

Effects of Different Drought and Salinity Levels on Seed Germination of *Citrullus colocynthis*

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Received: 15 February 2017 / Accepted: 19 July 2017 / Published Online: 23 September 2017

Background: Environmental stresses, especially salinity and drought, are effective on seed germination and growth.

Materials and Methods: After breaking seed dormancy by simultaneous use of leaching (48 hours) and gibberlic acid (1000 ppm), germination characteristics and seedling growth of *Citrullus colocynthis* from two ecotypes of Sistan-Baluchestan with six levels of drought (0, -0.3, -0.6, -0.9, -1.2, -1.5 Mpa) and salinity (0, 50, 100, 150, 200, 250 mM) were studied. This experiment was carried out in two different factorial experiments using completely randomized design with four replications. Statistical analysis was performed using version 18 of SPSS software and Factorial analysis of variance (general linear model) and mean comparisons were done using Duncan's multiple range test. **Results:** The results demonstrated that there were significant differences among different levels of drought and salinity in all measured traits ($P < 0.01$) and a significant decrease in seed germination and seedling growth were observed with increasing levels of drought and salinity ($P < 0.01$). The results revealed that there were significant interactions effect between ecotype and different levels of drought as well as salinity ($P < 0.01$), so seed vigor of Zabol' ecotype was higher than that of Saravan.

Discussion and Conclusions: This species has acceptable germination at -0.6 Mpa droughts and 50 mM salinity. These issues must be considered in its intense planting in Sistan-Baluchestan province and using of Zabol' ecotype seed is recommended.

Key words: *Citrullus colocynthis*, Drought, Germination, Salinity, Sistan and Baluchestan

1. Background

Drought and salinity, as the most influential factor limiting plant growth, are of particular importance among environmental stresses. On the other hand, Iran is characterized by an arid and semi-arid climate, with very low levels of precipitation, i.e. average annual rainfall of around 274 millimeters. Compared with the average global figure (approximately 860 mm), this very low precipitation indicates the dominance of aridity in the country (1). Germination is an important stage in most plants. Regarding the establishment and consolidation of plants in saline soils, salt tolerance at germination stage is of particular importance (2). Plant's resistance to drought is the outcome of various physiological, morphological, and phenological characteristics that, individually or in combination, determine plant response to drought stress (3). Drought affects various aspects of plant life and causes reduction delay in seed germination, shoot growth and dry matter production. Osmotic and total water potential reduction, wilting, stomatal closure and reduction in plant growth are the symptoms of drought stress. In the case of high water stresses, other symptoms such as remarkable reduction of photosynthesis, disruption of physiological processes, growth repression and eventually plant death is expected (4). Furthermore, drought stress reduces plant biomass and yield, (5). Examination of the effects of drought stress on wheat revealed reduced grain yield and weights by shortening the grain filling period (6).

Salinity reduces the potential of available water to plants in the root zone and some ions impose toxic effects on plant physiological and biochemical processes, both of which lead to perturbation in nutrient uptakes by root and reduction in plant growth. Plant sensitivity to salinity varies at the different growth stages (7), the highest of which is observed during the

germination and early seedling. Other secondary stresses like oxidative stress might appear as a result of salinity stress, through which the production and accumulation of active radicals lead to the oxidation of proteins and lipids, and hence cell death (8).

Numerous studies have been carried out about environmental stresses (9). Hanci and Cebeci (10) demonstrated that different cultivars of onion (*Allium cepa* L.) in their first growth stage had different responses to drought and salinity. Assessment of the germination indices on *Vicia villosa* L. under salinity and drought stresses demonstrated that both stresses had significant effects on the germination indices (11). Assessment of drought stress on germination indices of three rangeland species from *Bromus* genus revealed that all germination and growth traits of seedling decreased significantly by increasing drought level (12). Germination and growth indices of *Agropyron desertorum* significantly decreased under increased salinity and drought (13).

Medicinal plants are rich in active ingredients of many drugs. Although production of the active ingredients is primarily guided by genetic processes, environmental factors that cause changes in the growth of these plants have significant influences on the quantity and quality of their ingredients (14).

Fox melon, *Citrullus colocynthis*, also referred as "Hanzel", "Mararah el Sahari" and "Alqam" in ancient literature, is one of the medical plants in Cucurbitaceae family. This plant is native to Iran and can be found in Sistan-Baluchestan, South Khorasan and Kerman provinces. It is a widespread multifunctional plant that is used for medical and soil conservation purposes in desert areas. This species grows well in sandy soil with light texture, and its fruit is widely used to control blood sugar and diabetes (1). The fruit contains a bitter glycoside called klementin as well as

citrulline, resin, pectin, gum, alpha - alatrinsaponins, alkaloids, and tannins. Its seeds contain 53% oil and 28% protein and its roots contain alpha-alatryn, and saponins (15). The oil of this plant is similar to rapeseed oil (16) and can be used in dietary or for pharmaceutical and industrial purposes (17). Anti-viral, anti-microbial and anti-cancer effects of this plant are important, too (18). Different parts of this plant, especially fruits and seeds, are often used to treat urinary tract infections, in the Mediterranean countries (19).

2. Objective

Considering the medicinal importance of *C. colocynthis*, its protective role and resistance to sandy lands in southeastern Iran, and the lack of exact understanding of its response to salinity and drought stresses, the present study was conducted to investigate the tolerance threshold of *C. colocynthis* to drought and salinity stress in two ecotypes from Sistan-Baluchestan province.

3. Materials and Methods

In two separate experiments, *C. colocynthis* seed germination response to drought and salinity levels were evaluated in two ecotypes. Seeds were collected from Zabol and Saravan sites of Sistan-Baluchestan province in the summer 2015. Since the best treatment for breaking seed dormancy was obtained by simultaneous use of leaching for 48 hours and 1000 ppm gibberlic acid, all seeds were exposed to this treatment before testing the effect of different stress treatments (20). The first test consisted of six levels of aridity (0, -0.3, -0.6, -0.9, -1.2, and -1.5 MPa (Mega Pascal)), which was prepared using polyethylene glycol 6000. The second experiment consisted of six levels of salinity (0, 50, 100, 150, 200, 250 mM) and sodium chloride was used to simulate salt stress. To

induce a zero level stress, distilled water was used in both experiments. Before performing the tests, treatments were applied to break seed dormancy. The combined treatment of leaching for 24 hours with 1000 ppm gibberlic acid was identified as the best treatment, to be further carried out for all seeds. To this end, petri dishes and seedbed (Watman paper) were sterilized in the oven for 48 h at 20 °C (21). A total of 20 seeds were disinfected for 30 seconds with a solution of 10% sodium hypochloride. Once rinsed with distilled water, seeds were transferred on filter paper in petri dishes. Then to induce aridity and salinity stresses, five ml PEG (Polyethylene glycol) and five ml sodium chloride solutions were added to each petri dish. To keep the salt concentration in the petri dishes, the saline solution into each petri dish was changed every two days. Germination test was performed using factorial test in completely randomized design with four replications (20 seeds per petri dishes) in the germinator with 25°C temperature, 70% humidity and 5000 lux of light intensity. Germinated seeds were counted each day over a period of 20 days. Root length, shoot length and plant length were measured in the end of experiment. Germination percentage, germination speed, and vigor index of seed were calculated based on the following equations (21):

$$\text{Germination percentage } GP = \frac{\sum G}{N} \times 100$$

GP: germination percentage, G: number of germinated seeds, N: number of seeds.

$$\text{Germination rate } GR = \sum_{i=1}^n \frac{S_i}{D_i}$$

Si: germinated seeds at each counting, Di: days until n counting, n: numbers of counting.

plant length = root length + shoot length

$$\text{vigor index } V_i = \frac{\% Gr \times MSH}{100}$$

Vi: vigor index, MSH: mean of plant length (root length + shoot length) per mm, Gr: Germination percentage.

Statistical analysis was performed using version 18 of SPSS software and Factorial analysis of variance (general linear model). After checking the normality and homogeneity of data, the comparisons among treatments were made using Duncan's multiple range tests (DMRT) at 1% level of significance.

4. Results

4.1. Drought stress test

Results demonstrated significant effects ($P < 0.01$) of drought on all the measured indices (Table 1). The ecotype effect was just significant ($P < 0.01$) for germination percentage, germination rate, and seed vigority

indices. The ecotype and drought interaction was significant ($P < 0.01$) for only germination percentage, germination rate, and seed vigority indices (Table 1).

4.2. Germination percentage and rate

There were significant differences in germination percentage between different levels of stress, so that the highest germination percentage was observed in control treatment and the lowest was in -1.2 MPa treatments. Germination percentage significantly decreased with the increase in stress level. There was a significant difference germination percentage of the control treatment between the two ecotypes (Figure 1). In both the ecotypes germination rate was significantly different across varying levels of stress. The germination rate significantly decreased with increasing levels of drought. There was a significant difference between germination rates in control treatment of the two ecotypes (Figure 2), as Zabol ecotype had more favorable germination.

Table 1 Factorial variance analysis effect of drought on *C. colocynthis*

Source of variation	f	Germination percentage	Germination rate	Root length (cm)	Shoot length (cm)	Seedling Length (cm)	Seed vigor
Ecotypes	1	258**	24.1**	3.25 ^{ns}	0.39 ^{ns}	1.3 ^{ns}	77.7**
Drought	5	1490**	1339**	3851**	627.6**	2184**	6778**
Ecotypes*							
Drought	5	35.6**	4.5**	0.3 ^{ns}	0.08 ^{ns}	0.12 ^{ns}	12.9**
Error	33	0.6	0.02	0.07	0.03	0.04	146.2

** Significant differences between treatments at 1% level. ns: non significant differences between treatments.

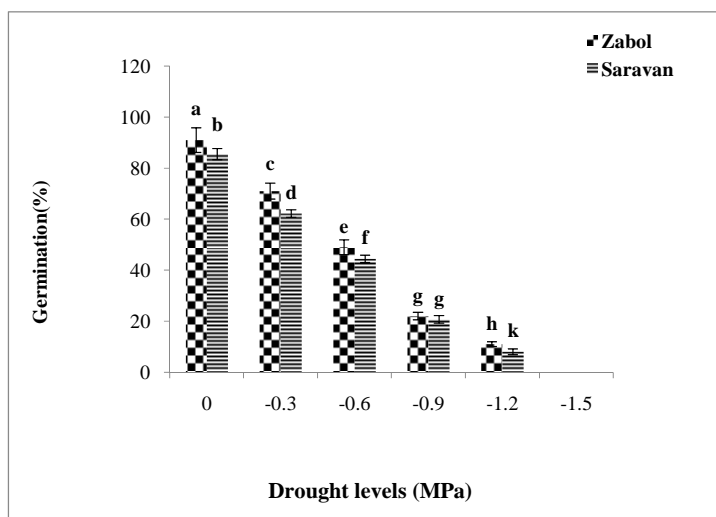


Figure 1 Effect of drought on germination of *C. colocynthis*. Means followed by the same letters in each level are not significantly different ($P>0.01$)

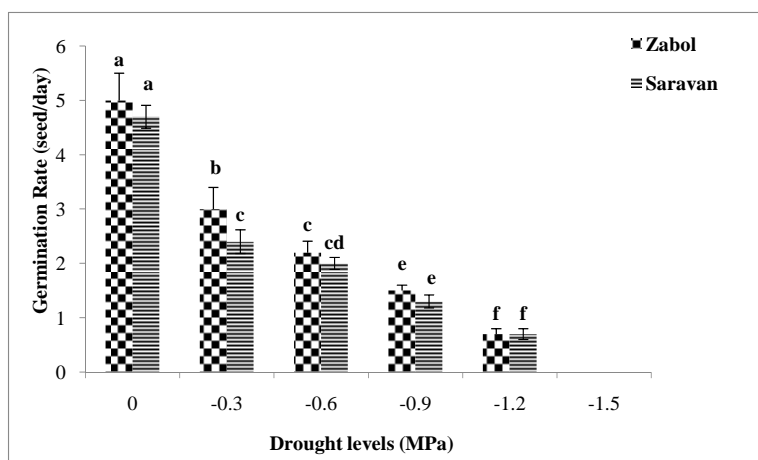


Figure 2 Effect of drought on germination rate of *C. colocynthis*. Means followed by the same letters in each level are not significantly different ($P>0.01$)

4.3. Root, shoot and seedling length

As it can be observed in Figures 3, 4 and 5, different drought levels had significant effects on root, shoot and seedling lengths. These indices decreased with increase in drought

level, so that the highest and lowest root lengths were observed in control and -1.2 MPa treatments, respectively. However, no significant difference was observed between the two ecotypes.

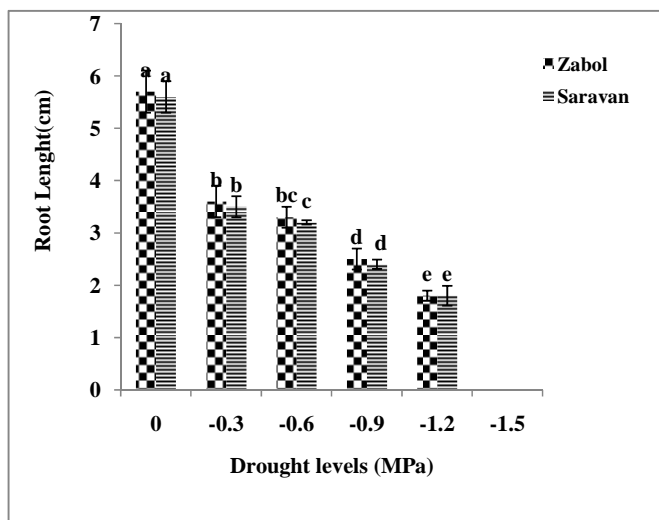


Figure 3 Effect of drought on root length of *C. colocynthis*. Means followed by the same letters in each level are not significantly different ($P>0.01$)

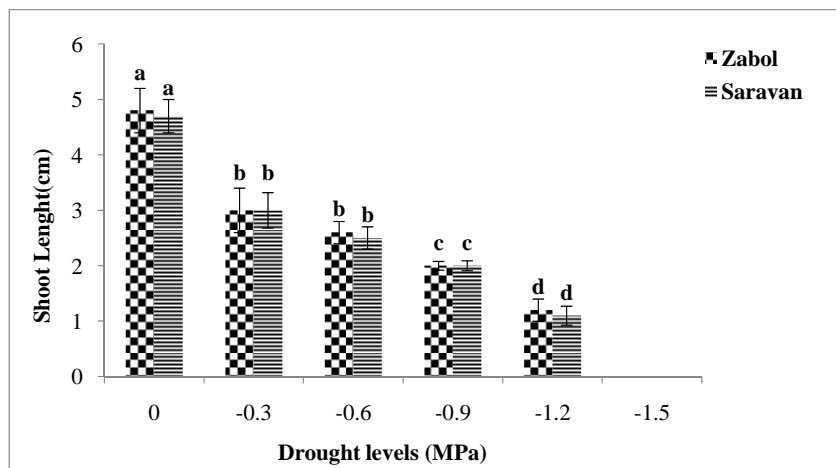


Figure 4 Effect of drought on shoot length of *C. colocynthis*. Means followed by the same letters in each level are not significantly different ($P>0.01$)

4.4. Seed vigor index

Drought treatments had a significant effect on the seed vigor index that decreased with increase in drought level (Figure 6). There were

significant differences between the two ecotypes with better seed vigor for the Zabol ecotype.

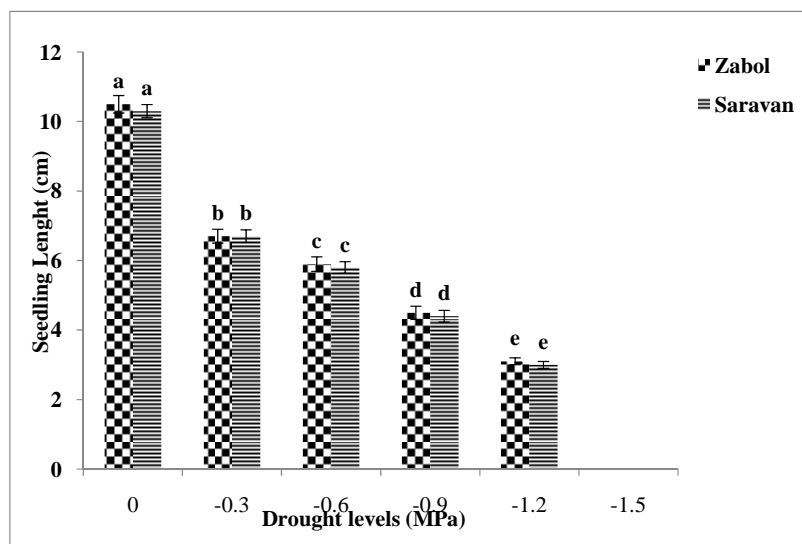


Figure 5 Effect of drought on seedling length of *C. colocynthis*. Means followed by the same letters in each level are not significantly different ($P>0.01$)

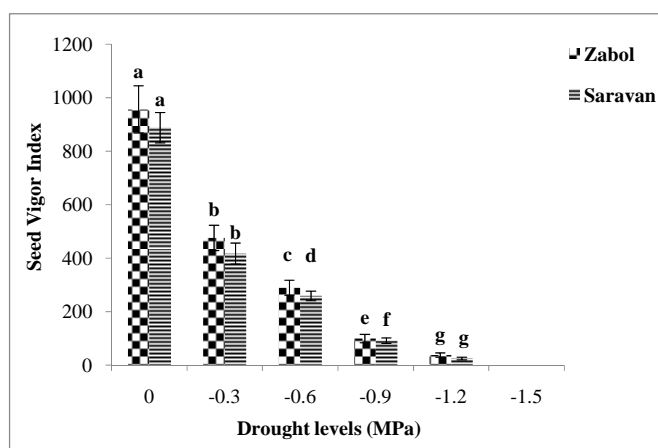


Figure 6 Effect of drought on seed vigor of *C. colocynthis*. Means followed by the same letters in each level are not significantly different ($P>0.01$). The

4.5. Salinity stress test

Results demonstrated significant effects ($P<0.01$) of different salinity treatments on all of the studied indices (Table 2). The ecotype effect was just significant ($P<0.01$) for germination percentage and seed vigour indices. The ecotype and salinity interaction was significant ($P<0.01$) for only germination percentage, shoot length, seedling length and seed vigour indices.

4.6. Germination percentage and rate

There were significant differences in germination percentage and rate between different levels of salinity stress; the highest germination percentage observed in control treatment and the lowest observed in 200 mM treatments (Figure 7 and 8). Germination percentage and rate were significantly decreased with increase in the stress level. There was a significant difference between germination percentages in the control treatment of the two ecotypes. (Figure 7), as Zabol ecotype had more favorable germination.

Table 2 Factorial variance analysis effect of salinity on *C. colocynthis*

Source of variation	df	Germination percentage	Germination rate	Root length (cm)	Shoot length (cm)	Seedling Growth (cm)	Seed vigor
Ecotypes	1	106 ^{**}	0.23 ^{ns}	3.6 ^{ns}	7.08 ^{ns}	1.02 ^{ns}	18.6 ^{**}
Salinity	5	9790 ^{**}	276.9 ^{**}	1697 ^{**}	699.5 ^{**}	1933 ^{**}	2988 ^{**}
Ecotypes*							
Salinity	5	9.9 ^{**}	0.8 ^{ns}	0.58 ^{ns}	7.6 ^{**}	5.6 ^{**}	10.5 ^{**}
Error	33	0.9	0.02	0.01	0.03	0.05	316.5

^{**} Significant differences between treatment at 1% level. ns: no significant difference between treatments

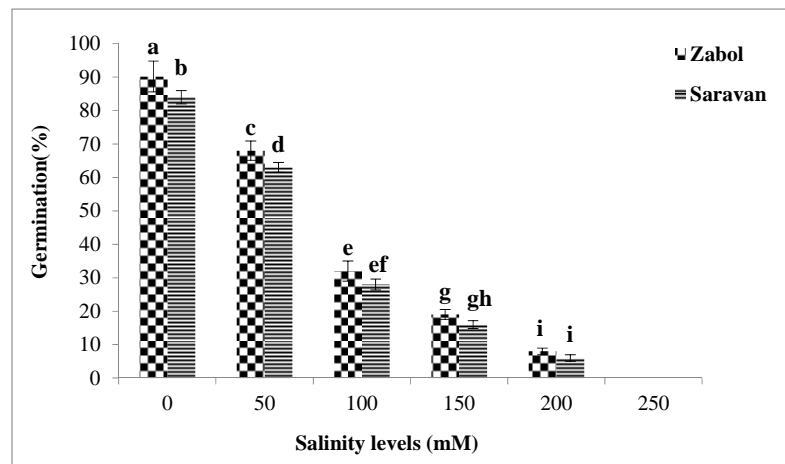


Figure 7 Effect of salinity on germination percentage of *C. colocynthis*. Means followed by the same letters in each level are not significantly different ($P>0.01$)

4.7. Root, shoot and seedling length

As it can be observed in Figures 9, 10 and 11, different salinity levels had significant effects on root, shoot and seedling lengths. These indices decreased with increase in salinity level, so that the highest and lowest

rates were observed in control and 200 mM treatments, respectively. However, no significant difference was observed between the two ecotypes.

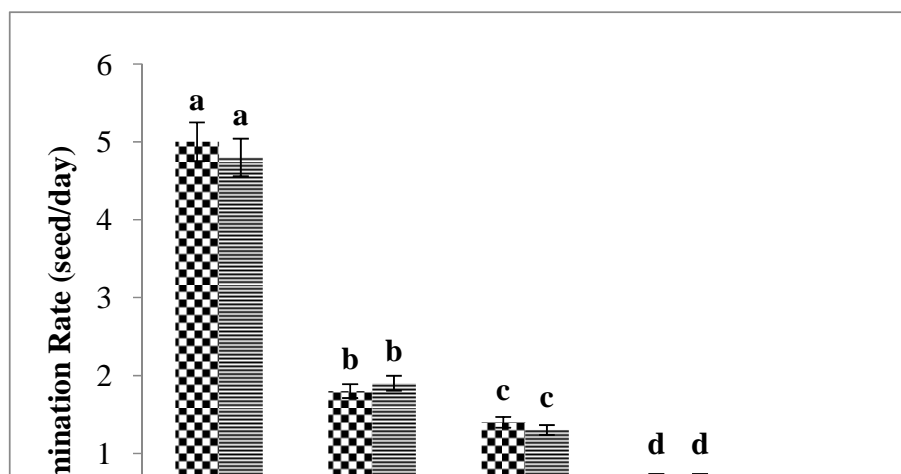


Figure 8 Effect of salinity on germination rate of *C. colocynthis*. Means followed by the same letters in each level are not significantly different ($P>0.01$)

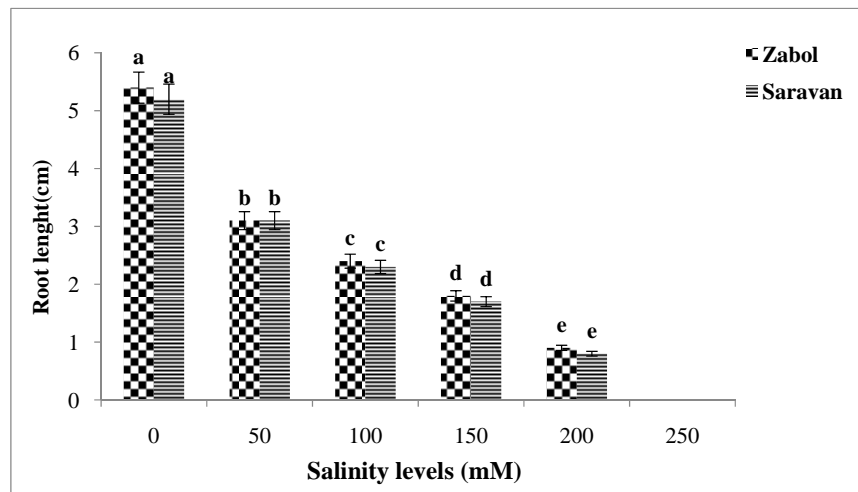


Figure 9 Effect of salinity on root length of *C. colocynthis*. Means followed by the same letters in each level are not significantly different ($P>0.01$)

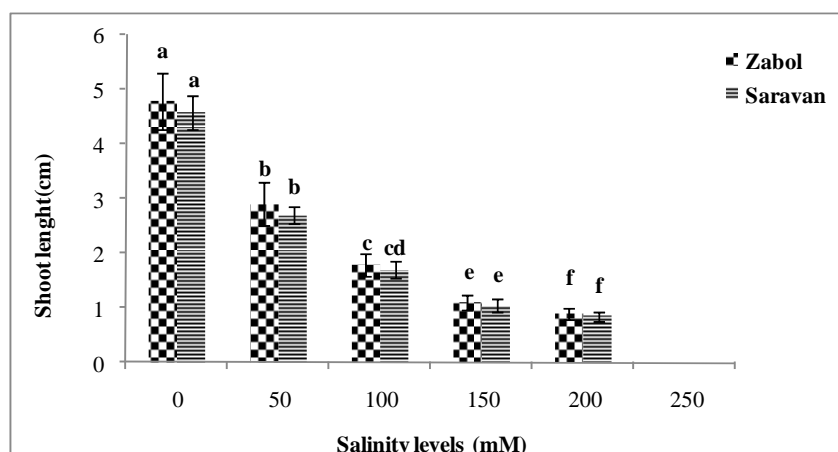


Figure 10 Effect of salinity on shoot length of *C. colocynthis*. Means followed by the same letters in each level are not significantly different ($P>0.01$).

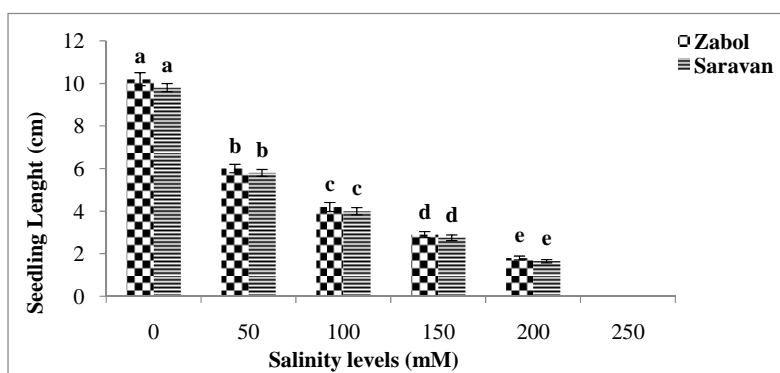


Figure 11 Effect of salinity on plant length of *C. colocynthis*. Means followed by the same letters in each level are not significantly different ($P>0.01$).

4.8. Seed vigor index

Seed vigor was reduced with increase in the salinity levels and this reduction was significant at all levels (Figure 12). There were also significant differences between the two ecotypes. Salinity treatment showed significant differences in the case of seed vigority index

that was decreased with increase in salinity level (Figure 12). The lowest and highest values were observed in control and 200 mm treatments, respectively. There was a significant difference between the seed vigority index in the control treatment of the two ecotypes, as Zabol ecotype had more favorable germination.

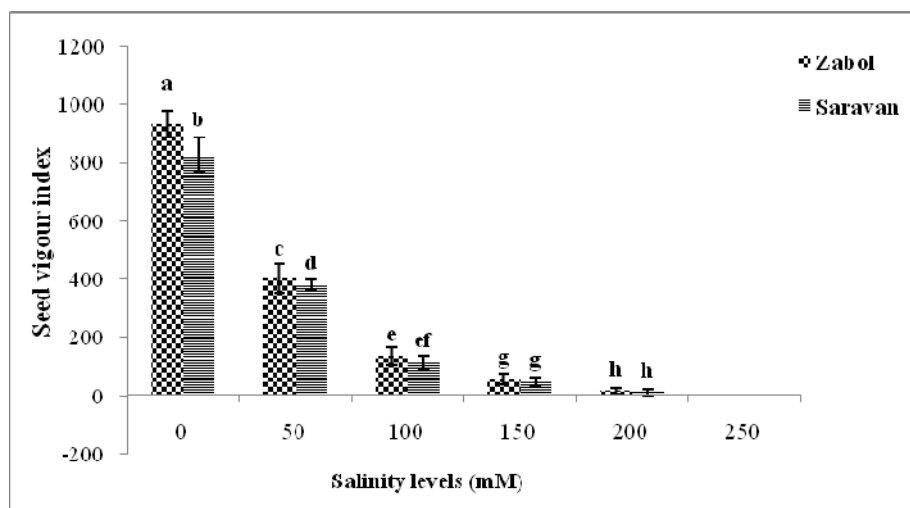


Figure 12 Effect of salinity on seed vigor of *C. colocynthis*. Means followed by the same letters in each level are not significantly different ($P > 0.01$)

5. Discussion

The results of this study revealed that in both the ecotypes of *C. colocynthis*, different drought and salinity treatments had significantly reduced all of the studied indices. The effect of drought was significant on germination percentage, germination rate and seed vigor, while the effect of salinity was significant only on germination percentage and seed vigor. Similarly, several works demonstrated that high levels of salinity and drought could create a harsh environment for seed germination with higher aridity and salinity that could weaken seed germination characteristics (22).

Reduction of internal osmotic potential of plants as the result of salt and water stress had already been mentioned (23), which had been attributed to a change in the balance between organic and mineral ions present in the cellular sap and ion absorption (24). The interruption of nutrients uptake was also reported to lead to the loss of germination percentage (25). Decrease in germination percentage and rate under the effects of drought stress, which had already been observed (26), could also be due to

decrease or lack of germination under drought stress and increase in the time to a minimum level of dewatering (27). Restrictive seeds water content because of drought stress will lead to decrease the activity of hydrolytic enzymes that are in charge for hydrolyzing cotyledons reserves needed for furnishing energy in the early stages of seeds growth by respiration.

Drought stress influences seed germination by limiting the seed water uptake, the movement and transfer of seed stocks or direct effect on fetal organic structure and protein synthesis (22). The lack of nutrient delivery from seed storage tissues to the embryos is considered as a reason for the reduced root length, shoot length and seedling length under drought stress (28).

The accumulation of minerals, physiological drought effect, and ion toxicity are considered as the main negative effects of salinity (17). Restraining absorbable water by seeds due to salinity stress can lead to decreased germination percentage and seedling growth (17). Decrease in seedling germination and seedling growth was also assumed to be due to the direct

influence of slower breakdown of endosperm materials in cotyledons accompanied by the slower transfer of materials broken down to seedling (13).

Decreased root and shoot length under salinity stress may be explained by turgescence pressure limitation or the accumulation of dry materials in root storage tissues (13). Seedling growth suppression occurs due to high levels of salinity, and thus the negative effects of sodium chloride on the cell membrane, ionic toxicity, and cytoplasmic membrane destruction. Salt can also damage the activity of some enzymes, hence effecting seedling and plant growth (2).

6. Conclusion

Our results indicated that Zabol ecotype of *C. colocynthis* could tolerate the examined stress better than the Saravan ecotype. *C. colocynthis* had acceptable germination at -0.6 Mpa droughts and 50 mM salinity and, therefore, as a medicinal and soil conservating plant, it is recommended for the rehabilitation of rangelands with such drought and salinity conditions.

Conflict of Interest

The authors have no conflict of interest.

Acknowledgement

This work was funded by the Gorgan University of Agricultural Sciences and Natural Resources in Iran as a PhD thesis. The authors are grateful to the university for this generous support.

Author's Contributions

All authors contributed extensively to the work presented in this paper.

Funding/Support

This research received no specific grant from any funding agency in the public, commercial, or not for profit sectors.

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اثرات سطوح مختلف خشکی و شوری بر جوانه زنی بذر هندوانه ابوجهل (*Citrulluscolocynthis*)

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تاریخ دریافت: ۲۷ بهمن ۱۳۹۵ / تاریخ پذیرش: ۲۸ تیر ۱۳۹۶ / تاریخ چاپ: ۱ مهر ۱۳۹۶

مقدمه: تنش‌های محیطی، خصوصاً شوری و خشکی بر جوانه‌زنی و رشد بذر اثرگذار می‌باشند.

مواد و روش‌ها: پس از شکست خواب بذر از طریق اعمال تیمار تلفیقی آبیویی (به مدت ۴۸ ساعت) و اسید جبرلیک (۱۰۰۰ پی پی ام)، ویژگی‌های جوانه‌زنی و رشد بذر گونه هندوانه ابوجهل (*Citrullus colocynthis*) از ۲ اکوتیپ سیستان- بلوچستان با ۶ سطح خشکی (۰، ۰/۳، ۰/۶، ۰/۹، ۱/۲، ۱/۵- مگاپاسکال) و ۶ سطح شوری (۰، ۵۰، ۱۰۰، ۱۵۰، ۲۰۰، ۲۵۰ میلی مولار) در دو آزمایش جداگانه به‌صورت فاکتوریل در قالب طرح کاملاً تصادفی با ۴ تکرار انجام شد. تجزیه و تحلیل آماری داده‌ها با استفاده از نرم‌افزار SPSS نسخه ۱۸ و تجزیه واریانس فاکتوریل (مدل خطی عمومی) انجام شده و مقایسه میانگین‌ها با استفاده از آزمون چنددامنه دانکن صورت پذیرفت.

نتایج: نتایج نشان داد که بین سطوح مختلف خشکی و شوری تفاوت معنی‌داری در کلیه صفات اندازه‌گیری شده وجود دارد (۰/۰۱ < P). با افزایش سطوح خشکی و شوری از جوانه‌زنی و رشد گیاهچه‌ها به طور معنی‌داری کاسته شد (۰/۰۱ < P). اثر متقابل اکوتیپ و سطوح مختلف خشکی و شوری نیز، نشانگر تفاوت معنی‌داری در سطح ۵ درصد بود، به‌طوری که بذر اکوتیپ زابل از قدرت جوانه‌زنی و رشد گیاهچه بهتری نسبت به بذر اکوتیپ سراوان داشتند.

بحث و نتیجه‌گیری: این گونه گیاهی درخشکی ۰/۶- مگاپاسکال و شوری ۵۰ میلی مولار جوانه زنی قابل قبولی دارد که این امر می‌بایست در برنامه‌های کشت انبوه این گونه در استان این موضوع مورد توجه قرار گیرد. استفاده از بذر اکوتیپ زابل نیز توصیه می‌گردد.

کلمات کلیدی: *Citrulluscolocynthis*، جوانه‌زنی، خشکی، سیستان و بلوچستان، شوری، هندوانه ابوجهل