that both stresses resulted in a physiologic water deficiency which more or less affect all plant organs. However, salt stress gradually imposes in a long period in addition to water loss, plant response to ionic and osmotic stresses as well. In addition to the changes occur in photosynthesis and cell growth, both salinity and drought stress, when slowly imposed, influence osmotic adjustment which is considered as an important mechanism in preserving and maintaining water uptake and cell turgor under stress condition. Effects of salinity and drought stress on photosynthesis range from the limitation of CO₂ diffusion into chloroplast by restrictions generated on stomatal opening mediated by shoot and root-generated phytohormone and on the mesophyll transport of CO₂ to alterations in leaf photochemistry and carbon metabolism. These effects are different depending on severity and duration of stress, leaf age (older leaves are influenced by drought stress and accumulation of salt more than younger leaves) and type of species (Flexas et al., 2004).

Photosynthesis and cell growth are the first processes which are influenced under drought (Chaves, 1991) and salinity stress (Munns et al., 2006). The impacts of salinity and drought could directly reduce available CO₂ owing to restriction of diffusion through stomatal and mesophyll (Flexas et al., 2004; Flexas et al., 2007) or alteration of photosynthetic metabolism (Lawlor and Cornic, 2002) or secondary effects called oxidative stresses appear which generally generated under multiple stresses (Chaves and Oliveira, 2004) and can drastically affect leaf photosynthesis mechanism (Ort, 2001).

Plant photosynthesis responses faced to salinity and drought stress are complex including interactions of restriction in different part of cell/leaf and different time related to development of plant. Severity, duration and rate of stress expansion affect plant responses in water deficiency and salinity stress condition on account of the fact that these factors determine whether reducing processes regarding acclimation will happen or not. Acclimation responses under drought condition which indirectly influence photosynthesis consist of those factors restricting growth or leaf abscissions which will help to preserve and maintain water with restricting water consumption through source tissues leading to preservation of carbon uptake. Osmotic compounds, which are gradually built by slowly imposed dehydration, are also affective in sustainability of tissue metabolism. Acclimation responses against salinity include synthesis of compatible solutes along with ion transportation (such as uptake, extrusion and ion sequestration). These responses lead to cellular homeostasis, toxic elicitation and finally plant survival in stress condition.

**Differences among vegetative parameters**

The result showed that severity in which aerial organs such as leaf number and plant height are affected under the salinity and drought stress is higher than root length. Root is the first organ of the plant which is affected by salinity and drought stress resulted in decreasing plant growth and production in short and long term due to reduction of available water and ionic toxicity caused by acclimation of salt (Munns, 2005).

After imposing salinity and drought stress particularly in higher levels, abscission of leaves was appeared continuously. Many mature plant species respond to drought stress with accelerating senescence and abscission of the older leaves (Gepstein and Glick, 2013). This process is also known as leaf area adjustment while root system continues its growth (Sharp et al., 1988).
Leaves growth has higher sensitivity facing abiotic stresses than root growth. For instance, osmotic stress in moderate level can restrict stems and leaves growth (Bartels and Sunkar, 2005). Although the reduction in leaf area under dehydration might lead to decrease of photosynthesis rate, decline of transpiration in plant is considered as a beneficial occurrence. On the other hand, in a climate which is proportionally arid, this strategy used by the plant is not always practical on account of evaporation occurred in soil surface leading to lack of water available for root system (Tardieu, 2005).

It has been proved by many researches that plants aerial organs are affected by salinity more than underground organs. For instance, reduction in leaf number or leaf abscissions could be considered as the main morphological characteristic of a plant affected by salinity. Formation of a certain type of Cl in leaves under salinity stress could stimulate synthesis aminocyclopropane-1-carboxilic acid (ACC) and convert it to ethylene with high efficiency which cause tremendous amount of stimulating phytohormone of leaf abscission (Tudela and Primo-Millo, 1992; Dodd, 2005). This conversion of ACC into ethylene has been approved in case of halophytes plants as well (Chrominski et al., 1998). Studies have also shown that salinity stress in primary step caused increasing of leaf abscission to prevent acclimation of toxic ions. An indirect effect related to initial acclimation of abscisic acid (ABA) and reduction of indol-3-acetic acid (IAA) and cytokinin (CK) lead to increasing of leaf senescence under salinity stress. However, ACC is the main phytohormone signal related to oxidative damage and reduction of chlorophyll fluorescence causing extreme acclimation of Na volume (Albacete et al., 2008; Ghanem et al., 2008).

One of the reasons of leaf number reduction is protective reaction of the plant with the aim of reducing maximum transpiration from aerial organs (Savé et al., 1994; Ruiz-Sánchez et al., 2000). This reaction favors acclimation of toxic ions in the roots which leads to reduction of aerial organs particularly leaves (Munns and Tester, 2008; Colmer et al., 2005). Alteration of cell wall properties is a normal phenomenon under salinity stress resulting in the declining of leaf turgor and photosynthesis rate, ultimately reducing leaf number and leaf area (Franco et al., 1997; Rodríguez et al., 2005). Furthermore, stem growth (as an aerial organ) decreases under increasing of salinity concentration. Reduction in leaf number and stem length stimulates the decline of other aerial organs sizes, finally decreasing plant height. Increment of the root-to-shoot length ratio or decreasing the shoot-to-root length ratio is a normal response from plant side to salinity stress which is more related to water deficiency rather than certain type of salt (Hsiao and Xu, 2000). Higher amount of root-to-shoot length ratio could be beneficial for maintaining toxic ions in this organ and controlling the transportation of this material into aerial organs. This response is a natural mechanism response from the plant for surviving under salinity stress (Cassaniti et al., 2012; Cassaniti et al., 2009).

**Combined effects of salinity and drought stresses**

The result showed that in all parameters, the effects of drought and salinity stress were high when they were conducted together. In other words, *L. depressum* L. was affected by combined effects of salinity and drought more than individual state. It has been proved by a huge body of research that combination of salinity and drought stress has higher negative effect on vegetative parameters than their individual effects (Sahin et al., 2018; Suzuki et al., 2014; Zandalinas et al., 2018) especially in natural ecosystems where condition cannot be gotten under control and many factors simultaneously affect the plant growth.
Conclusion
It is a widely accepted fact that cultivation and reproduction of plants in natural circumstances are quite different from under controlled condition such as agricultural lands or greenhouses. The effect of biotic and abiotic stresses does not impose separately and individually; in fact, many factors are effective on plant growth. Among all the limiting factors existing in Turkmen Sahra rangelands, salinity and drought stresses are the most apparent factors which can have restricting effects on flora of this region. Since *Lycium depressum* L. had been planted in the region in different sites with the aim of increasing the vegetation cover and rehabilitation projects used this plant had failed, this study was conducted to assess restricting factors affecting plant establishment and growth.

As it was shown through the findings, *Lycium depressum* L. is more sensitive to drought stress than salinity stress and it does not have the ability to tolerate high amount of salinity. Effects of drought stress on plant had been multiplied with salinity stress and reduced plant growth and production significantly. Therefore, two types of strategies are recommended in this situation:

a) Increasing of plant tolerance to salinity and drought stress which is possible through genetic improvement of the plant in natural lands and using plant growth-promoting bacteria. Additionally, improvement of plant tolerance to stress is highly necessary owing to the fact that the lower a plant is tolerant against stress, the higher consumption of water and nutrient it has.

b) Second strategy could be improvement of soil and water condition in the plantation site which can have a wide range of physical, biological and physiobiological operations. Regarding the fact that some area of Turkmen Sahra has altered into desert status, part of government budget is allocated to enhance the vegetation cover in the region with the aim of combating erosion hazards. It has been shown by reports that Golestan Natural Resources and Watershed Management office, Iran has the plan of plantation of more than one million plants in the following years to undertake erosion and dust storm challenges and improving livelihood sustainability of local people (pastoralists, farmers and watershed dwellers). Hence, it is recommended to assess desired plant adaptation ability with local condition and affecting factors restricting or improving their growth and production.

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پاسخ ویژگی‌های رویشی گیاه Lycium depressum L. به تنش‌های شوری و خشکی
منفرد و ترکیبی

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چکیده
گونه Lycium depressum L. تنها گونه درختچه ای بومی مستقر در مناطق شور و قلیایی ترکمن صحرا در استان گلستان کشور ایران است. در سال‌های گذشته، تلاش‌های زیادی برای افزایش پوشش گیاهی منطقه با کشت گونه L. depressum L. صورت گرفته است تا از این طریق چالش‌های ناشی از فرسایش آبی و بادی و همچنین ریزگرد با پیش‌بینی افزایش در آینده نزدیک، کاهش یابد. با این حال، کشت این گونه به طور کامل موفق نبوده است. البته منظور درک اثرات جدایگانه و ترکیبی تنش‌های شوری و خشکی بر روی ویژگی‌های رویشی این گیاه، آزمایش گلخانه‌ای در دی ماه 1396 با سطوح مختلف شوری (شامان (4 دسیزیمنس)، 14، 23 و 44 دسیزیمنس بر متر) با استفاده از محلول NaCl و سطوح مختلف خشکی (شامان (آب معمولی)، 42/0، 20/0، 52/0 و 64/0 مگاپاسکال) با استفاده از محلول پلی اتیلن گلایکول 8000 و تیمارهای ترکیبی آنها در قالب آزمایش فاکتوریل بر پایه طرح کاملاً تصادفی (ANVA) با هدف مقایسه تیمارهای مختلف خشکی و شوری و اثرات ترکیبی آنها بر استفاده شد. یافته‌ها نشان داد که تنش خشکی اثر منفی بیشتری بر روی عملکرد گیاهی نسبت به تنش شوری دارد که به طور چشمگیری موجب کاهش عوامل و توقف طول ریشه و ارتفاع گیاه شد (P<0.05). همچنین مشاهده شد که تیمارهای ترکیبی شوری و خشکی نسبت به حالت منفرد اثر منفی بیشتری بر عملکرد گیاهی دارد. علاوه بر این، نتایج تحقیقی ما اینکه اعمال هواپیمایی و زیرزمینی در خیزه تأثیرپذیری‌تر از نشان داد. به طور کلی، اینگونه مشاهدات شد که سطح دارد که به طور دوباره لیف‌گیاهی (هشدار از 30 دسیزیمنس بر متر) و در نتیجه این مشاهده شد که L. depressum L. به طریقی در برخی موارد حتی مقدار کامل گیاه می‌شود. در نهایت، این نتیجه حاصل که نسبت به خشکی اثر بیشتری دارد تا تنش شوری. همچنین، می‌توان از روش RSM به عنوان روش دقیق به منظور تعیین پیشینی سطح تنش پیش‌بینی شده بر روی گیاه استفاده کرد.

کلمات کلیدی: Lycium depressum L., شوری، خشکی، RSM, پاسخ ویژگی‌های رویشی گیاه Lycium depressum L.