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Effect of drought and salinity stresses on morphological and physiological characteristics of canola

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Abstract Environmental stresses such as salinity and drought are the most important factors affecting yield reduction and crops productivity. In order to investigate the effects of drought and salinity stresses on morphological and physiological characteristics of canola, an experimental campaign was conducted as a split plot based on a randomized complete block design with three replications at greenhouse of Agriculture Research Center and Natural Resources of East Azarbaijan in 2014. Main plots were including drought stress at four levels (0, -4, -8 and -12 bar polyethylene glycol solution) and subplots including salinity stress at four levels of sodium chloride (0, 75, 150 and 225 mM). The results analysis of variance indicated that the interaction of drought and salinity stresses was significant on leaf area and relative water

content of leaf. The most leaf area (383.03 cm⁻²) was obtained at non-stress treatments. The results showed that drought stress conditions led to significant reduction in relative water content in leaf. The highest proline (0.08 μm/g fresh weight) and soluble sugars (0.12 mg/g fresh weight) contents were observed at treatments of -12 bar polyethylene glycol. Also, the least proline (0.04 μm/g fresh weight) and soluble sugar (0.06 mg/g fresh weight) contents were achieved at treatments of drought non-stress.

Keywords Stress · Plant height · Proline · Leaf area · Soluble sugar · Relative water content

Introduction

Canola (*Brassica napus*) is one of the most important oil-seed plants that have high compatibility in resistance to drought and salinity stresses. After soybean, the largest cultivation area of oilseed plants is accounted to canola, and in terms of oil providing, after soybean and oil palm it is in third place (FAO 2005). Like many of the oilseed plants, canola is effected stress caused by the water deficits. Studies have shown that the incidence of water deficit at different growth stages, especially reproductive growth, is the effect of quantity and quality of oil (Angadi and Cutforth 2003). Salinity stress is one of the most important abiotic stresses, and its negative impacts on crop's growth led to increase in research in the field of tolerance to salinity with the objective of improving plant's tolerance (Zhao et al. 2007). Salinity with sodium chloride caused decrease in sugars that is necessary for cells growth and main steps of photosynthesis process and its velocity. Sugars supported main steps of photosynthesis process and

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its velocity, and usually the lowest of photosynthesis rate has been observed in plants under salinity stress, especially with salinity of sodium chloride (Parida and Das 2005).

Photosynthesis is the main determinant of growth and crops yield, and its maintainability under environmental stresses condition is important for preservation of yield stability. The decrease in photosynthesis could be due to lack of stomatal conductance that reduced under stress conditions. Although stomatal closure under stress conditions takes place in order to decrease the water loss, the prevention of the entrance of CO₂ could reduce the photosynthesis below the compensation point (Ashraf and Harris 2004). Turan et al. (2009) in investigating the effect of salinity stress on corn reported negative relevance between stomatal resistance and sodium chloride; they also stated that the total chlorophyll content reduced under salinity, as well as its stomatal resistance increases. One of the effects of salinity stress in plants is decrease in photosynthesis activity that lead to reducing the chlorophyll content, absorption of CO₂ and photosynthetic capacity (Francis et al. 2002). The reduction of the photosynthesis velocity induced by salinity stress is due to various factors such as cell membrane dehydration and consequently decrease in CO₂ permeability, toxicity caused by salt, reducing the amount of CO₂ due to stomatal closure, accelerate the aging process as a result of salt, enzymes activity change due to structural changes in the cytoplasm and negative feedback due to reduction in source activity. Also, salinity stress acts in inhibiting the photosynthetic electron transport, stomatal conductance reduction and increase in production of reactive oxygen species that cause oxidative damage to photosystem (Munns 2002).

According to report of Cha-um and Kirdmanee (2009), salinity reduces total chlorophyll concentration of corn. Chlorophyll is one of the main and important components of chloroplast, and the chlorophyll content with the photosynthesis rate has positive relation. Chlorophyll content of leaves is one of the key factors in determining of photosynthesis amount and dry matter production (Ghosh et al. 2004). In the stress conditions, dry matter reduction could be due to cell turgor pressure induced by reducing the leaf area and also photosynthetic rate decrease because of the biochemical restrictions due to water deficit such as decrease in photosynthetic pigments, especially chlorophylls (Lawlor and Cornic 2002). Zhao et al. (2007) reported that salinity led to reducing the chlorophyll amount in oat. Yari et al. (2005) stated that drought stress reduced the chlorophyll content in leaf. Change of relative water content is used as a short-term reaction to stress, and a criterion from ability maintains vigor of source in drought condition (Claussen 2002). The increase in salinity intensity reduces the relative water content of tissues. Reducing the relative water content could lead to reduce the cell

turgor potential and in result decrease in leaves and roots development irreversible till severe damage and even cell plasmolysis step and subsequently protein crystallization (Kasier 1992).

Hall (2001) reported that proline and soluble sugars accumulation shows prompt reaction that changes relative water content and leaf water potential. A higher relative water content means the ability of leaves to maintain water content under stress conditions. When plants exposed under drought stress, the water potential in leaves decreases, and then, considerable amount of relative water content was decreased in both conditions (drought stress and increase in leaf temperature). One of the key factors in plants protection against abiotic stress is osmotic (Slama et al. 2006). Proline as a compatible metabolite is responsible of important roles such as energy and storage compound for nitrogen, elimination of hydroxyl radicals, protection of enzymes, pH reduction and turgidity maintenance and cell volume (Ahmad and Hellebust 1988). Also, proline leads to stability of protein natural forms and prevents from disruption for the normal form of enzymatic compositions (Paul and Hasegawa 1996). The accumulation of soluble sugars in response to environmental stresses is associated with osmotic adjustment or cell membranes protection. The soluble sugars content may be a useful method in selecting varieties resistant to drought and salinity. The physiologic action of these sugars is to prevent the connection between the adjacent membranes during the stress period, lipid maintenance and protein stability through the creation of hydrogen bonds with proteins linear sequences, gene regulation and osmotic adjustment (Ho et al. 2001). Rozrokh et al. (2012) in experiments on pea reported that the soluble sugar content increased under drought stress. This experiment was conducted in order to investigate the effects of drought and salinity stresses on morphological and physiological characteristics of canola.

Materials and methods

In order to investigate the effects of drought and salinity stresses on morphological and physiological characteristics of canola, a pot experiment was carried out as split plot based on RCD with three replications at greenhouse of Agriculture Research Center and Natural Resources of East Azarbaijan in 2014. Main plots were including drought stress at four levels (0, -4, -8 and -12 bar polyethylene glycol solution) and subplots including salinity stress at four levels of sodium chloride (0, 75, 150 and 225 mM). Experimental units were included pots with dimensions of 40 × 40 cm and height of 35 cm. Ten seeds were planted in pots at depth of 3 cm.

From sowing stage until germination stage, irrigation was conducted with water, and after the date of emergence (50% emergence), pots were irrigated with a solution containing half the concentration of nutrient of Hoagland solution. At the four-leaf stage, salinity application started with the addition of gradual sodium chloride, so that all the pots except the control unit were irrigated by adding gradual of salt to 25 mM at each level (for adapting of plants).

After a week, the salinity treatments at each level were applied. Salinity treatments application continued until the end of vegetative stage with the ratios mentioned. At the same time, plant water requirement, the time of observing salt in the pots and the level of dan-druff in the pots were checked to prevent salt accumulation. In addition to the pot drainage, leaching uniformly with water was ensured in all the treatments. The chlorophyll content in leaves was measured by the methods of Arnon (1949). To determine the relative water content in the flowering stage and 1 day before the irrigation, five fully developed young leaves were harvested from each plot and immediately transported to the laboratory. Leaves were divided into 2-cm parts and weighed (FW). Then, in order to determine the turgor weight, the samples were placed for 4 h in low light intensity within distilled water and their saturation weight (SW) was measured. Finally, leaves dry weight (DW) was determined after oven heating for 48 h at 75° Celsius. Relative water content is calculated by using the following formula (Ritchie et al. 1990):

$$RWC = (FW - DW / SW - DW) * 100.$$

where RWC is relative water content.

The proline content was measured by the method of Bates (1973) and soluble sugars content by the method of Irigoyen et al. (1992). Data analysis was performed by using SAS software, and mean comparisons were made by Lsmeans test at the 5% probability level.

Results and discussion

Plant height

Results of data analysis showed that the effect of drought and salinity stresses was significant ($P \leq 0.01$) on plant height. The most plant height equal to 81.73 cm was obtained at non-stress treatments, as well as no significant difference was observed between non-stress and -4 bar PEG treatments, but with increasing stress intensity, especially by treatments of -12 bar PEG, plants height decreased (71.98 cm). Also among the salinity stress treatments, the highest plant height equal to 82.07 cm was achieved at non-stress treatments, although the mentioned treatments were not significant different from treatments at 75 mM sodium chloride. Thus, by increasing drought and salinity stress, plant height intensively decreased. Plant height reduction due to drought stress can be attributed to disruption at photosynthesis through the water deficit and decreasing of photo-assimilates production for transfer to growing parts of plant. According to some researchers under drought stress, the abscisic acid leads to induction of shoot growth due to cessation of protons secretion of the auxin induce (Rao and Mendham 1991). Because reduction in plant height is the effect of salinity on leaf area, this reduction is particularly evident at the end of vegetative growth, and after entering the plant to flowering stage, the leaves have started to fall gradually from the down (Nabizadeh marost 2002).

Leaf area

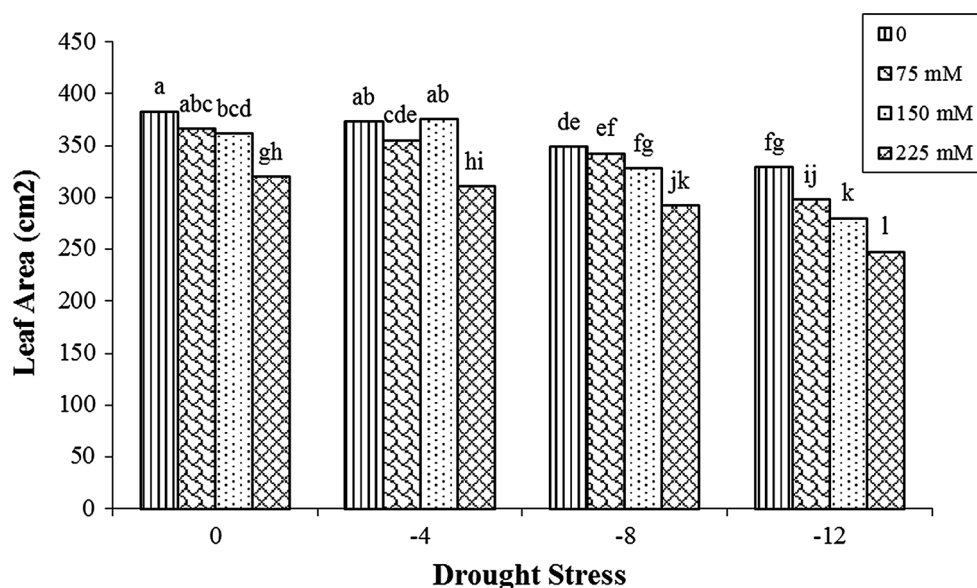
The results of data analysis indicated that the interaction of drought and salinity stresses was significant ($P \leq 0.05$) on leaf area (Table 1). The largest leaf area was obtained from treatments of drought stress and non-stress equal to 383.03 cm² (Fig. 1). The smallest leaf area was achieved at treatments of -12 bar PEG and 225 mM sodium chloride,

Table 1 Analysis of variance for some morphological and physiological characteristics of canola under the effect of drought and salinity stresses

Source of variation	df	Plant height	Leaf area	Number of lateral branch	Chlorophyll a	Chlorophyll b	Relative water content	Proline	Soluble sugar
Replication	2	4.36	147.46	0.03	0.00038	0.00077	23.72	0.00002	0.00030
Drought stress (D)	3	228.52 **	12064.52**	0.34 ns	0.00941**	0.01014**	1193.91**	0.00319**	0.00789**
Rep × D	6	16.17	53.83	0.10	0.00005	0.00002	3.76	0.00001	0.00001
Salinity stress (S)	3	387.12**	9409.32**	0.46**	0.00757**	0.02327**	245.52**	0.00276**	0.00360**
D × S	9	15.33 ^{ns}	296.72*	0.05 ^{ns}	0.00027 ^{ns}	0.00038 ^{ns}	32.71*	0.00001 ^{ns}	0.00016 ^{ns}
Error	24	15.86	105.71	0.05	0.00014	0.00022	12.11	0.00001	0.00007
Coefficient of variation (%)	-	5.18	3.09	8.54	12.67	6.91	5.37	5.67	8.34

** , * and ns are significant at 0.01 and 0.05 probability level and nonsignificant, respectively



Fig. 1 Interaction of drought and salinity stresses on leaf area

equal to 247.41 cm². Dry matter production in plant shows a positive correlation with leaf area and leaf photosynthetic rate; thus, for achieving higher rates of dry matter production, it is necessary to increase the photosynthesis rate by maintaining a high leaf area at all growth seasons. It seems that the decrease in leaf area in plant is due to the reduction in the water absorption capability of the plant due to osmotic stress caused by salinity.

Number of lateral branch

The results of variance analysis indicated that the effect of salinity stress was significant ($P \leq 0.01$) on the number of lateral branch. The highest number of lateral branch was obtained at treatments of non-stress and 75 mM sodium chloride, equal to 2.85 and 2.79, respectively (Table 2). Also, the least the number of lateral branch among the

stress treatments was achieved from 150 and 225 mM sodium chloride equal to 2.57 and 2.42, respectively. Sadeghinejad et al. (2014) reported that the effect of drought stress was significant on the number of lateral branch.

Chlorophyll a and b

The results of data analysis indicated that the effect of drought and salinity stresses was significant ($P \leq 0.01$) on chlorophylls a and b (Table 1). The highest chlorophylls a and b were obtained at treatments of drought non-stress equal to 0.12 and 0.24 mg/g fresh weight, respectively (Table 2). Also among drought different levels the least chlorophylls a and b was achieved at treatments of -12 bar equal to 0.05 and 0.18 mg/g fresh weight, respectively (Table 2). The highest chlorophyll a and b treatments were

Table 2 Mean comparison of the effects of drought and salinity stresses on some morphological and physiological characteristics of canola

Experimental factors	Plant height (cm)	Number of lateral branch	Chlorophyll a (mg g ⁻¹ fresh weight)	Chlorophyll b (mg g ⁻¹ fresh weight)	Proline (μM g ⁻¹ fresh weight)	Soluble sugar (mg g ⁻¹ fresh weight)
Drought stress						
0	81.73 ^a	2.87 ^a	0.12 ^a	0.24 ^a	0.04 ^d	0.06 ^d
-4 bar	79.70 ^a	2.67 ^{ab}	0.11 ^b	0.23 ^b	0.06 ^c	0.10 ^c
-8 bar	75.46 ^b	2.64 ^{ab}	0.09 ^c	0.20 ^c	0.07 ^b	0.11 ^b
-12 bar	71.98 ^b	2.46 ^b	0.05 ^d	0.18 ^d	0.08 ^a	0.12 ^a
Salinity stress						
0	82.07 ^a	2.85 ^a	0.12 ^a	0.27 ^a	0.05 ^d	0.08 ^d
75 mM	80.25 ^{ab}	2.79 ^a	0.09 ^b	0.22 ^b	0.05 ^c	0.10 ^c
150 mM	77.33 ^b	2.57 ^b	0.08 ^c	0.20 ^c	0.06 ^b	0.11 ^b
225 mM	69.22 ^c	2.42 ^b	0.07 ^d	0.16 ^d	0.08 ^a	0.12 ^a

In each column and treatment, similar letter or letters are indicative of no significant difference in the level of 5% based on the LSD test

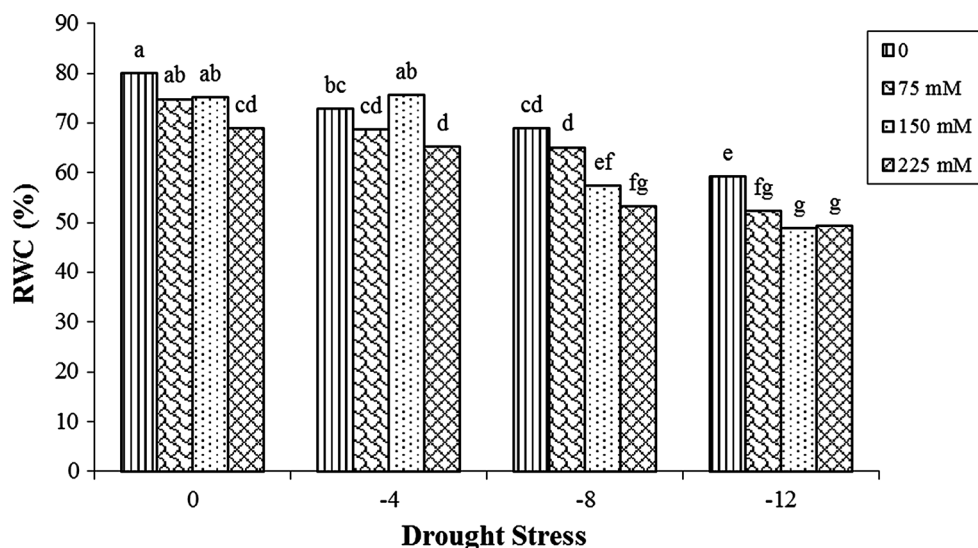
at salinity non-stress equal to 0.12 and 0.27 mg/g fresh weight, respectively (Table 2). Also among the salinity different levels, the least chlorophyll a and b was observed at treatment of 225 mM sodium chloride equal to 0.07 and 0.16 mg/g fresh weight, respectively. Yari et al. (2005) stated that drought stress decreases chlorophyll content in leaves. According to results of Zafar et al. (2014), the highest chlorophyll content was related to normal irrigation. Drought stress at growth different stages led to decrease in chlorophyll content in leaves. Jabari et al. (2006) stated that under the effect of drought stress cells are destroyed, because under drought stress conditions the stomata are closed, resulting in the reduction of carbon dioxide, while light reactions and electron transfer take place in normal state. In such conditions, the NADP available for electron accepting is limited. Thus, oxygen could be an alternative electron acceptor that leads to accumulation of toxic oxygen species such as superoxide radicals (O_2^-), peroxide hydrogen (H_2O_2) and hydroxyl radicals (OH^-). Accumulation of reactive oxygen species, which are produced under stress, caused damage to cellular ingredients such as lipids, proteins, sugars and nucleic acids (Jiang and Huang 2001). Salinity stress led to reduction of chlorophyll content, and this reduction could be due to destruction of chloroplasts structure and photosynthetic apparatus, chlorophylls photo-oxidation, their reaction with singlet oxygen, demolition of chlorophyll synthesis precursors, prevention of chlorophyll biosynthesis, prevention of the new chlorophylls biosynthesis and activating the chlorophyll-degrading enzymes such as including chlorophyllase and hormonal disruptions (Neocleous and Vasilakakis 2007). However, the accumulation of sodium and chlorine ions in leaves under salinity had the negative impact on chlorophyll concentrations. In the

meantime, those plants that are salinity tolerant could improve the decrease in chlorophyll content. Also, leaf aging as a result of decrease in chlorophyll content was affected by salinity stress. Kaya et al. (2006) by comparing the strawberries and spinach cultivars found that salinity stress decreases the chlorophyll content.

Relative water content (RWC)

The results of data analysis indicated that the interaction of drought and salinity stresses was significant ($P \leq 0.05$) on RWC (Table 1). The highest RWC was obtained at treatments of drought and salinity non-stresses equal to 79.99% (Fig. 2). Also among the interactions, the lowest RWC was obtained at drought of -12 bar PEG and salinity of 150 mM sodium chloride treatments equal to 48.88% (Fig. 2). In general by increasing drought stress intensity, the reduction in RWC can be due to decrease in water potential of leaves. Sadeghipour and Aghaei (2012) stated that drought stress conditions led to significant reduction in RWC. When plants are under drought stress, the water potential in leaves decreases. The obtained results of this study are in agreement with results of Watson et al. (2003). Water deficit in plants could happen by decrease in leaf stomatal opening, because water potential of saline soil is in the negative level by osmotic potential of dissolved solutions. Osmotic potential within cell can be more negative via increasing the soluble substance content. It can be said that osmotic adjustment is not a direct and indirect response to water deficit, but has occurred in result of other factors such as reducing the growth rate. The increase in sodium ion content at cell organelles such as chloroplasts and vacuoles led to structural changes and increasing membrane leakage.

Fig. 2 Interaction of drought and salinity stresses on leaf relative water content



Relative water content is considered as a reliable criterion for measuring the water status in the plant tissues and hence is superior to cell water potential since the relative water content through a direct connection with cell volume could better demonstrate the balance between the plant water and transpiration rate.

Proline and soluble sugar contents

The results of data analysis indicated that the effect of drought and salinity stresses was significant ($P \leq 0.05$) on proline and soluble sugar contents (Table 1). Among the drought stress levels, the most proline and soluble sugar contents were obtained at treatments –12 bar PEG equal to 0.08 $\mu\text{m/g}$ fresh weight and 0.12 mg/g fresh weight, respectively (Table 2). Also, the least proline and soluble sugar contents were observed at non-stress treatments equal to 0.04 $\mu\text{m/g}$ fresh weight and 0.06 mg/g fresh weight, respectively (Table 2). Plants in the face of drought stress are trying to maintain its osmotic pressure and the work performed by increasing osmolytes such as proline and soluble carbohydrates that help the pressure and plant cells turgidity to be maintained. Research results by Heuer (1994) showed that proline accumulation in all plant organs increases during drought stress; however, the proline accumulation in the leaves is higher than in other organs. Proline is stored amino acid at the cytoplasm and probably is effective in the protection of macromolecules structure within cells during the drought stress. Good and Zaplachinski (1994) reported that ingredients such as proline and amino acids in green tissues of canola under drought stress could provide partially necessary conditions for water absorption from the roots environment for plant, but dependence plants to organic ingredients are costly for osmotic adjustment and lead to decrease in yield. Among the salinity different levels, the highest proline and soluble content was obtained at treatments of 225 mM sodium chloride equal to 0.08 $\mu\text{m/g}$ fresh weight and 0.12 mg/g fresh weight, respectively (Table 2). Also the lowest proline and soluble content was observed in treatments of salinity non-stress equal to 0.05 $\mu\text{m/g}$ fresh weight and 0.08 mg/g fresh weight, respectively. Soluble sugars are another class of osmotic protectors.

The accumulation of soluble sugars in response to environmental stresses is associated with osmotic adjustment or cell membranes protect. The soluble sugars content may be a useful method in selecting of resistant varieties to drought and salinity. Soluble sugars are among the other compatible organic osmolytes those that accumulate in drought stress conditions and may act as an osmotic factor or osmotic guards (Brugnoli, and Lauteri 1991). In the first case, the increase in sugars under impact was stressed by osmotic adjustment and turgidity maintenance, and in the

second case it is associated by stable membranes and proteins. The increase in proline under salinity stress proves that proline plays an important role in osmotic adjustment and occurrence of resistance to stress in these conditions. Proline leads to maintenance of proteins hydration in dehydrated tissue and cell activities survival. Increase in proline in bean plants under salinity stress leads to osmotic potential regulation in the cell cytoplasm (Zayed and Elamry 2006). Javadipour et al. (2012) reported that with increase in salinity stress intensity increased accumulation of soluble sugars in leaves safflower. Also sucrose-phosphatase enzyme activity increases after application of salinity treatments. The increase in soluble sugars may be due to increase in the activity of this enzyme in plants. Along with the increase in sodium chloride concentration and ion accumulation in plant tissues for maintaining water balance and osmotic conditions, it is necessary to increase in the soluble sugars. It seems that plants are under salinity stress; the increase in proline amino acid led to maintain the turgidity pressure and continuing growth of cell, and it is an antioxidant role in the protection of biological membranes (Patakas et al. 2002). Increase of proline caused by the amount of sodium chloride can be due to the fact that glutamate pathway enzymes, under salinity stress of sodium chloride, activate and increase the proline synthesis, because sodium chloride stimulates the synthesis genes of these enzymes. About the accumulation of proline in the plant have been presented various reasons. Some assume that the reason is the effect of abscisic acid regulatory on light processes in the proline metabolism (Serraj and Sinclair 2002) and existence of high-energy ingredients derived from photosynthesis that led to stimulate the proline synthesis (Mattioni 1997). Ashraf and McNeilly (2004) stated that total soluble sugars are reduced in Brassica species of resistant to salinity.

Conclusion

The results of this study suggest that the interaction effect of drought stress and salinity on canola plant decreased the properties such as leaf surface, relative water content of leaves, while it had no impact on other properties studied. Treatments of drought stress and salinity reduced all of the properties. The highest and lowest contents of proline and soluble sugars in applied drought stress treatments were related to treatments of 12 bar polyethylene glycol and control (no stress), respectively. In addition, the highest and lowest proline and soluble sugar contents in different salinity treatments were related to treatments of 225 mM sodium chloride and control (no stress), respectively. Finally, it can be concluded that the drought stress and salinity had negative impact on most of the morphological

properties, but they increased the content of proline and soluble sugar in shoots.

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