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The Effect of Proline and Salt Stress on Growth Characteristics of Three Olive Cultivars at Three Different Stages of the Growing Season

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KEYWORDS

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ABSTRACT: Proline is a vital amino acid, commonly distributed in all plants. It is widely accumulated in plants under salt stress. It has been suggested that foliar application of proline has an important effect in reducing destructive effects of non-living stresses on plants. On the other hand, an excessive amount of free proline has negative effects on cell growth as well as protein functions. In this study, the six-months-old plantlets of three olive cultivars, including Arbequina, Arbosana and Koroneiki were sprayed with proline at 0, 100, and 200 mg/L for three times in intervals of 10 days. In addition, the samples were subjected to salinity at 0, 50, 100 and 200 mM sodium chloride for five months. Measurement of morphological characteristics of stems and leaves was conducted in three stages (4, 12 and 20 weeks after treatment). The results showed that stem length and number of nodes gradually increased over time at all concentrations of proline. Furthermore, at stage 3, stem diameter, number of leaves and branch number increased and leaf width decreased. The highest leaf thickness was observed at stage 1. However, no significant difference was found among the proline concentrations in the mentioned traits in any experimental stage. Plants sprayed with proline were later encountered the increased leaf necrosis. At stage 3, the control plants had a lower percentage of abscission than proline-treated plants. At stage 2, plants sprayed with proline had lower leaf thickness than control plants. Throughout the experiment, salinity, especially 200 mM, reduced cumulative stem length, number of shoots, internode length, number of nodes and number of leaves. The highest percentages of leaf abscission and necrosis, as well as the highest leaf thickness were observed at 200mM NaCl treatment. In general, despite the fact that proline increased in the plants under stress conditions, its external application was not significantly effective.

INTRODUCTION

Higher plants mainly synthesize proline from glutamine in response to stress [1]. Proline plays a significant role in plant protection against osmotic stress. Under these conditions, proline is usually synthesized from glutamate via two successive reductions catalyzed by 1-pyrroline-5-carboxylate synthetase (P5CS) and1-pyrroline-5

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carboxylate reductase (P5CR) [2, 3]. Proline is one of the vital amino acids, signaling molecules, and compatible organic solutions, acting as a growth regulator by activating various signaling processes [4, 5]. Proline accumulates in the cytoplasm without any detrimental effect on cytosolic enzyme activities [6]. The main function of the accumulated proline is osmotic regulation, an adaptation mechanism to environmental stress and salinity [7]. Increasing proline concentration results from preventing proline degradation and inhibiting proline entering into the protein production cycle that may be associated with decreasing growth [8]. In recent years, many papers have been published on the role of proline in promoting salt tolerance of crops, such as watermelon [9], olive [7], tobacco [10] and bean [11]. There has been a gradual increase in proline concentration with increase in salinity in two cultivars of *Vigna radiata* L. including Pusa Bold and CO 4 [3]. Proline contents in salt-tolerant varieties of rice were increased higher than in salt-sensitive varieties. Proline accumulation in different genotypes under salinity depends on the variety of crop species [12]. However, there are contradictions in proline external application, and despite the protective functions of intracellular proline in plants under stress, its external application may have toxic and harmful effects. It has been reported that the external application of proline at a concentration of 10 mM to the wild species of *Arabidopsis* is toxic [13]. Excessive accumulation of intracellular proline significantly inhibited the activity of several genes involved in the synthesis of other amino acids in *Arabidopsis* [14]. Applying proline to rice under sodium chloride-induced stress caused an excessive accumulation of intracellular proline and a significant reduction in root growth [15]. The inhibitory effect of external proline may be due to the proline toxicity or the stimulation of salt absorption [16]. Therefore, intracellular proline should be at a specific level to provide resistance to stress [11]. The effect of proline used in foliar spraying depends on the species, plant development stage, time of utilization and its concentration [17].

Olive (*Olea europaea* L.) is one of the most important species of fruit trees grown in the Mediterranean region, and its popularity is increasing all over the world [18]. Olive leaf, fruit and oil have a rich history of food, medicine and ceremonial utilization. Olive has been widely used in traditional treatments in the Mediterranean islands and countries such as Spain, Italy, France, Greece, Israel, Morocco, Tunisia and Turkey. Olive leaves have the highest amount of antioxidants compared to other parts of the olive tree [19]. The anticancerous effect of olive is due to the presence of phenolic compounds [20]. The concentration of these compounds in the plant can be changed under different stresses [21].

Among non-living stresses, salinity is one of the special concerns common in arid and semi-arid areas around the world and responsible for a significant reduction in crop yields [22]. Soil salinity is increasing due to irrigation, inappropriate drainage, sea advance in coastal areas, and salt accumulation in the desert and semi-arid areas. Since causing nutritional constraints through reducing the absorption of phosphorus, potassium, nitrate and calcium, increasing intracellular ion concentration and inducing osmotic stress, salinity is a limiting factor for plant growth [23].

Irrigation is necessary in certain stages of growth to produce fruit, but access to high quality water is limited and the use of salt water is inevitable in different areas [24]. Olive is a semi-resistant glycophyte species to salt stress and its resistance depends on cultivar [25]. Typical signs of salt stress in olive plants include growth reduction, leaf tip blight, leaf chlorosis, leaf tubing, flower wilting, root necrosis, branch wilt and leaf abscission [26]. The amount of growth reduction showed a significant variation based on the cultivar as well as the duration of exposure to salt [27].

As mentioned before, several studies have been so far performed on the benefits of applying proline to plants under stress conditions. However, its negative effects are less discussed. The aim of this study was to investigate the vegetative characteristics of different olive cultivars under conditions of salt stress during the growing period and the effect of proline under these conditions.

MATERIALS AND METHODS

Samples and treatments: This research was performed at Gorgan University of Agricultural Sciences and Natural Resources in 2015. The six-month-old plantlets, produced through cuttings, were supplied from "Olive Production and Development" nursery, located on Golestan province, north of Iran. The experiment was carried out in a factorial design based on a completely randomized design with three replications in three olive cultivars. Each replicate contained three pots. In each pot, a plantlet was cultivated in sandy loam (a mixture of field soil, leaf mould and sand in a ratio of 2:1:1). The treatments consisted of proline in three concentrations of 0, 100 and 200 mg/L and salinity in 4 concentrations of 0, 50, and 100 and 200 mM sodium chloride, all of which were applied to three olive cultivars of Arbequina, Arbosana and Koroneiki A38 (afterward simply called Koroneiki). Measurement of traits was performed in three stages including 4, 12 and 20 weeks after the onset of stress. The rooted plantlets were first transferred to larger pots and irrigated with urban water for one week to adopt the new conditions. Then, proline foliar was applied and repeated two times at intervals of 10 days. At the same time, in order to generate the stress, plants were irrigated with saline water containing different concentrations of NaCl for five months from May to October. The pots were irrigated once a week using 120 ml of saline water (the volume of water required for full irrigation of the soil content of each pot was determined at the beginning of the experiment).

Morphological and physical characteristics: Morphological characteristics were measured at three stages. The first stage was conducted simultaneously with the emergence of the initial symptoms of salt stress, i.e., four weeks after the initiation of stress; and subsequent measurements were performed 12 and 20 weeks after that. A millimeter precision ruler was used to measure the total plant height. The cumulative stem length was obtained through the difference between plant height and its initial height. The number of shoots, green leaves and necrotic leaves were separately counted in each plant. The abscised leaves in each pot were collected and counted. In order to calculate the percentage of leaf necrosis, the ratio of the necrotic leaves to the existing leaves was used. The percentage of leaf abscission was obtained from the ratio of the abscised leaves to the total leaves. The length and width of a leaf in the fourth node (from above) were measured with a millimeter precision ruler. The number of main stem nodes was counted and the internode length between the fourth and fifth nodes was measured by a ruler. The leaf thickness was measured in the fourth node and the stem diameter was measured approximately two centimeters above the crown by means of a digital caliper.

Data Analysis: Data obtained from this research were statically analyzed by GenStat Ver. 9.2 and the means were compared using the least significant difference test (LSD). Data were converted to angles or square roots before the statistical analysis, if needed.

RESULTS AND DISCUSSION

The results of this experiment showed that proline had no significant effect on the cumulative stem length at any stage (Table 1). However, the independent effects of cultivar and salinity on this trait were highly significant at all three stages of measurement (P<0.001). At all stages, Koroneiki and Arbosana had the highest and the lowest increases in stem length, respectively. Moreover, at stages 1 and 2, the lowest increase in stem length was observed at 200 mM and the difference between other concentrations was not significant. At the last stage of measurement, the salinity of 0 and 200 mM showed the highest and the lowest cumulative stem length, respectively. Furthermore, in all treatments, the effect of the growing stage on stem length was highly significant (P<0.001). The highest and lowest amounts were observed in stages 3 and 1, respectively. However, in Koroneiki, the difference between stages 1 and 2 was not significant; however, an enhancement was observed at stage 3.

Losing water from plant tissues is one of the primary effects of salinity. In other words, salinity increases the amount of energy needed to maintain the normal condition of the cell; and as a result, less energy remains for

developmental necessities [28]. Plant growth (e.g. enhancement of root and stem length, leaf area and dry weight) is inhibited by medium and high salinity levels [29]. The reduction of growth was found to be significant in terms of cultivar and the duration of exposure to salt [27]. According to the results of this study, the stem length of proline-treated plants did not show any significant difference from the control plants. The cumulative stem length was reduced under salt stress, and Arbosana had the lowest cumulative stem length compared to other two cultivars. This result is similar to another study in which the foliar application of proline (10 and 20 mM) had no significant effect on the shoot length of eggplant and could not improve the adverse effects of salinity [22]. Furthermore, according to an observation on same cultivars used in this study, the main stem length in all the cultivars was lower at 200 mM NaCl than other concentrations [30]. In bean, salt stress reduced plant height, but applying 25 mM proline prevented the negative effect of salinity [11]. It is possible that the reduction of stem length caused by salinity be due to lessened photosynthesis [31]. In another study, it has been reported that proline is not involved in osmotic adjustment of salt-stressed rice. When seedlings were exposed to sodium chloride for two weeks, the mean relative growth rate of rice shoots was decreased. It seems that the mean relative growth of root and shoot has a negative relation with Na and Cl content as well as a positive correlation with K content [6].

Table 1. Independent effects of proline, salinity and cultivar in different stages of measurement on cumulative stem length, internode length, and number of nodes

	Cumulative stem length (cm)				Internode length (cm)				Number of nodes			
	Stage 1	Stage 2	Stage 3		Stage 1	Stage 2	Stage 3		Stage 1	Stage 2	Stage 3	
Proline (mg/L)	*P*=0.403	*P*=0.268	*P*=0.715		*P*=0.352	*P*=0.161	*P*=0.163		*P*=0.669	*P*=0.704	*P*=0.649	
0	5.23 ^{C}	6.89 ^{B}	8.76 ^{A}	*P*<0.001	1.53	1.52	1.33	*P*=0.090	13.48 ^{C}	14.48 ^{B}	15.70 ^{A}	*P*<0.001
100	5.77 ^{C}	7.60 ^{B}	9.29 ^{A}	*P*<0.001	1.47	1.43	1.39	*P*=0.100	13.74 ^{C}	14.80 ^{B}	16.08 ^{A}	*P*<0.001
200	5.21 ^{C}	6.86 ^{B}	9.06 ^{A}	*P*<0.001	1.43	1.40	1.28	*P*=0.053	13.80 ^{C}	14.66 ^{B}	16.00 ^{A}	*P*<0.001
Salt (mM)	*P*<0.001	*P*<0.001	*P*<0.001		*P*=0.028	*P*=0.194	*P*<0.001		*P*=0.526	*P*<0.001	*P*<0.001	
0	5.80 $^{a-C}$	8.53 $^{a-B}$	12.21 $^{a-A}$	*P*<0.001	1.51 $^{a-A}$	1.49 ^{A}	1.37 $^{a-B}$	*P*=0.014	13.81 ^{C}	15.63 $^{a-B}$	17.67 $^{a-A}$	*P*<0.001
50	5.63 $^{a-C}$	7.55 $^{a-B}$	9.53 $^{b-A}$	*P*<0.001	1.48 $^{ab-A}$	1.43 ^{AB}	1.34 $^{ab-B}$	*P*=0.020	13.85 ^{C}	15.00 $^{ab-B}$	16.38 $^{a-A}$	*P*<0.001
100	6.12 $^{a-C}$	7.43 $^{a-B}$	8.71 $^{b-A}$	*P*<0.001	1.57 ^{a}	1.51	1.45 ^{a}	*P*=0.253	13.75 ^{C}	14.26 $^{bc-B}$	14.82 $^{b-A}$	*P*<0.001
200	4.06 $^{b-C}$	4.97 $^{b-B}$	5.71 $^{c-A}$	*P*<0.001	1.34 ^{b}	1.37	1.17 ^{b}	*P*=0.688	13.27 ^{C}	13.70 $^{c-B}$	14.84 $^{b-A}$	*P*<0.001
Cultivar	*P*<0.001	*P*<0.001	*P*<0.001		*P*<0.001	*P*<0.001	*P*<0.001		*P*<0.001	*P*<0.001	*P*<0.001	
Arbequina	5.77 $^{b-C}$	7.24 $^{b-B}$	9.27 $^{a-A}$	*P*<0.001	1.44 ^{b}	1.48 ^{b}	1.33 ^{b}	*P*=0.148	15.12 $^{a-B}$	15.74 $^{a-B}$	17.09 $^{a-A}$	*P*<0.001
Arbosana	2.68 $^{c-C}$	5.03 $^{c-B}$	7.13 $^{b-A}$	*P*<0.001	1.22 $^{c-A}$	1.14 $^{c-A}$	1.06 $^{c-B}$	*P*<0.001	11.31 $^{b-C}$	13.10 $^{b-B}$	15.07 $^{b-A}$	*P*<0.001
Koroneiki	7.76 $^{a-B}$	9.08 $^{a-B}$	10.72 $^{a-A}$	*P*<0.001	1.76 ^{a}	1.73 ^{a}	1.60 ^{a}	*P*=0.931	14.58 $^{a-B}$	15.10 $^{a-AB}$	15.63 $^{b-A}$	P=0.025

The small letters within each column represent significant differences (at 0.01 or 0.05, LSD test) between treatments and large letters within each row show significant differences (at 0.01 or 0.05, LSD test) between stages.

According to the results, the effect of proline on the internode length was not significant at any stage (Table 1). The applied salinity had significant (P=0.028) and highly significant (P<0.001) effects on the internode length at stages 1 and 3, respectively; and the lowest length was observed at the highest salinity. At stage 2, however, the difference among various concentrations of NaCl was not significant. Furthermore, the effect of the growing stage on the internode length was significant at the concentrations of 0 and 50 mM (P=0.014 and P=0.020 respectively). The internode length was influenced later in the control treatment and decreased at stage 3. At the concentration of 50 mM, the highest and the lowest internode lengths were observed at stages 1 and 3, respectively. However, stage 2 did not differ significantly from other stages. At high concentrations, the duration of exposure to salt did not have

any significant effect on this trait. The effect of cultivar on the internode length was highly significant at all three stages (P<0.001). The highest and the lowest amounts were observed in Koroneiki and Arbosana cultivars, respectively. However, the difference among the measuring times was highly significant only in Arbosana (P<0.001), consequently the highest internode length was observed at stages 1 and 2 and the lowest amount obtained at stage 3.

Salinity inhibits the plant growth through affecting water absorption and biochemical processes, such as N and CO_2 assimilation and protein biosynthesis. Under salinity, plants lose their ability to maintain the balance of organic and inorganic components leading to suppressed growth and yield [32].

The results of this study showed that the effect of proline on the number of nodes was not significant at any stage (Table 1). However, increasing over time, the effect of the growing stage on the number of nodes was highly significant (P<0.001) at all three proline levels. At the first month when the plants were under stress, different concentrations of NaCl had no significant effect on the number of nodes; nevertheless, it had a highly significant effect (P<0.001) at stages 2 and 3; therefore, the number of nodes decreased by growing salt concentration. At stage 2, the highest and the lowest numbers of nodes were obtained at concentrations of 0 and 200 mM, respectively. At stage 3, a decline in the number of nodes was observed at 100 mM, which was not significantly different at 200 mM. As the plants were exposed to salt stress for a longer period of time, there was a significant difference between the four levels of NaCl in terms of this trait (P<0.001), and the highest and the lowest numbers of nodes were related to stages 3 and 1, respectively. The effect of cultivar on the number of nodes was highly significant at all stages (P<0.001). Arbequina and Koroneiki had the highest and Arbosana had the lowest numbers of nodes. In addition, the effect of the growing stage on this trait was highly significant (P<0.001) in Arbequina and Arbosana, but not significant (P=0.025) in Koroneiki. The highest number of nodes was obtained at stage 3. Arbosana showed an increase in the number of nodes throughout the experiment; however, the number of nodes increased in Arbequina only at stage 3. In Koroneiki, stage 2 had no significant difference from other stages.

As mentioned above, there was no significant difference between proline-treated and control plants in terms of the internode length and the number of nodes. Salinity reduced the amount of these traits in the studied cultivars. The number of nodes increased as the plants were more exposed to salinity, but the internode length decreased at low concentrations. In a study on rice, adding proline to salt nutrition solution did not have any positive effect on growth, and plant growth was reduced not only under salt stress, but also after adding proline [33]. It was previously suggested that the internode length decreased because of salt stress [34, 35].

According to the results, the effect of proline on stem diameter was not significant at any growing stage. However, at all three proline levels, the effect of duration of exposure to salt was highly significant (P<0.001) and the stem diameter increased at the final stage (Table 2). At stages 1 and 2, salinity did not have any significant effect on stem diameter; however, there was a highly significant difference between the salinity levels at stage 3 (P<0.001). It decreased at the concentration of 200 mM, while it was almost constant at other concentrations. The effect of the growing stage was highly significant (P<0.001) at the concentrations of 0, 50 and 100 mM NaCl, and the stem diameter was significantly increased at stage 3 but there was no significant difference between stages 1 and 2. There was a significant difference among the three stages at 200 mM salinity (P=0.002), and an inverse trend was observed compared to other concentrations; in other words, 200 mM NaCl significantly reduced stem diameter. During the whole experiment, the effect of cultivar on stem diameter was highly significant (P<0.001). At stage 1, this difference was observed among all the studied cultivars and the highest and the lowest values were related to Koroneiki and Arbosana, respectively. However, there was no significant difference between Arbequina and Arbosana at stage 2, and Koroneiki had a greater stem diameter. Arbequina and Koroneiki had the highest stem diameters at stage 3. The differences among the stages of measurement were highly significant in Arbequina and Arbosana. In both cultivars,

there was no significant difference between stages 1 and 2, but stem diameter increased at stage 3. The effect of the growing stage on this trait was not significant in Koroneiki.

Table 2. The independent effects of proline, salinity and cultivar in different stages of measurement on stem diameter, number of shoots and number of leaves

	Stem diameter (mm)				Number of shoots				Number of leaves			
	Stage 1	Stage 2	Stage 3		Stage 1	Stage 2	Stage 3		Stage 1	Stage 2	Stage 3	
Proline (mg/L)	*P*=0.477	*P*=0.852	*P*=0.5		*P*=0.579	*P*=0.696	*P*=0.393		*P*=0.234	*P*=0.102	*P*=0.342	
0	3.05 ^{B}	3.07 ^{B}	3.15 ^{A}	*P*<0.001	0.71 ^{B}	0.80 ^{B}	1.28 ^{A}	*P*<0.001	29.68 ^{B}	27.86 ^{B}	31.57 ^{A}	*P*<0.001
100	2.98 ^{B}	3.00 ^{B}	3.07 ^{A}	*P*<0.001	0.68 ^{B}	0.87 ^{AB}	1.08 ^{A}	*P*<0.001	28.81 ^{B}	28.81 ^{B}	30.21 ^{A}	*P*<0.001
200	3.07 ^{B}	3.08 ^{B}	3.14 ^{A}	*P*<0.001	0.80 ^{B}	0.91 ^{B}	1.19 ^{A}	*P*<0.001	30.16 ^{AB}	29.59 ^{B}	31.02 ^{A}	*P*<0.001
Salt (mM)	*P*=0.392	*P*=0.215	*P*<0.001		*P*=0.014	*P*<0.001	*P*<0.001		*P*<0.001	*P*<0.001	*P*<0.001	
0	2.96 ^{B}	2.97 ^{B}	3.24 $^{a-A}$	*P*<0.001	0.97 $^{a-B}$	1.21 $^{a-B}$	2.01 $^{a-A}$	*P*<0.001	31.58 $^{a-B}$	33.66 $^{a-B}$	45.81 $^{a-A}$	*P*<0.001
50	3.03 ^{B}	3.05 ^{B}	3.21 $^{a-A}$	*P*<0.001	0.77 $^{ab-B}$	0.91 $^{ab-B}$	1.34 $^{b-A}$	*P*<0.001	29.49 $^{ab-B}$	30.25 $^{b-B}$	35.28 $^{b-A}$	*P*<0.001
100	3.11 ^{B}	3.11 ^{B}	3.22 $^{a-A}$	*P*<0.001	0.59 $^{c-B}$	0.72 $^{b-A}$	0.76 $^{b-A}$	*P*<0.001	29.36 $^{ab-A}$	27.83 $^{b-B}$	25.44 $^{c-C}$	*P*<0.001
200	3.04 ^{A}	3.06 ^{A}	2.81 $^{b-B}$	*P*=0.002	0.58 $^{c-B}$	0.61 $^{b-A}$	0.63 $^{b-A}$	*P*<0.001	27.77 $^{b-A}$	23.28 $^{c-B}$	17.20 $^{d-C}$	*P*<0.001
Cultivar	*P*<0.001	*P*<0.001	*P*<0.001		*P*<0.001	*P*<0.001	*P*<0.001		*P*<0.001	*P*<0.001	*P*<0.001	
Arbequina	3.06 $^{b-B}$	2.95 $^{b-B}$	3.15 $^{a-A}$	*P*<0.001	0.94 $^{a-B}$	1.11 $^{a-B}$	1.56 $^{a-A}$	*P*<0.001	31.81 $^{b-B}$	31.57 $^{a-B}$	36.13 $^{a-A}$	*P*<0.001
Arbosana	2.73 $^{c-B}$	2.80 $^{b-B}$	2.88 $^{b-A}$	*P*=0.004	0.20 $^{b-B}$	0.27 $^{b-B}$	0.71 $^{b-A}$	*P*<0.001	22.73 $^{c-B}$	22.92 $^{b-B}$	25.20 $^{c-A}$	*P*<0.001
Koroneiki	3.31 ^{a}	3.31 ^{a}	3.33 ^{a}	*P*=0.365	1.05 $^{a-B}$	1.20 $^{a-AB}$	1.28 $^{a-A}$	*P*=0.031	34.11 $^{a-A}$	31.78 $^{a-B}$	31.47 $^{b-B}$	P=0.038

The small letters within each column represent significant differences (at 0.01 or 0.05, LSD test) between treatments and large letters within each row show significant differences (at 0.01 or 0.05, LSD test) between stages.

The reduction of stem diameter under salt stress compared to control is particularly due to the reduction of vascular tissue and parenchymal tissue of skin and stem [35]. Proline treatment had no significant effect on the studied cultivars. On the other hand, 200 mM sodium chloride had a negative effect on stem diameter, but it increased at other concentrations over time. Stem diameter was higher in Koroneiki than that in other cultivars. It was previously reported that stem diameter decreased under salt stress due to the reduction of stem wall thickness, and proline 25 mM (unlike 50 mM) increased the stem diameter [11]. According to the studies conducted on different almond cultivars, shoot diameter enhanced in all genotypes by increasing sodium chloride concentration in irrigation water [36]. In a study on pistachios [37] and *Agastache* [34], it was also found that the shoot diameter decreases under salt stress.

According to the results, the effect of proline on number of shoots was not significant at any stage (Table 2). However, at all concentrations of proline, the growing stage had a significant effect on number of shoots (*P*<0.001). At the concentration of 0 and 200 mg/L, number of shoots increased in stage 3. At 100 mg/L, the lowest and highest numbers were observed at stages 3 and 1, respectively. However, there was no significant difference between stage 2 and other stages. The effect of salinity on number of shoots was significant at stage 1 (*P*=0.014) and it was highly significant (*P*<0.001) at stages 2 and 3. At stages 1 and 2, the highest number of shoots was observed in control and the lowest number was in the concentrations of 100 and 200 mM. However, there was no significant difference between 50 mM and other concentrations. At stage 3, the number of shoots decreased from 0 mM to 100 mM; however, no significant changes were observed at 200 mM compared to 100 mM. The effect of the growing stage on this trait was highly significant at all salt concentrations (*P*<0.001). At 0 and 50 mM, there was no significant change in stage 2 compared to stage 1, but the number of shoots increased at stage 3. The number of shoots was affected at concentrations of 100 and 200 mM more rapidly than the lower concentrations and it increased at stage 2. However, there was no significant enhancement at stage 3

compared to stage 2. The effect of cultivars on number of shoots was highly significant at all stages (P<0.001). Arbequina and Koroneiki had the highest and Arbosana had the lowest number of shoots. The effect of the growing stage on this trait was highly significant (P<0.001) in Arbequina and Arbosana cultivars and it increased in stage 3, but the difference between stages 1 and 2 was not significant. In Koroneiki, the lowest and highest number of shoots were observed at stages 3 and 1, respectively (P=0.031). However, there was no significant difference in stage 2 with the other stages.

As the present study was conducted during the growing season, the number of shoots increased over time, especially in Arbequina and Koroneiki. The proline treatment had no significant effect on number of shoots compared to the control. However, the plants exposed to salt stress had lower number of shoots compared to control plants. In mung beans (*Vigna radiata*), high concentrations of 50 mM proline inhibited growth both in plants under salt stress and in non-stressed plants [38]. In another study, salt stress reduced the height and number of lateral branches in *Artemisia*, and the reduction was more severe by increasing the tension [39]. High branching under stress conditions is an undesirable trait since it increases the level of transpiration and water loss. Therefore, reducing the number and length of the lateral branches may be a kind of adaptive mechanism by which the plant tries to reduce water loss [34].

The results of this study showed that the effect of proline on number of leaves was not significant in the whole duration of the experiment (Table 2). However, the independent effects of cultivar and salinity on this trait were significant at all stages (P<0.001). At stage 1, the highest number was related to Koroneiki, at stage 2, it was obtained in Arbequina and Koroneiki, and at stage 3, it was observed in Arbequina. At all three stages, the lowest number of leaves was found in Arbosana. Furthermore, at all stages, the highest and lowest number of leaves was observed at 0 and 200 mM concentrations, respectively. The reduction was more pronounced at stage 3 and there was a highly significant difference among all the concentrations of NaCl. However, no significant difference was found between the concentrations of 50 and 100 mM at stages 1 and 2. According to the results, the effect of the growing stage on the number of leaves of Koroneiki was significant (P=0.038), indicating a quicker response than other cultivars, and the number of leaves decreased at stage 2. Although there was no significant difference between stages 2 and 3, the effect of the growing stage was highly significant in other cultivars and at all proline and saline levels (P<0.001). At all concentrations of proline, at salinity levels of 0 and 50 mM, and in Arbequina and Arbosana cultivars, the number of leaves increased at stage 3 and there was no significant difference between stages 1 and 2. At concentrations of 100 and 200 mM NaCl, the number of leaves reduced continuously until the end of the experiment; consequently, the highest and the lowest number of leaves were observed at stage 1 and 3, respectively.

The number of leaves in the plants treated with proline did not significantly differ from the control plants and the trend of changes over time in this trait was similar in all three concentrations. Koroneiki had the highest number of leaves compared to other cultivars, although it declined over time. Low levels of salinity did not have a negative effect on the number of leaves and it increased during the experiment. However, the number of leaves decreased throughout the experiment by irrigation of plants with high concentrations of sodium chloride. Based on a study performed on the bean, the addition of 25 mM proline increased the number of leaves, but no significant difference was observed at 50 mM concentration compared to the control plants [11]. In addition, in researches on many other plants under salt stress, such as olive [40], strawberry [28], almond [41] and pistachio [37] the reported results were similar to our findings. It probably is due to increased leaf abscission or reduced leaf production under salinity [42].

The results of this study also revealed that the independent effect of proline on leaf thickness was significant only at stage 2 (P=0.01) and the proline treated plants had a lower leaf thickness compared to the control plants, although there was no significant difference between the concentrations of 100 and 200 mg/L (Table 3). At all proline concentrations, the effect of the growing stage on

this trait was highly significant (P<0.001). In both control and proline-treated plants, the leaf thickness decreased over time and therefore, the highest and the lowest values were obtained at stages 1 and 2, respectively. The effect of salinity on leaf thickness was highly significant at all stages (P<0.001). At stage 1, low concentrations had no significant effect on this trait, but the concentration of 200 mM increased the leaf thickness. At stage 2, the leaf thickness increase was started at the concentration of 50 mM. However, the differences among concentrations of 50, 100 and 200 mM were not significant. At stage 3, the leaf thickness increased from 0 to 100 mM, and then remained constant at the concentration of 200 mM. The effect of cultivar on this trait was significant at stage 1 (P=0.028). The highest and the lowest leaf thickness were observed in Arbequina and Arbosana cultivars, respectively; however, there was no significant difference between Koroneiki and other cultivars. At stage 2, this effect was highly significant (P<0.001), so that the highest thickness was obtained in Koroneiki and the lowest effect was in Arbequina and Arbosana cultivars. At stage 3, the differences among the cultivars were highly significant (P<0.001). The highest and the lowest values were observed in Koroneiki and Arbosana, respectively.

Table 3. The independent effects of proline, salinity and cultivar in different stages of measurement on leaf thickness, leaf abscission and leaf necrosis

	Leaf thickness (mm)				Leaf abscission (%)				Leaf necrosis (%)			
	Stage 1	**Stage 2**	**Stage 3**		**Stage 1**	**Stage 2**	**Stage 3**		**Stage 1**	**Stage 2**	**Stage 3**	
Proline (mg/L)	P=0.379	P=0.010	P=0.234		P=0.013	P=0.298	P<0.001		P=0.868	P=0.306	P=0.282	
0	0.238 ^{A}	0.133 $^{a-C}$	0.166 ^{B}	P<0.001	1.92 $^{a-B}$	6.35 ^{A}	5.60 $^{b-A}$	P<0.001	0.36 ^{B}	0.72 ^{A}	0.94 ^{A}	P<0.001
100	0.237 ^{A}	0.123 $^{b-C}$	0.172 ^{B}	P<0.001	2.15 $^{a-B}$	6.71 ^{A}	9.30 $^{a-A}$	P<0.001	0.28 ^{B}	0.35 ^{B}	0.62 ^{A}	P<0.001
200	0.244 ^{A}	0.122 $^{b-C}$	0.173 ^{B}	P<0.001	1.01 $^{b-C}$	5.19 ^{B}	9.38 $^{a-A}$	P<0.001	0.32 ^{B}	0.47 ^{B}	0.81 ^{A}	P<0.001
Salt (mM)	P<0.001	P<0.001	P<0.001		P<0.001	P<0.001	P<0.001		P=0.004	P=0.006	P<0.001	
0	0.236 $^{b-A}$	0.107 $^{b-C}$	0.128 $^{c-B}$	P<0.001	0.34 $^{b-B}$	1.45 $^{c-A}$	0.32 $^{d-B}$	P<0.001	0.00 $^{b-B}$	0.11 $^{b-A}$	0.17 $^{b-A}$	P<0.001
50	0.229 $^{b-A}$	0.126 $^{a-C}$	0.150 $^{b-B}$	P<0.001	0.91 $^{b-B}$	3.26 $^{bc-A}$	2.95 $^{c-A}$	P<0.001	0.25 $^{ab-B}$	0.28 $^{b-B}$	0.36 $^{ab-A}$	P<0.001
100	0.234 $^{b-A}$	0.136 $^{a-C}$	0.200 $^{a-B}$	P<0.001	2.19 $^{a-B}$	5.25 $^{b-A}$	7.46 $^{b-A}$	P<0.001	0.56 $^{a-B}$	0.62 $^{ab-AB}$	0.76 $^{ab-A}$	P<0.001
200	0.259 $^{a-A}$	0.135 $^{a-C}$	0.204 $^{a-B}$	P<0.001	3.34 $^{a-C}$	14.38 $^{a-B}$	21.63 $^{a-A}$	P<0.001	0.47 $^{a-B}$	1.05 $^{a-AB}$	1.88 $^{a-A}$	P<0.001
Cultivar	P=0.028	P<0.001	P<0.001		P<0.001	P=0.004	P<0.001		P<0.001	P=0.024	P<0.001	
Arbequina	0.247 $^{a-A}$	0.109 $^{b-C}$	0.180 $^{b-B}$	P<0.001	1.09 $^{ab-B}$	4.19 $^{b-A}$	4.48 $^{c-A}$	P<0.001	0.16 ^{b}	0.19 ^{b}	0.42 ^{b}	P<0.001
Arbosana	0.232 $^{b-A}$	0.114 $^{b-C}$	0.137 $^{c-B}$	P<0.001	1.02 $^{b-C}$	6.56 $^{ab-B}$	13.22 $^{a-A}$	P<0.001	0.08 $^{b-C}$	0.87 $^{a-B}$	1.40 $^{a-A}$	P<0.001
Koroneiki	0.239 $^{ab-A}$	0.155 $^{a-C}$	0.195 $^{a-B}$	P<0.001	2.97 $^{a-B}$	7.50 $^{a-A}$	6.58 $^{b-A}$	P<0.001	0.72 ^{a}	0.48 ^{ab}	0.56 ^{b}	P=0.025

The small letters within each column represent significant differences (at 0.01 or 0.05, LSD test) between treatments and large letters within each row show significant differences (at 0.01 or 0.05, LSD test) between stages.

In another study, the estimation of proline accumulation and distribution during shoot and leaf development of two salt-tolerant genotypes of sorghum showed that proline accumulation is a reaction of the plant to salt stress and not a response associated to tolerance [43]. High concentrations of sodium chloride in plant growth medium create primary and secondary responses negatively affecting the growth and development of plants. Under salt stress, the leaves become smaller and thicker. These morphological changes are probably due to the reduction of turgor pressure in cells that have limited cell development [44]. The leaf of glycophyte plants usually becomes thicker under salinity conditions. In this study, at stage 2, the leaf thickness was lower in proline-treated plants compared to control plants and there was no significant difference between other stages. Therefore, in terms of this mechanism, proline was unable to help the plant confront with stress conditions. On the other hand, the leaves became thicker by increasing the duration of irrigation with sodium chloride. In bean, under 6.25 mM salinity conditions, the middle vein thickness

decreased and proline 25 mM increased the thickness [11]. Some previous studies also suggested that the leaf thickness of *Rubia* amplified by increasing salinity, which was consistent with the results of this study [45, 46].

The results of this study indicated that the effect of proline on the percentage of leaf abscission was significant (P=0.013) at stage 1 (Table 3). There was no significant difference between the proline concentrations of 0 and 100 mg/L; nevertheless, the abscission reduced at 200 mg/L. At stage 2, there was no significant difference among the concentrations of proline. At stage 3, proline had an inverse effect on leaf abscission and its effect was highly significant (P<0.001). Proline-treated plants had higher leaf abscission compared to control plants; however, the difference was not significant between the proline concentrations of 100 and 200 mg/L. At all proline concentrations, the growing stage also had a highly significant effect on leaf abscission (P<0.001). Over time, the percentage of abscission was increased in the proline-treated plants as well as in the control plants. In plants treated with proline at concentrations of 0 and 100 mg/L, the leaf abscission percentage increased from stage 2. However, there was no significant difference between stages 2 and 3. At 200 mg/L, the leaf abscission increase continued to the end of the experiment, so that the highest and the lowest abscissions were related to stage 3 and 1, respectively. Similarly, the effect of salinity on the leaf abscission percentage was highly significant throughout the salt stress (P<0.001). At stage 1, abscission increase was started from 100 mM salinity, although no significant difference was found between 100 and 200 mM concentrations. At stage 2, the highest and the lowest abscissions were observed at 200 and 0 mM, respectively. However, there was no significant difference between concentrations of 0 and 50 mM. Besides, the difference between concentrations of 50 and 100 mM was not significant. At stage 3, there was a significant difference among all concentrations of NaCl, so that leaf abscission increased by enhancing salt concentration. The highest and the lowest leaf abscissions were related to the concentrations of 200 and 0 mM, respectively. At all concentrations of NaCl, the effect of the growing stage on this trait was highly significant (P<0.001). The control plants showed the highest leaf abscission at stage 2 and the difference between stage 1 and 3 was not significant. At concentrations of 50 and 100 mM, an increase was observed at stage 2, but there was no significant difference between stages 2 and 3. At the highest salinity level, the highest and the lowest leaf abscissions were related to stages 3 and 1, respectively. The results suggested that the effect of cultivar on the percentage of leaf abscission was highly significant (P<0.001) at stage 1. The highest and the lowest abscissions were observed in Koroneiki and Arbosana, respectively, and Arbequina had no significant difference from other cultivars. At stage 2, there was a significant difference among the cultivars (P=0.004); the highest and the lowest abscissions were related to Koroneiki and Arbequina, respectively. These two cultivars were not significantly different from Arbosana. At stage 3, the effect of cultivars was highly significant (P<0.001). The highest and the lowest abscissions were obtained in Arbosana and Arbequina, respectively. In all cultivars, the effect of the growing stage on this trait was highly significant (P<0.001). In Arbequina and Koroneiki, the highest leaf abscission was observed at stages 2 and 3, respectively, and the leaves were more stable at stage 1. In Arbosana, the trend of increase in leaf abscission was observed at all stages, so that the highest and the lowest abscissions were related to stages 3 and 1, respectively.

According to the results, the effect of proline on the percentage of leaf necrosis was not significant at any stage (Table 3). Nevertheless, at all concentrations of proline, the growing stage had a significant effect on this trait. The percentage of leaf necrosis increased both in proline-treated plants and in control plants. In control plants, the percentage of leaf necrosis started increasing from stage 2. However, there was no significant difference between stages 2 and 3. In the proline concentrations of 100 and 200 mg/L, leaf necrosis increased at stage 3 and the difference between stages 1 and 2 was not significant. Moreover, the results showed that the effect of salinity on the leaf necrosis percentage was significant at stages 1 and 2 (P=0.004 and P=0.006, respectively). At stage 1, all leaves of the control plants were healthy and leaf necrosis symptoms were

observed at a concentration of 50 mM NaCl. There was no significant difference among the three salt concentrations. At stage 2, increasing necrosis rate started at higher concentrations (100 mM). However, there was no significant difference between the concentrations of 100 and 200 mM. At stage 3, salinity had a highly significant effect on this trait (P<0.001). The highest and the lowest percentages were related to the concentrations of 200 and 0 mM, respectively. The effect of the growing stage on leaf necrosis was significant at all salinity levels. In the control plants (P=0.004), the necrosis symptoms were observed at stage 2. However, there was no significant difference between stages 2 and 3. In plants treated with 50 mM salt (P=0.006), necrosis increased at stage 3 and the difference between stages 1 and 2 was not significant. At the concentrations of 100 and 200 mM, the highest and the lowest percentages were related to stages 3 and 1, respectively. However, stage 2 did not show a significant difference from other stages. The effect of cultivar on the leaf necrosis percentage was highly significant at stage 1 (P<0.001). Necrosis rate in Koroneiki was higher than other cultivars and there was no significant difference between Arbequina and Arbosana. The effect of cultivar on this trait was significant (P=0.024) at stage 2. The highest and the lowest percentages were observed in Arbosana and Arbequina, respectively. Koroneiki had no significant difference from the two other cultivars. At stage 3, the cultivar had a highly significant effect on leaf necrosis (P<0.001). The highest percentage was observed in Arbosana and there was no significant difference between other cultivars. The effect of the growing stage was significant only in Arbosana (P=0.004). Over time, the leaf necrosis percentage increased, so that the highest and the lowest necrotic leaves were observed at stages 3 and 1, respectively.

Necrosis and abscission symptoms appeared in leaves due to salinity, so that the symptoms were intensified at the highest concentration. In the first month of the experiment, proline at the concentration of 200 mg/L prevented from leaf abscission to some extent. However, the leaf abscission and necrosis percentage was higher in proline-treated plants compared to the control plants in the last days, and Arbosana showed a higher sensitivity in terms of these two traits. Necrosis symptoms increased later in proline-treated plants than control plants, and the leaf abscission reached its peak later in proline-treated plants at the concentration of 200 mg/L compared to other plants, indicating a positive effect of proline on this particular case. Application of compatible osmolyte may increase water absorption and improve plant growth due to its active role in osmotic regulation. Proline has a positive effect on plant growth, which may be due to its role as a nutrient and as osmoprotectant [11]. However, external proline (0, 5, 10, and 50 mM) significantly inhibited the growth of Arabidopsis and Petunia and accelerated leaf senescence [47]. Delaying growth and development, the severity of necrosis induced by salt stress can be used as a trait for assessing plant salinity tolerance [48]. In another study, the necrosis of almond leaves increased as salinity increased [49]. In a study on rose, necrosis symptoms appeared at leaf tip two months after the plants were exposed to salt stress. Leaf damage increased after increasing sodium chloride concentration as well as treatment duration. The damaged leaves eventually abscised [50]. Sodium prefers to accumulate in the basal and older leaves, rather than lateral and newer leaves. Sodium specific damage is also associated with sodium accumulation in leaf tissue, resulting in the necrosis of old leaves, which first begins at tip and margins, then extends to the center if it exacerbated [51]. The reason for tip burn of olive leaves is that it has much thinner cuticle at the top and, if exposed to salt stress, vascular tissue necrosis rapidly develops [28]. Leaf abscission is often observed when the salt stress increases [48]. Abscission may be caused by the accumulation of ions, especially in older leaves [52]. When the chloride concentration reaches a certain threshold (about 1.5% of dry weight), 1-amino-cyclopropane 1-carboxylic acid accumulates and oxidation of ethylene results in leaf abscission [53].

The results indicated that the effect of proline on leaf length was only very significant at stage 3 (P<0.001), so that proline 200 mg/L decreased the leaf length (Table 4). There was no significant effect on this trait at any proline concentrations. Similarly, the effect of salinity on leaf

length was significant (P=0.034) and highly significant (P<0.001) at stages 1 and 2, respectively. At both stages, the leaf length reduction began at a concentration of 100 mM and the lowest amount was observed at 200 mM. At stage 3, there was no significant difference among all salinity concentrations. The effect of cultivar on this trait was significant (P=0.008) and highly significant (P<0.001) at stages 1 and 2, respectively. At both stages, the leaves of Arbosana had the highest length and there was no significant difference between other cultivars. At stage 3, no significant differences were observed among the cultivars. Moreover, the growing stage had a highly significant effect on leaf length (P<0.001) in Arbosana. The leaf length at stage 2 did not significantly change compared to stage 1; but it decreased at stage 3. In other cultivars, the growing stage had no significant effect on this trait.

Table 4. Independent effects of proline, salinity and cultivar in different stages of measurement on leaf length, leaf width, and leaf length to width ratio

	Leaf length (cm)				Leaf width (cm)				Leaf length to width ratio			
	Stage 1	**Stage 2**	**Stage 3**		**Stage 1**	**Stage 2**	**Stage 3**		**Stage 1**	**Stage 2**	**Stage 3**	
Proline (mg/L)	P=0.312	P=0.433	P<0.001		P=0.206	P=0.632	P=0.62		P=0.764	P=0.09	P<0.001	
0	4.47	4.49	4.41 ^{a}	P=0.084	1.32 ^{A}	1.29 ^{A}	1.25 ^{B}	P=0.010	3.39	3.48	3.53 ^{b}	P=0.604
100	4.46	4.45	4.44 ^{a}	P=0.262	1.30 ^{A}	1.31 ^{A}	1.23 ^{B}	P=0.007	3.43	3.40	3.61 ^{a}	P=0.272
200	4.32	4.37	4.08 ^{b}	P=0.451	1.26 ^{A}	1.32 ^{A}	1.23 ^{B}	P=0.020	3.43	3.31	3.32 ^{c}	P=0.269
Salt (mM)	P=0.034	P<0.001	P=0.944		P<0.001	P=0.006	P=0.016		P=0.566	P=0.505	P<0.001	
0	4.53 ^{a}	4.60 ^{a}	4.33	P=0.158	1.35 $^{a\text{-}A}$	1.33 $^{a\text{-}A}$	1.27 $^{ab\text{-}B}$	P=0.013	3.56	3.46	3.41 ^{bc}	P=0.704
50	4.53 ^{a}	4.61 ^{a}	4.32	P=0.393	1.32 $^{a\text{-}A}$	1.35 $^{a\text{-}A}$	1.21 $^{bc\text{-}B}$	P=0.016	3.43	3.41	3.57 ^{ab}	P=0.220
100	4.41 ^{ab}	4.37 ^{ab}	4.32	P=0.802	1.30 ^{ab}	1.31 ^{ab}	1.28 ^{a}	P=0.058	3.39	3.34	3.38 ^{c}	P=0.349
200	4.20 ^{b}	4.18 ^{b}	4.27	P=0.991	1.21 ^{b}	1.24 ^{b}	1.19 ^{c}	P=0.178	3.47	3.37	3.59 ^{a}	P=0.185
Cultivar	P=0.008	P<0.001	P=0.518		P<0.001	P<0.001	P<0.001		P<0.001	P<0.001	P<0.001	
Arbequina	4.32 ^{b}	4.35 ^{b}	4.27	P=0.895	1.38 ^{a}	1.38 ^{a}	1.30 ^{a}	P=0.051	3.13 $^{c\text{-}B}$	3.15 $^{c\text{-}B}$	3.28 $^{b\text{-}A}$	P=0.014
Arbosana	4.62 $^{a\text{-}A}$	4.71 $^{a\text{-}A}$	4.37 ^{B}	P<0.001	1.23 $^{b\text{-}AB}$	1.27 $^{b\text{-}A}$	1.19 $^{b\text{-}B}$	P=0.035	3.76 $^{a\text{-}A}$	3.71 $^{a\text{-}AB}$	3.67 $^{a\text{-}B}$	P=0.038
Koroneiki	4.32 ^{b}	4.26 ^{b}	4.30	P=0.398	1.27 ^{b}	1.27 ^{b}	1.22 ^{b}	P=0.937	3.40 ^{b}	3.35 ^{b}	3.52 ^{ab}	P=0.371

The small letters within each column represent significant differences (at 0.01 or 0.05, LSD test) between treatments and large letters within each row show significant differences (at 0.01 or 0.05, LSD test) between stages.

According to the results, the effect of proline on leaf width was not significant at any stage, and proline spraying did not affect the leaf width (Table 4). However, the effect of the growing stage was significant at all three concentrations of proline (P=0.010, P=0.007, and P=0.020, respectively). The leaf width decreased at all concentrations over time and the reduction trend in proline-treated plants was exactly similar to that of the control plants. The width reduction was more evident at stage 3. Besides, the effect of salinity on leaf width was highly significant (P<0.001) and significant (P=0.006) at stages 1 and 2, respectively. At both stages, leaf width reduction occurred at 100 mM concentration and the lowest value was related to the plants treated with 200 mM salinity. However, leaf size at 100 mM was not significantly different from other concentrations. At stage 3, a significant difference was obtained among different concentrations of sodium chloride (P=0.016), so that the highest and the lowest widths were observed at concentrations of 100 and 200 mM, respectively. Nevertheless, there was no significant difference between those with the lower concentrations. The results showed that the effect of the growing stage on this trait was significant only at low concentrations (0 and 50 mM) (P=0.013 and P=0.016, respectively). At these concentrations, leaf width was affected by salinity and it decreased at stage 3; however, the difference between the early stages was not significant. The effect of cultivar on leaf width was highly significant at all stages (P<0.001). For the whole duration of the experiment, the highest width was observed in Arbequina and there was no significant

difference between other cultivars. The effect of the growing stage was significant only in Arbosana (P=0.035). The leaves had the highest and the lowest width at stages 2 and 3, respectively; but stage 1 was not significantly different from the next stages.

According to the findings of this study, the independent effects of proline and salinity on the leaf length to width ratio were highly significant only at stage 3 (P<0.001, Table 4). The highest and the lowest values were observed at concentrations of 100 and 200 mg/L proline, as well as 200 and 100 mM Sodium chloride, respectively. The effect of the growing stage on this trait was not significant at any concentrations of proline and salinity. The results revealed that the effect of cultivar on the leaf length to width ratio was highly significant (P<0.001). For the whole experiment duration, the highest and the lowest rates were observed in Arbosana and Arbequina, respectively. However, at stage 3, the Koroneiki cultivar did not show a significant difference from other cultivars. In Arbequina, the effect of the growing stage on this trait was also significant (P=0.014), so that the leaf length to width ratio increased at the last stage. In Arbosana, the growing stage had a significant effect on this ratio (P=0.038), and the leaves had the highest and lowest ratios at stages 1 and 3, respectively. Stage 2 did not show a significant difference from other two stages.

Arbosana had longer leaves than other two cultivars. The leaf length and width decreased at the end of the experiment; however, the leaf length to width ratio was higher at stage 3 compared to stages 1 and 2. The leaf length of the plants treated with proline 200 mg/L was lower at stage 2 than other concentrations. At this stage, the leaf length to width ratio was higher in plants treated with proline 100 mg/L; nevertheless, proline 200 mg/L reduced leaf size even compared to control plants. Compared to the control plants, the length and width of the leaves decreased under salt stress. Reduced leaf growth is the first reaction of glycophyte plants to salinity. This decrease may be due to direct salt effects on cell division or because of the decreased duration of cellular development. Furthermore, it seems that in glycophytes, the inability of leaves for the accumulation and the use of salt transferred from the root at a rate appropriate to its reception slows down leaf growth and eventually induces leaf death [34]. Reduced leaf growth rate following increased salinity is mainly due to increased osmotic pressure around the root and reduced water absorption efficiency. Increasing the salinity level makes the leaf cells gradually lose their water, and over time, the rate of division and prolongation of the cells decreases, and these changes lead to smaller leaf areas. Changing cell dimensions due to salt stress is associated with a more reduction in the surface than in the depth of the leaves, resulting in smaller and thicker leaves, and these structural changes increase chloroplast density per leaf area [49].

CONCLUSIONS

Proline is commonly distributed in all plants, playing a key role in metabolic processes. However, its external application may cause an excessive enhancement in intracellular proline concentration and prevent plant growth by inducing toxicity. As stated, proline not only had no significant effect on most traits at any stage, but also it had a negative effect on the percentage of leaf abscission, leaf thickness, leaf length, and leaf length to width ratio at the final stages. Moreover, the variations of studied traits in proline-treated plants were similar to those of control plants during three growth stages, except for the percentages of leaf abscission and necrosis; so that the necrosis symptoms increased later in proline-treated plants compared to control plants and the leaf abscission in proline-treated plants at 200 mg/L reached its maximum percentage later than the other plants.

Since olive is one of the semi-resistant plants against salinity, the salt stress, especially at high concentrations (100 and 200 mM), had a negative effect on the growth traits in all cultivars. Higher concentrations of sodium chloride reduced the growth of stems and leaves. The leaf thickness increased in order to confront with stress conditions; however, it was reduced by the continuity of stress. Higher concentrations of salinity led to necrosis symptoms and eventually leaf abscission. The number of leaves was reduced as plants were more exposed to stress.

One of the reasons for decreased number of leaves under stress conditions can be the increased abscission, as well as its reduced production. In general, Koroneiki was more resistant than other cultivars and Arbosana exhibited a higher sensitivity in terms of the studied traits. According to the results, olive plantlets are less resistant to the salinity of more than 100 mM, and it is recommended to avoid cultivating Arbosana in saline lands.

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