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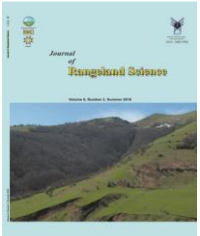

Research and Full Length Article:

Investigating the Effects of Pod Elimination on Salinity Tolerance in Annual Medic (*Medicago scutellata* L.)

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Abstract. In most southern provinces of Iran, Soil Salinity is a growing problem particularly in the irrigated agricultural areas and has been found to reduce crop yields. In order to investigate the role of pod removal in seed germination, seedling growth and antioxidant enzymes activity in annual medics (*Medicago scutella* L.) under salinity stress conditions, a factorial experiment was conducted at College of Agriculture, Shiraz University in 2014 based on the completely randomized design with four replications. The factors included: 1) the presence or removal of seed pod, and 2) salinity stress at five levels involving controls 3, 6, 9 and 12 dS m^{-1}. The results showed that salinity stress has caused the changes of germination, seedling growth and antioxidant enzymes activity. By increasing salinity stress levels, a significant reduction was observed in speed and percentage of germination as well as rootlet length, shoot length and seedling weight. However, the increased levels of salinity stress caused the increased antioxidant enzymes activity. Germination and seedling growth in seeds with no pod were more than seeds with pod, but about enzymes' activity, no significant difference was observed among seeds with and without pod, except for ascorbate peroxidase enzyme activity that was significantly more in seeds without pod as compared to the seeds with pod.

Keywords: Sodium chloride, Percentage of germination, Ascorbate peroxidase

Introduction

Salinity after drought is the most important and common environmental stress in Iran and the world and is one of major factors that currently reduce crop production. Because Iran is located in a region with arid and semiarid climate, close to 50% of agricultural products' cultivation area is faced with various degrees of salinity and alkalinity problems (Mirmohammadi Maibody and Qara Yazy, 2002).

In general, sodium chloride is the most common substance causing salinity through creating toxicity due to chloride and sodium ions and osmotic stress because of osmotic potential reduction in soil exposes plants to stress (Zhu, 2001). Studies show that salinity stress has led to reduce photosynthesis, seed germination percent, leaf number and finally leaf area reduction (Sadeghi and Nazemosadat, 2011). Salinity first causes reducing water absorption by seeds because of low osmotic potential of the environment and in the next step, it causes toxicity and a change in enzymatic activities. The seedlings that are susceptible to ions' absorption and are exposed to salinity sometimes die immediately after germination (Sadeghi and Khaef, 2011). Mass and Poss (1989) have shown that reducing turgor pressure, a result of salinity is the most important inhibitor of plant growth under salinity stress. It should be mentioned that the growth of cells is associated with turgor pressure. In plants sensitive to salinity, reducing turgor pressure has a negative impact on cell division, cell elongation and openings. By reducing turgor pressure, gas exchange (photosynthesis and respiration) is reduced that causes the reduced growth of a plant. Negative effects of salinity on a plant growth are due to low osmotic potential of soil solution (osmotic stress), special ionic effects and nutrients' imbalance or a combination of these factors (Ashraf and Harris, 2004).

Kaya *et al*. (2006) in their study observed that by increasing salinity stress, germination percentage, rootlet length, shoot length and weight of sunflower seedling were reduced. In a study by Singh (1995) on safflower response to soil salinity by increasing salinity rate from 1.8 to 30 dS m^{-1}, it was found that germination was negatively correlated with increasing salinity. So that up to 3 dS m^{-1} salinity 9 percent germination reduction was observed and in salinity of 19 dS m^{-1} 50% germination and in salinity of 30 dS germination was completely stopped (Singh, 1995).

Annual medics' species because of nitrogen fixation and proper quality of fodder have high crop values. Many annual medics have characteristics that make them particularly compatible with arid environments. The abilities to grow and adapt in the areas with poor distribution of rainfall, hard seed production and also much seed production are among the characteristics. Hard seed production allows a plant to survive under drought conditions (Sadeghi and Khani, 2012). Species without pod as compared to the ones with pod during a period of stress have high free proline content and enzymes' activity of catalase, peroxidase, dismutase and higher ascorbate peroxidase (Ghanaatiyan and Sadeghi, 2015). Since stresses are the most important factors of reducing the growth and yield of crop plants, identifying the plants resistant to stress, studying mechanisms of this resistance (Ghamari Zare *et al.*, 2009), understanding physiological and biochemical characteristics of plants affected by salinity stress and including toxic ions, osmotic potential, balance of elements, other physiological and chemical disorders as well as the interaction between different stresses are also important are important (Abdul Qados, 2011). In this context, our aim was to evaluate the effects of pod removal on seed germination, seedling early growth and antioxidant enzyme activity in annual medics (*Medicago scutellata* L.).

Materials and Methods

The study area

This study was carried out in a controlled environment of the laboratory at the College of Agriculture, Shiraz University, and Shiraz, Iran during 2014. The first factor included: 1) The presence or removal of seed pod, and 2) Salinity stress at five levels involving controls 3, 6, 9 and 12 dS m^{-1}. Salinity stress levels were prepared using distilled water and Sodium chloride salt. Seeds were prepared from Isfahan Pakan Bazr Co. Pod removal operation was performed before starting the experiment. Petri dishes were first put in sodium hypochlorite solution 2% for two minutes, then washed by water and disinfected with alcohol. In each of Petri dishes, 20 seeds of annual medics *(Medicago scutellata* L.) were put on two Whatman filter papers and treated with the related solutions. Seeds were put at 25 °C in a place with light intensity of 270 to 370 and relative humidity of 45% (Sadeghi and Khaef, 2011) and then, treated when necessary with the related osmotic solution.

The number of germinated seeds was recorded on a daily basis till the twentieth day and after the end of the experiment, plumule and rootlet length were measured. In order to determine the weight of seedling, 10 seedlings were randomly chosen and weighed using an accurate digital scale. Germination percent and rate were determined based on the equations 1 and 2 (Pirasteh-Anosheh *et al*., 2011).

$$G\% = \frac{GN}{N} \times 100 \qquad (1)$$

In this equation, G% is germination percent, GN is the number of germinated seeds and N is total number of planted seeds.

$$GR = \sum \frac{N}{DN} \qquad (2)$$

In this equation, GR is the germination rate, N is the number of germinated seeds in a day, and D is the number of days since the beginning of germination.

In order to determine peroxidase and catalase activity, Chanc and Maehly (1955) method was used in order to determine Super oxidase activity. Beauchamp and Fridovich (1971) method was used to determine ascorbic peroxidase activity. Nakano and Asada (1981) method was used. In these methods, spectrophotometer (Biochrom Ltd., Biowave S2100, and Cambridge, UK) was used.

Statistical analysis

This study was carried out as a factorial experiment based on the completely randomized design (CRD) with four replications with standard error using the statistical software SAS Program. Fisher's Least Significant Difference (LSD) was used to separate the means.

Results and Discussion

A. Percentage and Rate of Germination

The results showed that by increasing salinity, in seeds with and without pod, germination percent has been reduced (Fig. 1), but the reduction in seeds without pod was much more than seeds with pod. Under salinity stress, a significant difference was found between germination of seeds with pod and germination of seeds without pod so that maximum germination percent was related to the seeds without pod in control treatment (100%) and minimum germination percent (37.5%) was observed in the seeds with pod under osmotic treatment of 12 dS m^{-1}.

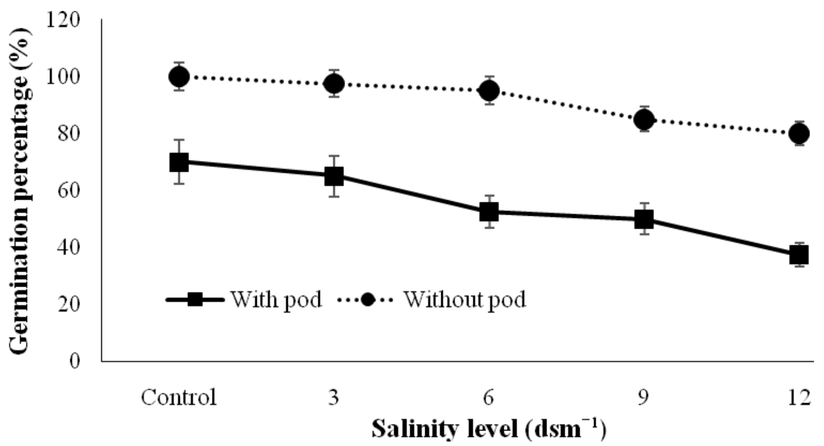

Fig. 1. Effect of different salinity levels on germination percent of seeds with and without pod in annual medics, The means with the same overlap had no significant difference (±SE)

B. Seedling Growth

Maximum rootlet length (72.3 mm) was related to the seeds without pod that were under control treatment while minimum rootlet length (14.7 mm) was seen in the seeds that were under salinity treatment of 12 dS m^{-1}. By increasing salinity stress, rootlet length was reduced in the seeds with and without pod (Fig. 3).

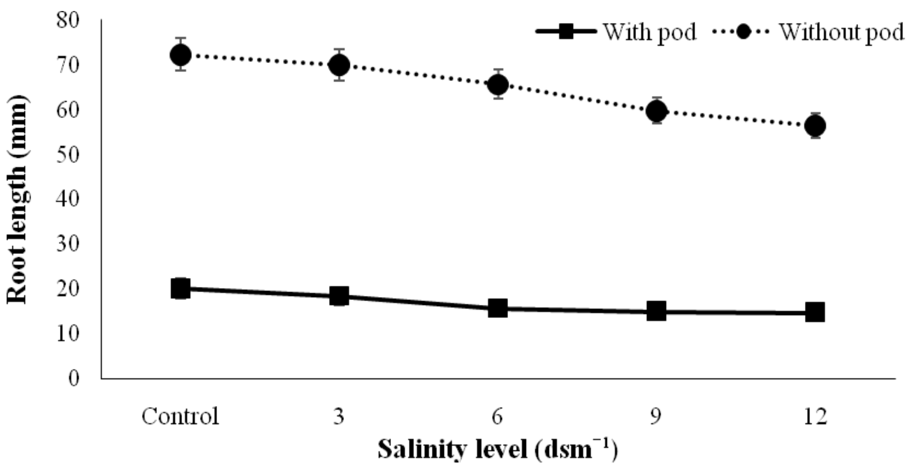

Fig. 3. Effects of different salinity levels on root length of seeds with and without pod in annual medics. The means with the same overlap had no significant difference (±SE)

The results showed that by increasing salinity stress, plumule length was reduced in the seeds with and without pod (Fig. 4). In terms of plumule length, a significant difference was found between seeds with and without pod. Maximum plumule length (31 mm) was related to seeds without pod that were under control treatment while minimum length (10.45 mm) was seen in seeds with pod that had been under salinity treatment of 12 dS m^{-1}.

By increasing salinity stress, seedling weight in the seeds with and without pod was reduced (Fig. 5). A significant difference was found between seeds with and without pod in terms of seedling weight. Maximum weight of seedling as 0.75 was related to the seeds without pod and in control treatment and minimum weight as 0.05 was related to seeds with pod that were irrigated with salinity of 12 dS m^{-1}. These results are consistent with the results reported by Sadeghi and Khani (2012) on (*Medicago polymorpha* L.).

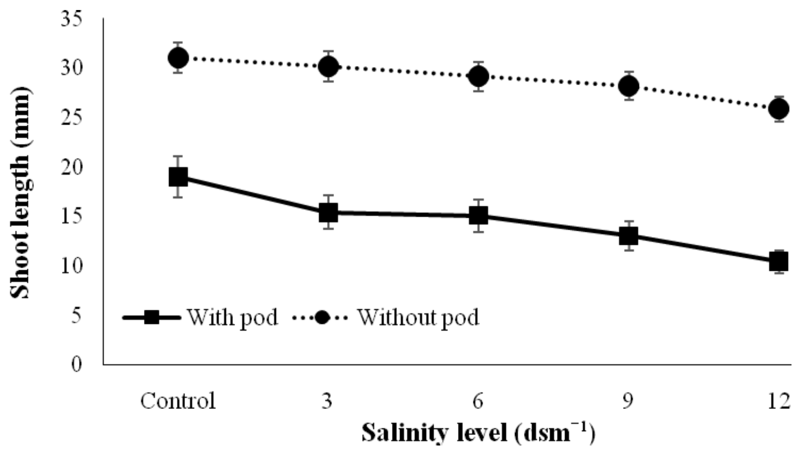

Fig. 4. Effects of different salinity levels on shoot length of seeds with and without pod in annual medics. The means with the same overlap had no significant difference (±SE)

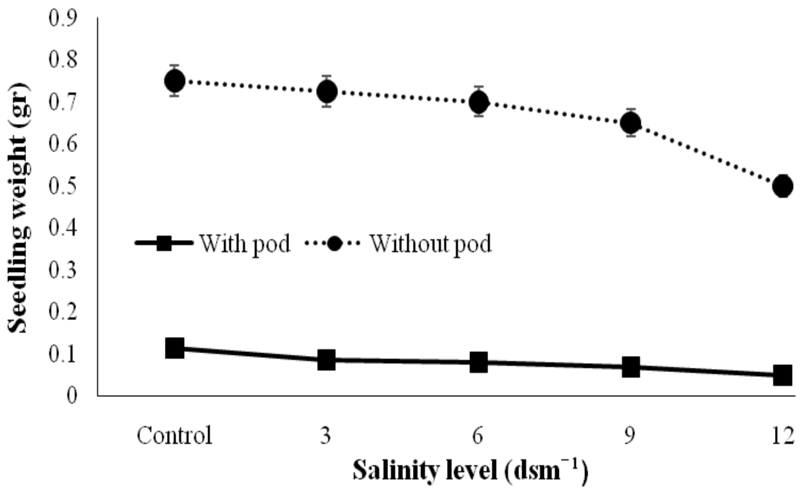

Fig. 5. Effects of different salinity levels on seedling weight of seeds with and without pod in annual medics. The means with the same overlap had no significant difference (±SE)

In this study, it was seen that by increasing stress, plumule and rootlet length were reduced that is consistent with the results reported by Hosseini and RezvaniMoghadam (2006) on fleawort and Miri and Mirjalali (2013) on *Echinacea purpurea*. Reduced rootlet length of canola and wheat by increasing water potential has also been reported by Tavakkol-Afshari and Majnoun-Hosseini (2002). They mentioned one of the causes of reduced plumule length under drought stress conditions as the reduction or lack of transfer of nutrients through seeds' storage tissues to the germ. In general, germination of seeds of plants in the environments exposed to stress is along with shorter plumules and rootlets and it is believed that the reduced seeds' germination characteristics can be attributed to slow and low initial uptake rate of water as well as negative effects of low osmotic potential and toxicity of chlorine and sodium ions on biochemical processes of germination catabolic and anabolic steps (Lynch and Lauchli, 1988).

C. Antioxidant Enzymes Activity

The results showed that by increasing salinity stress, catalase activity was increased in the seeds with and without pod (Fig. 6). At all levels of salinity stress, no significant difference was found between the seeds with and without pod in terms of catalase activity.

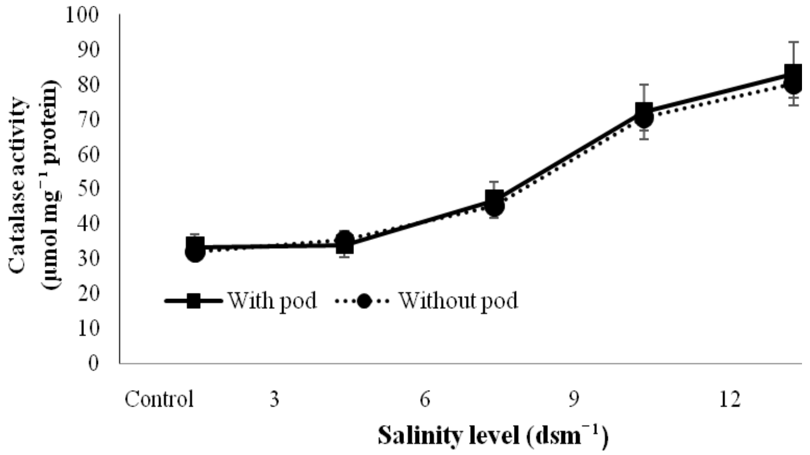

Fig. 6. Effects of different salinity levels on catalase enzyme activity of seeds with and without pod in annual medics. The means with the same overlap had no significant difference (±SE)

By increasing salinity stress, peroxidase activity rate was increased in both seeds with and without pod (Fig. 7). At all levels of stress, no significant difference is found between seeds with and without pod. Maximum enzyme activity rates given as 48.87 and 48.32 micromole/ mg of protein were related to seeds without pod and with pod related to treatment of 12 dS m^{-1}. Minimum enzyme activity rates as 11.35 and 12.22 micromole/ mg of protein were related to seeds with pod and without pod in control treatment, respectively.

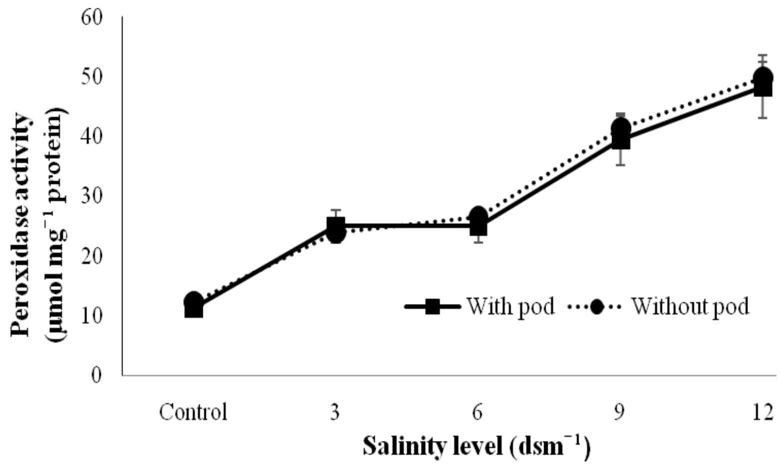

Fig. 7. Effects of different salinity levels on peroxidase enzyme activity of seeds with and without pod in annual medics. The means with the same overlap had no significant difference (±SE)

The results showed that by increasing salinity stress, dismutase super oxidase activity has been increased in seeds with and without pod (Fig. 8). At all levels of stress, no significant difference is found between seeds with and without pod. Maximum activities of this enzyme as 198.64 and 196 micromole/ mg of protein were seen in the salinity of 12 dS m^{-1} for seeds with pod and seeds without pod and minimum activity of the enzyme as 102.32 and 104.06 micromole/mg of protein was seen in seeds with pod and seeds without pod in control treatment.

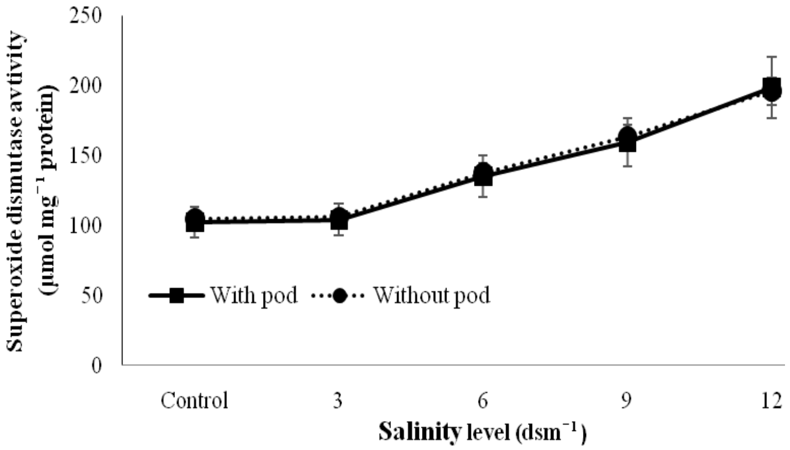

Fig. 8. Effects of different salinity levels on superoxide dismutase enzyme activity of seeds with and without pod in annual medics. The means with the same overlap had no significant difference (±SE)

Ascorbate peroxidase activity was increased in the seeds with and without pod (Fig. 9). A significant difference was found between seeds with and without pod in other treatments except control treatment. Maximum activity of this enzyme was 512.43 micromole/ mg of protein related to seeds without pod with salinity of 12 dS m^{-1} and its minimum activity was 311.21 micromole/ mg of protein related to seeds with pod and in control treatment. As the results presented in this study, by increasing salinity stress, the activity of antioxidant enzymes of catalase, peroxidase, ascorbate peroxidase and superoxide dismutase was increased in both seeds with and without pod that was consistent with the results reported by Aghaei *et al.* (2009) on potato and Munir and Aftab (2013) on sugarcane and Gao *et al.* (2008) on *Jatropha curcas* L.

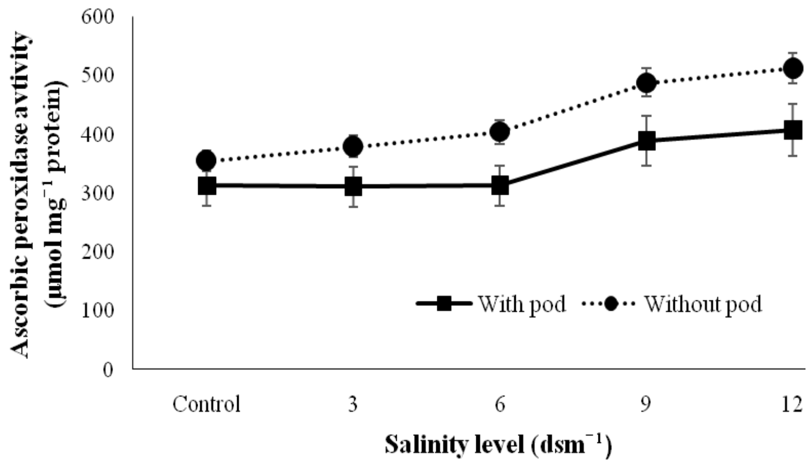

Fig. 9. Effects of different salinity levels on ascorbic peroxidase enzyme activity of seeds with and without pod in annual medics. The means with the same overlap had no significant difference (±SE)

By increasing salinity level, plant antioxidant enzyme was activated and increased the activity of the enzyme superoxide. This enzyme acts as the first line of defense against oxygen free radicals causing the increased resistance against damages caused by salinity stress (Ghanaatiyan and Sadeghi, 2015). Dehghani and Mostajeran (2011) by studying ginger reported that catalase activity was increased on the plumule and leaf under salinity stress conditions of 4 dS m^{-1} and was reduced in salinity of 6 dS m^{-1}and 8 dS m^{-1}. Esfandiari *et al.* (2007)

by studying wheat reported that super oxidase enzyme activity was increased by increasing salinity stress and was constant in Alvand variety at all levels of stress.

Conclusion

In general, salinity stress caused the reduced germination percent, the length of shoot and rootlet and seedling weight. So, maximum values were observed for above features under control conditions and the lowest ones were observed under maximum stress conditions (salinity of 12 dS m^{-1}). Also, highest rates of catalase, peroxidase; superoxide dismutase and ascorbate peroxidase were observed under maximum stress conditions and the lowest activity rate of mentioned enzymes was observed under control conditions. In terms of enzyme activity rate, no significant difference was observed between seeds with and without pod, except for APX at all stress levels. It was also observed that pod removal causes increasing germination percent and better growth of annual medics; so, in the case of planting under salinity stress conditions, it is better to use seeds without pod to make faster and more germination and stronger seedlings in the farm.

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اثر از بین بردن غلاف بر افزایش مقاومت به شوری در یونجه یکساله (*Medicago scutellata* L.)

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چکیده. شوری خاک را می‌توان جزء عمده‌ترین عوامل محدود کننده رشد و عملکرد گیاهان در اکثر مناطق جنوبی ایران دانست به منظور بررسی نقش حذف غلاف بر جوانه‌زنی بذر، رشد گیاهچه و فعالیت آنزیم‌های آنتی اکسیدان در بذر یونجه یکساله (*Medicago scutella* L.) در شرایط تنش شوری، آزمایش فاکتوریل دو عاملی بر پایه طرح کاملاً تصادفی با چهار تکرار در آزمایشگاه بخش مدیریت مناطق بیابانی دانشگاه شیراز در سال ۱۳۹۳ انجام شد. فاکتورهای مورد مطالعه عبارت بودند از ۱) وجود یا حذف غلاف بذرو ۲) تنش شوری در پنج سطح: شاهد، ۳، ۶، ۹ و ۱۲ دسی زیمنس بر متر. نتایج نشان داد که تنش شوری سبب تغییر در ویژگی‌های جوانه زنی، رشد گیاهچه و فعالیت آنزیم‌های آنتی اکسیدان شد. با افزایش سطوح تنش شوری کاهش معنی‌داری در سرعت و درصد جوانه‌زنی و همچنین در طول ریشه چه، طول ساقه‌چه و وزن گیاهچه مشاهده شد. با این وجود افزایش سطوح تنش شوری سبب افزایش فعالیت آنزیم‌های آنتی‌اکسیدان گردید. جوانه‌زنی و رشد گیاهچه در بذرهای بدون غلاف بیشتر از بذرهای دارای غلاف بود؛ اما در مورد فعالیت آنزیم‌ها بین بذرهای دارای غلاف و بذرهای بدون غلاف تفاوت معنی‌داری مشاهده نشد؛ به جز فعالیت آنزیم آسکوربات پراکسیداز که در بذرهای بدون غلاف به طور معنی‌داری بیشتر از بذرهای دارای غلاف بود.

کلمات کلیدی: کلرید سدیم، رشد گیاهچه، آنزیم آسکوربات پراکسیداز