

Germination and morphophysiological responses of flax (*Linum usitatissimum* L.) ecotypes to salinity stress

Batool Mahdavi* and Farnaz Alasvandyari

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Department of Genetics and Crop Production, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran.

*Corresponding Author; Email: b.mahdavi@vru.ac.ir

Abstract

Salinity is one of the most important factors that limit plant growth in many regions of the world. In order to evaluate the effects of salinity stress on germination, growth and some physiological characteristics of flax, two separate experiments were carried out in the laboratory and greenhouse. The experimental factors included three ecotypes of flax (Kurdistan Native, L18, E37) and four salinity levels (0, 50, 100 and 150 mM NaCl). The results showed that with increasing salinity, germination percentage, germination rate, seedling vigor index, length and dry weight of hypocotyl and radicle decreased as compared to the control. At 150 mM salinity, Kurdistan Native and E37 ecotypes had the highest and lowest germination and seedling growth, respectively. Also, salinity decreased shoot and root length, shoot and root dry weight and relative water content, whereas, increased electrolytes leakage and soluble sugars. Kurdistan Native had the highest shoot and root dry weight, relative water content and total soluble sugar at 150 mM salinity followed by L18 and E37 ecotypes. At salinity of 150 mM, Kurdistan Native had the lowest electrolyte leakage and L18 and E37 ecotypes had the highest electrolyte leakage. In general, the most sensitive and most tolerant ecotypes were E37 and Kurdistan Native, respectively. Therefore, Kurdistan Native has potential for cultivation in areas with saline water.

Keywords: Electrolyte leakage; Growth; NaCl; Proline; Total soluble sugar

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Introduction

Flax (*Linum usitatissimum* L.) is cultivated for its seed, which is used for bread, extraction of oil, human consumption (nutraceutical and pharmaceutical industries), and feeding poultry. Furthermore, flaxseed oil is used as industrial oil in varnishes and paints (Berti *et al.* 2010). Flax is also planted for its fibers, which are made into linen and other cloths (El-Nagdy *et al.* 2010). The main fatty acids of the flax seeds are alpha-linolenic acid, oleic acid and linolenic acid (El-Nagdy *et al.* 2010).

Salinity as one of the main problems in many arid and semi-arid regions of the world, leads to a decrease in agricultural production (Pessaraki and

Szabolcs 1999). Salinity damage in plants is due to the effects of water absorption, ionic toxicity, and nutrient absorption disorder. In most cases salinity tension leads to delay in germination, reduction of germination percentage, reduction of seed vigor, delaying root and stem emergence and consequently decreasing seedlings growth (Shiri *et al.* 2009). Salinity stress changes many physiological characteristics of the plant, including relative water content (RWC), leaf osmotic potential (Qin *et al.* 2010), transpiration rate and leaf temperature (Sharma *et al.* 2005) through ionic toxicity, disturbance of ionic balance and osmotic potential. In fact, free radicals produced by oxygen disrupt the

permeability of cell membrane by attacking to lipids and proteins under salinity stress. Therefore, measurement of electrolyte leakage is a good criterion for identifying quantitative damages due to salinity (Zhao *et al.* 2007).

Germination and early seedling growth is an important stage in the life of most plants and salinity tolerance is very important for the establishment, germination and growing of plants that grow in saline soils. There are limited studies about the effects of salinity stress on flax ecotypes. Therefore, the aim of this research was to study the effect of different levels of salinity on germination, seedling and morpho-physiological characteristics of different ecotypes of flax.

Materials and Methods

Two separate experiments were carried out in a laboratory and greenhouse of Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Iran.

First experiment

This experiment was conducted as factorial based on completely randomized design with three replications in the laboratory. Factors included salinity (0 (non-stress), 50, 100 and 150 mM NaCl) and three flax ecotypes (Kurdistan Native, L18, E37). Salinity was applied using sodium chloride salt. Seeds were first sterilized with 95% alcohol for 30 seconds and with sodium hypochlorite for 2 minutes and washed with distilled water. Then, 25 seeds were treated in sterile petri dishes containing filter paper by different salinity concentrations. After that, petri dishes were placed in a growth chamber at 20 ± 1

°C for six days (Saeidi and Rowland 1999). Germinated seeds were counted daily (every 24 hours). Seeds with a root length of one millimeter were considered as germinated. After six days, characteristics such as germination percentage, germination rate (GR), seedling vigor, hypocotyl length, radicle length, hypocotyl dry weight and radicle dry weight were measured. The germination rate was calculated using the following equation:

$$GR = \sum \left(\frac{ni}{Di} \right)$$

where, n is the number of the germinated seeds in day i and Di is the number of days after start of the experiment (Agrawal 2004).

Seedling vigor was obtained from the following equation (Abdul-Baki and Anderson 1970):

$$\text{Seedling vigor} = (\text{germination percentage} \times \text{mean seedling length (cm)})/100$$

Second experiment

This experiment was also conducted as factorial using completely randomized design with three replications. Experimental factors were salinity (0 (non-stress), 50, 100 and 150 mM NaCl) and three flax ecotypes (Kurdistan Native, L18, E37). Plastic pots (diameter: 20 cm, height: 30 cm, volume: 8 liters) were used for this purpose, with holes in the bottom for drainage. The seeds of each ecotype were planted in pots filled with cocopite and perlite (1:1). After planting, all pots were irrigated with a nutrient solution with half strength Hoagland's solution (Hoagland and Arnon 1950). After the appearance of fourth true leaves, 50, 100 and 150 mM NaCl was added to

half strength Hoagland's solution. Control plants (non-stress) were only irrigated with half strength Hoagland's solution. After 45 days of planting, the plants were harvested and shoot height, root length, root and shoot dry weight, RWC, electrolyte leakage, total soluble sugar and proline were measured.

RWC was measured by Ritchie *et al.* (1990) and electrolyte leakage was measured by Agarie *et al.* (1995). Proline was measured as described by Bates *et al.* (1973). Total sugars were determined by the method of Dubois *et al.* (1956).

Statistical analysis

After analysis of variance, means were compared by LSD test at 5% probability level using SAS software.

Results and Discussion

First experiment

Germination percentage, germination rate and seed vigor were affected by ecotype, salinity and their interaction (Table 1). Germination percentage decreased as salinity increased. All three ecotypes had no significant difference in terms of germination percentage under non-stress condition, however, under salinity condition, E37 ecotype showed a significant decrease in germination percentage as compared to the other two ecotypes. There were no significant differences between two Kurdistan Native and L18 ecotypes except at the salinity of 100 mM (Table 2).

With increasing the salinity up to 100 mM, the germination rate of the studied ecotypes did not have a significant difference with the non-stressed

level, whereas at 150 mM salinity, E37 ecotype showed lower germination rate than other two ecotypes, however, its difference with L18 was not significant (Table 2).

Seed vigor decreased in all ecotypes with increasing salinity. In non-stressed condition and all salinity levels, seed vigor was higher in Kurdistan Native than other ecotypes. There was no significant difference between L18 and E37 ecotypes at salinity of 50 mM and non-stressed level. The lowest seed vigor was observed in E37 under salinity condition (Table 2).

According to Alasvandyari *et al.* (2017), salinity stress reduced the germination rate of safflower. It has been stated that the inhibitory effect of sodium chloride on germination of sunflower seeds depends on absorption of chlorine and sodium ions by hypocotyls (Turhan and Ayaz 2004). Reports show that the increase in salt concentration decreases germination index (El Goumi *et al.* 2014), germination percentage (Bordi 2010), rate of seed germination (Habtmu *et al.* 2013) and seedling vigor (Zapata *et al.* 2003) in different plants.

The effect of ecotype, salinity and the interaction them were significant on seedling growth characteristics (Table 1). The plumule and radicle length and dry weight of plumule and radicle decreased with increasing salinity in all ecotypes. In non-stressed conditions and all stressed levels, Kurdistan Native had the highest plumule length, radicle length, dry weight of plumule and radicle, which was followed by L18 and E37 (Table 3). High salinity may inhibit root and shoot growth due to slowing down the water uptake by the plant (Puvanitha and Mahendran

Table 1. Analysis of variance for germination and seedling growth characteristics of flax ecotypes under salinity condition.

SOV	d.f	Germination percentage	Germination rate	Seed vigor	Plumule length	Radicle length	Plumule dry weight	Radicle dry weight
Ecotype	2	841.36**	3.79*	992.30**	2.15**	3.34**	0.000023**	0.00083**
Salinity	3	1065.87**	10.43**	840.70**	0.97**	2.04**	0.000030**	0.00065**
Salinity * Ecotype	6	104.21*	2.06*	14.54*	0.07*	0.06*	0.0000009*	0.00006**
Error	24	35.05	18.50	5.70	0.02	0.02	0.0000003	0.000009
CV (%)		7.09	7.74	9.51	12.67	10.95	12.72	9.84

* and ** significant at 5% and 1% probability levels, respectively; CV: coefficient of variation

Table 2. Effects of salinity stress on germination characteristics of flax ecotypes.

Salinity (mM)	Ecotype	Germination percentage (%)	Germination rate (seed/day)	Seed vigor
0	Kurdistan Native	100±0.00a	12.42±0.06a	47.87±0.07a
	L18	95±4.00ab	12.39±0.06a	34.07±0.76bc
	E37	94.33±4.67ab	11.89±0.71ab	33.00±0.58bc
50	Kurdistan Native	92.33±3.71ab	12.02±0.04a	47.87±0.07a
	L18	90±0.00bc	11.99±0.16a	34.07±0.76bc
	E37	75.66±1.20d	11.65±0.59ab	33.00±0.58bc
100	Kurdistan Native	91.33±3.76abc	11.99±0.12ab	35.79±3.15b
	L18	77.33±2.67d	11.69±0.18ab	25.31±0.90e
	E37	75±0.58d	11.44±0.59ab	15.44±0.30g
150	Kurdistan Native	81.66±1.20cd	11.03±0.18ab	30.28±3.04cd
	L18	75.33±7.54d	10.59±1.05bc	19.59±1.28f
	E37	53.66±2.73e	7.77±0.58c	12.62±0.26gh

In each column, means with the same letters are not significantly different ($p \leq 0.05$) based on LSD test; values are means ± SE for three replications.

Table 3. Effects of salinity stress on seedling growth characteristics of flax ecotypes.

Salinity (mM)	Ecotype	Plumule length (cm)	Radicle length (cm)	Plumule dry weight (g)	Radicle dry weight (g)
0	Kurdistan Native	2.19±0.15a	2.60±0.03a	0.009±0.0000a	0.055±0.0035a
	L18	1.81±0.04b	1.88±0.01bc	0.008±0.0003b	0.036±0.0009bc
	E37	1.32±0.02d	1.65±0.05c	0.005±0.0003de	0.033±0.0033c
50	Kurdistan Native	1.69±0.27bc	2.01±0.26b	0.006±0.0003c	0.040±0.0003b
	L18	1.64±0.03bc	1.14±0.05d	0.005±0.0003cd	0.034±0.0003c
	E37	0.91±0.03e	1.09±0.06de	0.004±0.0003efg	0.031±0.0003c
100	Kurdistan Native	1.61±0.08bc	1.71±0.05c	0.005±0.0003de	0.035±0.0003c
	L18	1.48±0.06cd	1.06±0.04de	0.004±0.0006ef	0.034±0.0003c
	E37	0.81±0.01ef	0.87±0.06e	0.003±0.0003gh	0.019±0.0003d
150	Kurdistan Native	1.52±0.03cd	1.62±0.04c	0.004±0.0003efg	0.032±0.0010c
	L18	0.80±0.09ef	0.88±0.04de	0.003±0.0003fg	0.019±0.0032d
	E37	0.61±0.04f	0.24±0.09f	0.002±0.0003h	0.013±0.0018e

In each column, means with same letters are not significantly different ($p \leq 0.05$), according to LSD test; values are means ± SE for three replications.

2017). The adverse effect of salinity on plant growth varies among species (Bolarian *et al.* 1991) and among varieties within species (Ghoulam *et al.* 2002). The combination of osmotic effects and ion toxicity of Cl⁻ and Na⁺

may have contributed to the reduction of root dry weight as a result of increased salinity (Taffouo *et al.* 2010). Ionic toxicity may cause root injury and death which affects water uptake adversely and increases water deficit leading to decreased net

photosynthesis and shoot growth (Puvanitha and Mahendran 2017).

Second experiment

The effect of ecotype, salinity and their interaction were significant on stem length, root length and stem and root dry weight (Table 4). With increasing salinity, stem length, root length and stem and root dry weight decreased in all ecotypes. In the non-stressed condition, no significant difference was obtained between Kurdistan Native and L18 in terms of stem length but Kurdistan Native had higher stem length at all salinity levels (Table 5). The root length of ecotypes was not significantly different at non-stressed and all salinity levels, except that at 150 mM, E37 ecotype showed a significant decrease in root length at the salinity of 150 mM as compared to other ecotypes. Kurdistan Native had the highest stem and root dry weight at all levels

of salinity (except at 100 mM salinity for root dry weight) and non-stressed level (Table 5). The root length and shoot length are important traits involved in the response of plants to salt stress because roots contact with soil directly and absorb water from the soil. (Jamil and Rha 2004). Ashraf and Ahmad (2000) reported a decrease in stem and root dry weight due to salinity in common flax.

Electrolyte leakage was affected by the ecotype, salinity and the interaction of these factors (Table 4). Electrolyte leakage increased in all three ecotypes as salinity increased (Table 5). In the non-stressed condition, the lowest electrolyte leakage was related to Kurdistan Native followed by the L18 and E37. At the salinity of 50 mM, the lowest electrolyte leakage was observed in Kurdistan Native, but two ecotypes of L18 and E37 showed no significant difference. Under salinity level of 100 mM, three

Table 4. Analysis of variance for growth and some physiological characteristics of flax ecotypes under salinity condition.

SOV	d.f	Shoot length	Root length	Shoot dry weight	Root dry weight	Electrolyte leakage	RWC	Total soluble sugars	Proline
Ecotype	2	32.74**	14.17**	0.0024**	0.00028**	402.71**	586.92**	0.537**	0.027**
Salinity	3	21.94**	61.85**	0.0026**	0.00094**	575.46**	1332.24**	0.890**	0.137**
Salinity * Ecotype	6	1.77*	5.81*	0.00009*	0.00002*	47.91*	115.54*	0.098*	0.004 ^{ns}
Error	24	0.67	54.05	0.00003	0.000008	17.41	40.46	0.036	0.054
CV (%)		9.54	8.60	9.22	10.26	7.25	9.52	9.62	13.66

^{ns}, * and ** not significant and significant at 5% and 1% probability levels, respectively; CV: coefficient of variation; RWC: relative water content.

ecotypes had no significant difference for electrolyte leakage. However, at the salinity of 150 mM, Kurdistan Native ecotype had the lowest electrolyte leakage and L18 and E37 ecotypes had the highest electrolyte leakage (Table 5). Increased electrolyte leakage in rice plants was observed with increasing salinity (Lutts *et al.* 1996). Similarly, an increase in electrolyte

leakage was observed by increasing salinity level in cucumber (Kaya *et al.* 2001) and sugar beet (Ghoulam *et al.* 2002). Mandhania *et al.* (2006) reported that salinity enhanced membrane damage, electrolyte leakage and oxidative damage. The effect of ecotype, salinity and their interaction were significant on RWC and total soluble sugar content (Table 4). All ecotypes

showed no significant change in RWC from non-stressed condition to the salinity of 50 mM. At the salinity of 100 mM, a significant reduction in RWC was observed in all ecotypes compared to the non-stressed condition. The highest RWC was found in Kurdistan Native and the lowest was

observed in E37 (Table 6). In a study, salt treatment significantly decreased the RWC of sugar beet varieties (Ghoulam *et al.* 2002). Based on Sairam *et al.* (2002), the reduction in RWC of a salt-sensitive wheat cultivar was higher than the tolerant cultivar under salt stress condition.

Table 5. Effects of salinity stress on growth characteristics of flax ecotypes.

Salinity (mM)	Ecotype	Shoot length (cm)	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)
0	Kurdistan Native	11.15±0.38a	20.57±0.12a	0.09±0.003a	0.05±0.001a
	L18	10.40±0.15a	20.47±0.20ab	0.08±0.001bcd	0.04±0.005b
	E37	9.21±0.24bcd	20.12±0.48ab	0.07±0.006de	0.04±0.002cd
50	Kurdistan Native	11.14±0.78a	18.73±0.41abc	0.08±0.005ab	0.04±0.000bc
	L18	8.43±0.18cde	18.70±0.16abc	0.07±0.001cde	0.03±0.001d
	E37	8.10±0.15de	17.99±1.60bcd	0.06±0.001fg	0.02±0.001e
100	Kurdistan Native	10.07±0.88ab	17.39±0.63cde	0.08±0.003ab	0.03±0.000e
	L18	7.49±0.11e	16.47±0.28cde	0.07±0.001def	0.03±0.001e
	E37	7.24±0.48e	16.16±1.04de	0.06±0.002g	0.02±0.001e
150	Kurdistan Native	9.52±0.60bc	16.907±1.22cde	0.07±0.004efg	0.02±0.000e
	L18	5.12±0.57f	15.033±0.91e	0.04±0.006h	0.01±0.001f
	E37	5.10±0.31f	10.767±1.45f	0.03±0.004h	0.01±0.000f

In each column, means with same letters are not significantly different ($p \leq 0.05$), according to LSD test; values are means \pm SE for three replications.

Table 6. Effects of salinity stress on some physiological characteristics of flax ecotypes.

Salinity (mM)	Ecotype	Electrolyte leakage (%)	RWC (%)	Total soluble sugar (mg/g FW)
0	Native Kurdistan	41.25±0.31e	84.13±5.57a	1.88±0.197cde
	L18	47.90±7.03e	83.50±6.68ab	1.75±0.023cde
	E37	54.97±2.78d	73.06±3.68c	1.58±0.063e
50	Native Kurdistan	42.33±1.17e	73.48±3.69abc	2.01±0.002bcd
	L18	59±0.00cd	73.32±3.10bc	1.87±0.034cde
	E37	61.99±0.58bcd	72.57±5.31c	1.75±0.013de
100	Native Kurdistan	59.1±2.93cd	71.58±0.23c	2.02±0.025bcd
	L18	61.853±0.18bcd	55.28±1.87d	1.90±0.057cde
	E37	62.02±1.06bc	54.53±0.42d	1.84±0.015cde
150	Native Kurdistan	61.34±0.68cd	66.97±0.81c	2.98±0.009a
	L18	68.48±0.09ab	52.59±3.83d	2.29±0.025b
	E37	69.64±0.72a	40.19±1.49e	2.07±0.314bc

In each column, means with same letters are not significantly different ($p \leq 0.05$), according to LSD test; values are means \pm SE for three replications; RWC: relative water content.

Decreased RWC can potentially retard plant growth under salt stress (Ghoulam *et al.* 2002). A decrease in RWC is the indication of turgor loss that limit water availability for cell extension (Katerji *et al.* 1997). Electrolyte leakage had negative correlation with stem dry weight, root dry weight and RWC in our study (Table 7). In

contrast, the correlation between RWC and stem and root dry weight was positive (Table 7). This shows that the increase in electrolyte leakage is interpreted as a damage to the membrane associated with a decrease in RWC under salinity condition, and this might decrease plant growth.

The salinity levels of 50 and 100 mM did not

change soluble sugar content in ecotypes compared to the non-stress condition. However, under salinity of 150 mM, the increase in total soluble sugar was observed and the Kurdistan Native ecotype had the highest rate of this trait, (Table 6). Increased total soluble sugars under salinity stress have been reported in some safflower cultivars by Jabeen and Ahmad (2013). The change in soluble sugars, such as sucrose and fructan, are related with photosynthesis, translocation and respiration (Housley and Pollock 1993; McKersie and Leshem 1994). The correlation of total soluble sugar content with root dry weight was negative and significant, but was not significant with shoot dry weight (Table 7).

Proline was affected by ecotype and salinity (Table 4). The proline content increased with

increasing salinity. Kurdistan Native had significantly higher proline content than L18 and E37, but difference between L18 and E37 was not significant (Figure 1). The correlation between proline and shoot and root dry weight was negative and significant (Table 7). According to Ashraf and Foolad (2007), proline is involved in regulating the osmotic pressure in the cells. However, the contribution of soluble sugars to osmotic adjustment is possibly higher than that of proline (Watanabe *et al.* 1999).

Conclusions

Results showed that salinity stress reduced germination and seedling growth characteristics of different flax ecotypes. With increasing salinity to 150 mM, Kurdistan Native and E37 ecotypes

Table 7. Correlation coefficients among growth and some physiological characteristics of flax ecotypes under salinity condition.

Trait	Root length	Shoot length	Root dry weight	Shoot dry weight	Electrolyte leakage	RWC	Total soluble sugars	Proline
Root length	1							
Shoot length	0.74**	1						
Root dry weight	0.81*	0.79**	1					
Shoot dry weight	0.76*	0.86**	0.82**	1				
Electrolyte leakage	-0.67*	-0.74**	-0.83**	-0.74**	1			
RWC	0.76*	0.77**	0.75**	0.78**	-0.67**	1		
Total soluble sugars	-0.28*	-0.06 ^{ns}	-0.36*	-0.23 ^{ns}	0.23 ^{ns}	-0.24 ^{ns}	1	
Proline	-0.51**	-0.28 ^{ns}	-0.57**	-0.34*	0.46**	-0.51**	0.81**	1

^{ns}, * and ** not significant and significant at 5% and 1% probability levels, respectively.

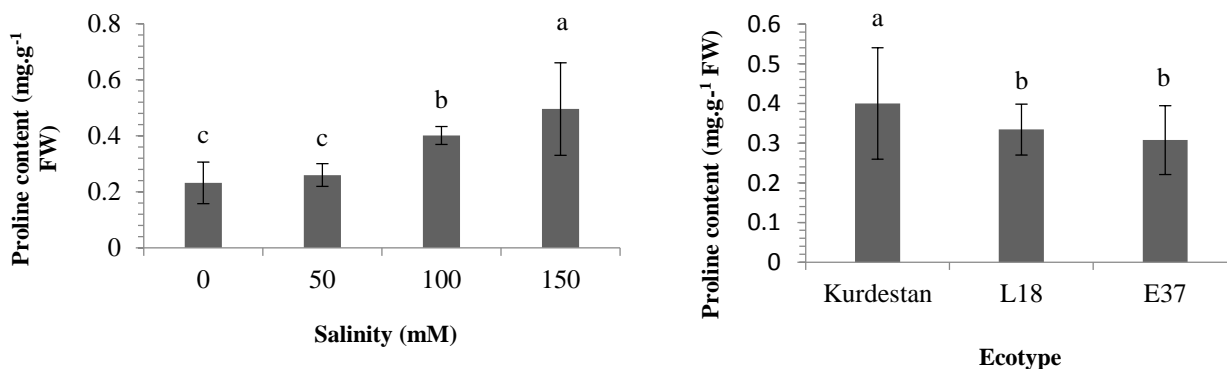


Figure 1. Proline content of flax ecotypes and different salinity levels; means with the same letters are not significantly different ($p \leq 0.05$) based on LSD test; values are means \pm SE for three replications.

had the highest and lowest germination and seedling growth, respectively. Salinity with its negative effect on the plant, by reducing the osmotic potential resulted in the decreased water absorption, growth and RWC and increased electrolyte leakage, thereby causing inhibition of root and stem growth and decreasing their length, which ultimately led to the reduction of the dry

weight of root and stem in different flax ecotypes. The main characteristics which indicated the tolerance of flax ecotypes to salinity stress were electrolyte leakage, RWC and total soluble sugar content. Based on these characteristics Kurdistan Native was determined as the most tolerant flax ecotype to salinity.

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واکنش جوانه زنی و مورفوفیزیولوژیک اکوتیپ‌های کتان روغنی به تنش شوری

بتول مهدوی* و فرناز علاسوندیاری

گروه ژنتیک و تولید گیاهی، دانشکده کشاورزی، دانشگاه ولی عصر رفسنجان، رفسنجان.

*مسئول مکاتبه؛ Email: b.mahdavi@vru.ac.ir

چکیده

در بسیاری از نواحی جهان شوری از مهمترین فاکتورهای محدود کننده رشد گیاه است. برای ارزیابی اثرات تنش شوری روی جوانه زنی، رشد و برخی ویژگی‌های کتان روغنی دو آزمایش جداگانه در آزمایشگاه و گلخانه انجام شد. فاکتورهای آزمایشی شامل سه اکوتیپ کتان روغنی (بومی کردستان، L18، E37) و چهار سطح شوری (0، 50، 100 و 150 میلی مولار) بودند. نتایج نشان داد که با افزایش شوری درصد جوانه‌زنی، سرعت جوانه‌زنی، بنیه گیاهچه، طول و وزن خشک ساقه‌چه و ریشه‌چه نسبت به شاهد در هر سه اکوتیپ کاهش یافت. در شوری 150 میلی مولار، اکوتیپ بومی کردستان و E37 به ترتیب بالاترین و پایین‌ترین جوانه زنی و رشد گیاهچه را داشتند. همچنین شوری طول ساقه و ریشه، وزن خشک ساقه و ریشه و محتوی نسبی آب را کاهش داد درحالی که نشأت الکترولیت‌ها و قندهای محلول را در هر سه اکوتیپ افزایش داد. اکوتیپ بومی کردستان بیشترین وزن خشک ریشه و ساقه، محتوی نسبی آب و قندهای محلول را داشت و بعد از آن L18 و E37 قرار گرفتند. در شوری 150 میلی مولار اکوتیپ بومی کردستان پایین‌ترین و اکوتیپ‌های L18 و E37 بالاترین نشأت الکترولیت-ها را دارا بودند. به طور کلی، حساس‌ترین و متحمل‌ترین اکوتیپ‌ها به ترتیب E37 و بومی کردستان بودند. بنابراین، اکوتیپ بومی کردستان دارای پتانسیل برای کاشت در مناطق با آب شور است.

واژه‌های کلیدی: پرولین؛ جوانه زنی؛ قندهای محلول کل؛ نشأت الکترولیت؛ NaCl