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# Ion content and its correlation with some physiological parameters in olive cultivars in response to salinity

**F. Olyaei, B. Baninasab*, C. Ghobadi, M. Gholami**

Department of Horticulture, College of Agriculture, Isfahan University of Technology, Isfahan, I.R. Iran

* Corresponding Author: bbanin@cc.iut.ac.ir

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**ABSTRACT-** Olive (*Olea europaea* L.) is one of the most valuable and widespread fruit trees in Iran. Salt stress-induced changes in membrane stability, photosynthesis and antioxidant enzyme activity were examined on four olive cultivars (Dakal, Shiraz, Zard and Amigdalifolia) by emphasizing the correlation between measured parameters and ion ($K^+$, $Na^+$) accumulation. Plants were subjected to four salt treatments (0, 100, 150 or 200 mM NaCl) under greenhouse conditions. The exposure of the olive plants to increased salinity resulted in a decline in relative leaf chlorophyll content (RLCC), photosynthesis rate ($P_n$), transpiration rate (E) and leaf and root $K^+$ content. NaCl increased superoxide dismutase (SOD) and peroxidase (POX) activity of olive leaves. Increasing the concentrations of NaCl in soil increased the concentration of $Na^+$ in the leaves and roots. Differences in the effectiveness of $Na^+$ exclusion mechanism among cultivars at high salinity reflected differences in salt tolerance. 'Zard', the better-adapted cultivar, displayed tolerance to high internal salt concentrations without apparent cell damage. Relationships between parameters involved in salinity response are discussed in relation to ion accumulation in leaves and roots of olive cultivars.

## INTRODUCTION

Salinity is one of the most important environmental factors, limiting crop production in arid and semi-arid regions (Sepaskhah and Yarami, 2010). The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution (water stress), nutritional imbalance, specific ion effect (salt stress), or a combination of these factors (Ashraf, 1994; Marschner, 1995). Toxicity of $Na^+$ in metabolic processes results from its ability to compete with $K^+$ for binding sites and to inactivate enzymes and essential cellular functions and, consequently, crops growing in saline soils may suffer the dual injury of $Na^+$ toxicity and low $K^+$ concentrations (Munns and Tester, 2008). For most plants to tolerate salinity, $Na^+$ and $Cl^-$ uptake must be restricted while maintaining the uptake of macro nutrients such as $K^+$, $NO_3^-$ and $Ca^{2+}$ (Tavakkoli et al., 2011). One of the harmful changes possibly occurring when plants are subjected to salt stress conditions is the production of reactive oxygen species (ROS) such as superoxide ($O_2^-$), singlet oxygen ($^1O_2$), hydroxyl radicals (OH) and hydrogen peroxide ($H_2O_2$) (Misra and Gupta, 2006). These ROSs have the potential to initiate destructive processes such as damage to membrane, nucleic acids and other cellular structures, and consequently photosynthesis reduction and growth inhibition (Agarwal and Shaheen, 2007; Gao et al. 2008). Plants have developed enzymatic and nonenzymatic defense systems against ROSs (Parida and Das, 2005). According to Menvielle-Bourg (2005) superoxide dismutase (SOD) is a powerful antioxidant enzyme that inactivates the $O_2^-$ by transforming it into $H_2O_2$; therefore, it constitutes the first and one of the main links of the defense process against free radicals.

Electrolyte leakage (EL) from leaf tissues has been reported as an indirect mean of assessing membrane stability (Franklin and Zwiazek, 2004; Goreta et al., 2007). Therefore, such a technique has also been applied to quantify damages to cell membranes in various abiotic stress conditions (Bajji et al., 2002) such as salt stress (Sreenivasulu et al., 2000).

It has been reported that under salinity conditions, excessive accumulation of ions in the cytoplasm or chloroplast of mesophyll cells reduces the photosynthesis rate (Dubey, 2005). On the other hand Jha et al. (2010) found that the ecotype accumulating the lowest shoot $Na^+$ exhibits the lowest salinity tolerance whereas the ecotype accumulates significantly higher concentrations of $Na^+$ maintaining near-normal levels of growth. In addition, salt-induced changes in physiological, morpho-anatomical and biochemical traits are different depending on the crop species (Baum et al., 2000). As with other plants, differences could exist among different own-rooted cultivars of olive in response to salinity stress (Chartzoulakis et al., 2002; Perica et al., 2004). The relation between ion accumulation because of salt stress and diminished growth is not consistent among studies. The use of plant ionic status to identify salt tolerance has been shown to be applicable and its relationship with salt tolerance is considered strong enough to be exploited as a selection tool in the breeding of salt tolerant cultivars (Omielon et al., 1991).

The cultivation of olive in Iran is highly encouraged because of its limited water requirement and its

tolerance towards water salinity. Although there are numerous studies on the salt tolerance of olive cultivars, there is a lack of studies that compare Iranian olive cultivars.

In this paper, the effect of different concentrations of NaCl on some physiological and biochemical parameters and cultivar variability of the response was studied by emphasizing the correlation between measured parameters and ion ($K^+$, $Na^+$) accumulation. Our aim was to test the differences among the studied cultivars under salinity stress conditions and the relation between ion distribution in leaves and roots and membrane stability, photosynthesis and enzymatic antioxidant system.

## MATERIALS AND METHODS

One-year-old rooted cuttings of Iranian olive cultivars ('Dakal', 'Shiraz', 'Zard') and non-Iranian cultivar 'Amigdalifolia' were grown in the research greenhouse of Agricultural College, Isfahan University of Technology of Iran. Plants were grown in plastic pots filled with a mixture of soil, fine sand and leaf mould (1: 1: 1, v/v/v). The minimum and maximum temperatures during the experiment period were 19 and 37˚C, respectively. A month after sticking the cuttings, the pots with uniform plants were subjected to the treatment with 0 (control), 100, 150 or 200 mM NaCl. The electrical conductivities of these solutions were 0.003, 10.52, 15.43 and 19.55 dS $m^{-1}$, respectively. To avoid osmotic shock, the NaCl concentration was increased gradually. The layout was a 4×4 factorial experiment in a complete randomized design, with four replications. The experimental measurements were carried out 8 months after beginning the treatments.

### Superoxide dismutase (SOD) and peroxidase (POX)

Fresh leaves (0.5 g) of plants were ground in 8 mL of 50 mM cold phosphate buffer (pH 7.8) and centrifuged at 15000 g for 20 min at 4 °C. The supernatant was used for the determination of the activities of antioxidant enzymes. SOD activity was measured according to Van Rossun et al.'s (1997) method with a slight modification. Briefly, 3 ml of the reaction mixture contained 13 mM methionine, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.0), 100 µl enzyme extract, 1 mM nitroblue tetrazolium chloride (NBT) was added to 2 µM riboflavin. The tubes were shaken and placed below two 30-W fluorescent lamps. The reaction was stopped by turning off the lamps. A tube without enzyme extract was taken as control and a non-irradiated complete reaction mixture served as blank. The absorbance was recorded at 560 nm by Shimadzu, UV-160A spectrometer (Shimadzu Corporation, Kyoto, Japan).

The activity of POX was determined spectrophotometrically according to Rodriguez and Sanchez (1982). POX activity was analyzed in 50 mM phosphate buffer (pH 6.5) containing 40 mM guaiacol and 26 mM $H_2O_2$. The increase of absorbance at 420 nm was recorded within 180 sec after adding $H_2O_2$. Total soluble protein was measured according to the method of Bradford (1976). The enzyme activity was expressed in µmol $min^{-1}$ mg $protein^{-1}$.

### Electrolyte leakage (EL)

EL was used to estimate membrane permeability. It was measured according to the method of Lutts et al. (1996). Five leaf discs were taken from the youngest fully-expanded leaves on one plant per replicate. After three washes with distilled water to remove surface contamination, the discs were then placed in a test tube containing 15 ml deionized water. Test tubes were vibrated on a shaker at room temperature for 24 h. After incubation, electrical conductivity (EC) of the solutions ($EC_1$) was recorded. The samples were autoclaved for 20 min, cooled to room temperature and the conductivity of solutions was read again ($EC_2$). EL was calculated from the following formula (Eq: 1)

$$EL = (EC_1/EC_2) \times 100 \quad (1)$$

### Relative leaf chlorophyll content (RLCC)

RLCC of the youngest fully-expanded leaf was determined just before the harvest by using a chlorophyll content meter (Hansatech Instrument Ltd., King's Lynn, UK). The chlorophyll meter readings were used as relative values for chlorophyll content.

### Photosynthetic rate ($P_n$) and transpiration (E)

$P_n$ and E were monitored with a portable photosynthesis system LCi (English instrument Ltd, UK). The measurements were performed on young fully expanded sun exposed leaves between 10:00-14:00 h.

### Ion Content

To determine ion content, leaves and roots were washed with distilled water and were oven dried at 75˚C for 72 h. A 0.5 g sample of dried tissue was ashed in a muffle furnace at 500˚C for 5 h. The ash was then dissolved in 10 ml 2 N HCl and the volume was adjusted to 100 ml with deionized water. $Na^+$ and $K^+$ concentrations were measured using flame photometerry (PEP7, Jenway, Dunmow, UK).

### Statistical analyses

Statistical analysis was performed using the SAS program version 9.1 (SAS Institude, Crag, NC). Means were compared using the least significance difference (LSD) test at 5% probably level.

## RESULTS AND DISCUSSION

The salinity and cultivar significantly affected SOD activity (Table 1). Salinity stress increased SOD activity of salt-treated olive plants. The highest SOD activity was obtained at 200 mM NaCl, which was 17.18% higher than that of the untreated control. Significant variability was found between cultivars on SOD activity. The highest (229.09 µmol.$min^{-1}$.mg $protein^{-1}$) SOD activity was

obtained in 'Zard' whereas the lowest (195.50 µmol.min$^{-1}$.mg protein$^{-1}$) one was observed in 'Dakal' (Table 1). Similar to SOD activity, salt stress increased POX activity significantly, with maximum increase observed in plants grown with 200 mM NaCl (Table 1). A significant difference was observed between cultivars concerning POX activity (Table 1). The highest POX activity was obtained in 'Zard' whereas the lowest one was observed in 'Dakal' (Table 1).

The identification of cultivars that show differences in the photosynthetic response, exclusion of ions from leaf blades and photo-assimilates use under salt stress conditions is a necessary starting point to study the key regulatory steps in response to salt stress and the involvement of genes related to salt stress tolerance (Nebauer et al., 2013).

**Table 1.** Effects of salinity, cultivar and their interactions on superoxide dismutase (µmol min$^{-1}$ mg protein$^{-1}$) and peroxidase activity (µmol min$^{-1}$ mg protein$^{-1}$) of one-year olive cuttings.

| NaCl (mM) | Cultivars | | | | Mean |
|---|---|---|---|---|---|
| | Dakal | Shiraz | Zard | Amigdalifolia | |
| Superoxide dismutase (SOD) activity | | | | | |
| 0 | 18.04$^{h\dagger}$ | 181.85$^{h}$ | 193.43$^{g}$ | 183.01$^{h}$ | 185.33$^{D}$ |
| 100 | 197.26$^{fg}$ | 203.61$^{ef}$ | 217.91$^{cd}$ | 197.22$^{fg}$ | 204.00$^{C}$ |
| 150 | 201.55$^{fg}$ | 223.92$^{c}$ | 245.94$^{b}$ | 203.87$^{ef}$ | 218.82$^{B}$ |
| 200 | 216.14$^{cd}$ | 239.41$^{b}$ | 259.09$^{a}$ | 211.57$^{de}$ | 231.55$^{A}$ |
| Mean | 195.50$^{C}$ | 212.20$^{B}$ | 229.09$^{A}$ | 198.92$^{C}$ | |
| Peroxidase (POX) activity | | | | | |
| 0 | 51.41$^{h}$ | 181.85$^{h}$ | 193.43$^{g}$ | 183.01$^{h}$ | 185.33$^{D}$ |
| 100 | 77.54$^{g}$ | 203.61$^{ef}$ | 217.91$^{cd}$ | 197.22$^{fg}$ | 204.00$^{C}$ |
| 150 | 108.06$^{ef}$ | 223.92$^{c}$ | 245.94$^{b}$ | 203.87$^{ef}$ | 218.82$^{B}$ |
| 200 | 122.19$^{bcd}$ | 239.41$^{b}$ | 259.09$^{a}$ | 211.57$^{de}$ | 231.55$^{A}$ |
| Mean | 89.80$^{C}$ | 212.20$^{B}$ | 229.09$^{A}$ | 198.92$^{C}$ | |

Values followed by the same letters within columns and rows are not significantly different at P ≤0.05. Upper-case and lower - case letters are used for the main and interaction effects, respectively.

The increase in antioxidant enzymes activities show that salinity causes oxidative stress in olive plants. SOD is one of the most important scavengers of ROS which play a primary role in preserving cells via the scavenging of the $O_2^-$, a precursor of all other ROSs. The increase in SOD activity leads to enhanced production of $H_2O_2$. POX isozymes are able to scavenge the $H_2O_2$ produced by SOD (Asada, 2006). Thus, in olive cultivars, the possible $H_2O_2$ build-up could be attended by an increase in the activity of POX. It has been reported that plants with high levels of antioxidants have greater resistance to oxidative damage (Young and Jung, 1999). Our results are in agreement with this finding. The cultivars 'Zard' and 'Shiraz' were the most tolerant ones, with the highest SOD and POX activities. Meneguzzo et al. (1999) proposed that the mechanism by which salinity affects the antioxidant responses might be via the change in membrane integrity caused by high $Na^+$ to $Ca^{2+}$ ratio.

EL was affected by both salt stress and cultivar (Table 2). Salinity stress induced a dramatically increased leaf electrolyte leakage in the leaves of salt-treated olive plants. At 200 mM NaCl, leaf electrolyte leakage was the maximum compared to those of the control and other salt levels (Table 2). Significant variability was found among cultivars on leaf electrolyte leakage. The lowest increase in leaf electrolyte leakage was obtained in 'Dakal', which was reached by 2.9 times at 200 mM NaCl whereas the highest one was observed in 'Amigdalifolia' with a 4.8 times increase when compared to untreated control (Table 2).

Salinity stress impaired membrane permeability by inducing electrolyte leakage in leaves (Kaya et al., 2013). EL of all cultivars increased with increasing salinity, but the differences could be due to genetic differences between cultivars. High $Na^+$ accumulation have been reported to result in an enhanced membrane damage, electrolyte leakage and oxidative damage (Mandhania et al., 2006). The 'Amigdalifolia' cultivar was found to be salt sensitive in terms of higher leaf EL and $Na^+$ accumulation under salinity conditions. A linear relationship between $Na^+$ concentration in olive leaves with EL ($r^2$= 0.87) suggests that this process is related to $Na^+$ accumulation, and could be used for the early detection of membrane injury of olive cultivars under salinity stress (Perica et al., 2008).

Salinity stress decreased RLCC of salt-treated olive plants. The lowest RLCC was observed at 200 mM NaCl, which was 66.79% lower than that of the untreated control (Table 2). The effect of salinity on RLCC showed a significant cultivar variation. RLCC was significantly reduced in 'Amigdalifolia' at 100 mM NaCl while the negative effect of salt treatments on the RLCC of other cultivars was significant at the 150 mM NaCl. However, results showed that RLCC reduction ranged between 50.38% in 'Zard' to 85.21% in 'Amigdalifolia' at 200 mM NaCl compared to the untreated control plants (Table 2).

Salt stress significantly reduced the net photosynthetic rate ($P_n$) and transpiration rate (E), with maximum reduction observed in plants grown with 200 mM NaCl (Table 1). Plants of all cultivars exposed to salt stress showed a significant decrease in $P_n$ at the end

of the experiment. The highest reduction in $P_n$ was observed in 'Dakal', which reachedy 50.88% at 100 mM NaCl whereas the lowest one was observed in 'Zard' with a 34.99 % decrease when compared to untreated control (Table 3). Significant variability was found between cultivars on E. The highest (1.16 mmol $m^{-2}$ $s^{-1}$) transpiration rate was observed in 'Zard' whereas the lowest (0.79 mmol $m^{-2}$ $s^{-1}$) one was observed in 'Amigdalifolia' (Table 3).

**Table 2.** Effects of salinity, cultivar and their interactions on electrolyte leakage (%) and relative leaf chlorophyll content of one-year olive cuttings.

| Mean | Cultivars | | | | Mean |
|---|---|---|---|---|---|
| | Dakal | Shiraz | Zard | Amigdalifolia | |
| Electrolyte leakage (EL) | | | | | |
| 0 | $14.62^{h\dagger}$ | $14.29^{h}$ | $13.37^{h}$ | $11.70^{h}$ | $13.49^{D}$ |
| 100 | $25.39^{fg}$ | $27.59^{ef}$ | $26.04^{e-g}$ | $24.00^{g}$ | $25.76^{C}$ |
| 150 | $29.21^{de}$ | $31.94^{d}$ | $31.36^{d}$ | $44.08^{c}$ | $34.15^{B}$ |
| 200 | $42.40^{c}$ | $53.93^{ab}$ | $50.95^{b}$ | $56.52^{a}$ | $50.95^{A}$ |
| Mean | $28.07^{D}$ | $31.94^{C}$ | $30.27^{B}$ | $34.08^{A}$ | |
| Relative leaf chlorophyll content (RLCC) | | | | | |
| 0 | $201.67^{a}$ | $213.10^{a}$ | $213.92^{a}$ | $207.10^{a}$ | $208.95^{A}$ |
| 100 | $176.60^{a}$ | $199.38^{a}$ | $206.88^{a}$ | $162.50^{bc}$ | $186.34^{B}$ |
| 150 | $101.65^{d}$ | $153.75^{c}$ | $154.38^{c}$ | $77.50^{e}$ | $121.82^{C}$ |
| 200 | $43.83^{f}$ | $96.95^{de}$ | $106.15^{d}$ | $30.63^{f}$ | $69.39^{D}$ |
| Mean | $130.94^{B}$ | $165.79^{A}$ | $170.33^{A}$ | $119.43^{C}$ | |

†Values followed by the same letters within columns and rows are not significantly different at $P \leq 0.05$.
Upper-case and lower -case letters are used for the main and interaction effects, respectively.

**Table 3.** Effects of salinity, cultivar and their interactions on photosynthesis rate (mmol $m^{-2}$ $s^{-1}$) and transpiration rate (mmol $m^{-2}$ $s^{-1}$) of one-year olive cuttings.

| NaCl (mM) | Cultivars | | | | Mean |
|---|---|---|---|---|---|
| | Dakal | Shiraz | Zard | Amigdalifolia | |
| Photosynthesis rate ($P_n$) | | | | | |
| 0 | $15.62^{b\dagger}$ | $18.03^{a}$ | $18.49^{a}$ | $14.67^{b}$ | $16.70^{A}$ |
| 100 | $7.68^{e}$ | $10.82^{d}$ | $12.02^{c}$ | $7.42^{e}$ | $9.48^{B}$ |
| 150 | $3.20^{g}$ | $4.96^{f}$ | $5.30^{f}$ | $2.42^{gh}$ | $3.97^{C}$ |
| 200 | $00.66^{i}$ | $1.18^{i}$ | $1.41^{hi}$ | $0.44^{i}$ | $0.92^{D}$ |
| Mean | $6.79^{C}$ | $8.75^{B}$ | $9.31^{A}$ | $6.24^{C}$ | |
| Transpiration rate (E) | | | | | |
| 0 | $1.46^{c}$ | $1.62^{b}$ | $1.72^{a}$ | $1.45^{c}$ | $1.56^{A}$ |
| 100 | $1.10^{e}$ | $1.21^{d}$ | $1.22^{d}$ | $1.01^{ef}$ | $1.14^{B}$ |
| 150 | $0.68^{g}$ | $0.98^{f}$ | $1.01^{ef}$ | $0.42^{h}$ | $0.77^{C}$ |
| 200 | $0.45^{h}$ | $0.65^{g}$ | $0.71^{g}$ | $0.28^{i}$ | $0.52^{D}$ |
| Mean | $0.92^{C}$ | $1.12^{B}$ | $1.16^{A}$ | $0.79^{D}$ | $1.56^{A}$ |

†Values followed by the same letters within columns and rows are not significantly different at $P \leq 0.05$. Upper-case and lower-case letters are used for the main and interaction effects, respectively.

The salt stress period was paralleled by a decrease in RLCC, $P_n$ and E. Reduction of chlorophyll content by salinity has been reported by several researchers such as Erturk et al. (2007) and Mousavi et al. (2008). There was a relationship between salt accumulation and photosynthesis reduction in leaves. Thus, photosynthetic sensitivity to salt depended on salt exclusion or compartmentalization in the leaves of the olive cultivars investigated. According to Tavakkoli et al. (2010) high $Cl^-$ concentration reduces the photosynthesis capacity due to chlorophyll degradation which may result from a structural impact of high $Cl^-$ concentration on PSII. The lower RLCC and $P_n$ in 'Amigdalifolia' and 'Dakal' can be explained by the lower SOD and POX activities, leading to peroxidation of membrane lipids and thus the cooxidation of chlorophyll (Candan and Tarhan, 2003). The Salt tolerant cultivars as well as the salt sensitive cultivars were similar in photosynthetic capacity. These findings suggest that cultivar variation for salt tolerance in olive was not due to differences in photosynthetic rate and thus it cannot be used as an effective selection criterion for salt tolerance in olive. This has earlier been observed in different crops such as radish (Noreen et al., 2012). The transpiration rate was reduced with the addition of NaCl. The reduction was highest in 'Amigdalifolia' (80.7%) compared to other cultivars. Sharma et al. (2005) reported higher reduction in E in salt sensitive cultivar of wheat under salinity stress. Since $K^+$ is the specific ion involved in stomatal opening, one of the reasons that 'Zard' was less affected by $Na^+$ may be due to its higher $K^+$ concentration and

greater control of stomatal regulations under such stress compared with other cultivars.

The $Na^+$ of the root and leaf increased by increasing NaCl in all cultivars, although in the root, it showed a saturation trend at 200 mM in 'Zard' and 'Amigdalifolia' (Table 4). Root $Na^+$ concentration in all cultivars was higher than that of leaves. Root $Na^+$ concentration was the highest in 'Zard' while the lowest leaf $Na^+$ was noticed in this cultivar. 'Amigdalifolia' showed exactly the opposite pattern of $Na^+$ accumulation. In 'Dakal', 'Shiraz', 'Zard' and 'Amigdalifolia', $Na^+$ concentration in roots increased by 493, 550, 425 and 600 percent, respectively (Table 4). Salinity in root zone led to a significant decrease in $K^+$ concentration in the root and leaf in all olive cultivars, but no significant differences were observed in root $K^+$ concentration between 150 and 200 mM NaCl treatments except in 'Zard' (Table 5). At the highest NaCl concentration (200 mM), there were no significant differences in leaf $K^+$ among the three cultivars of 'Shiraz', 'Zard' and 'Amigdalifolia' (Table 5).

**Table 4.** Effects of salinity, cultivar and their interactions on $Na^+$ content (mg $g^{-1}$) of root and leaf of one-year olive cuttings.

| NaCl (mM) | Cultivars | | | | Mean |
|---|---|---|---|---|---|
| | Dakal | Shiraz | Zard | Amigdalifolia | |
| | | | Root $Na^+$ content | | |
| 0 | $5.57^{g\dagger}$ | $5.34^{g}$ | $6.46^{g}$ | $3.37^{h}$ | $5.19^{D}$ |
| 100 | $15.81^{f}$ | $16.77^{f}$ | $19.60^{e}$ | $15.27^{g}$ | $16.86^{C}$ |
| 150 | $22.92^{d}$ | $24.97^{c}$ | $33.04^{a}$ | $22.35^{d}$ | $25.82^{B}$ |
| 200 | $30.03^{b}$ | $34.70^{a}$ | $33.90^{a}$ | $23.58^{cd}$ | $30.55^{A}$ |
| Mean | $15.58^{C}$ | $20.44^{B}$ | $23.25^{A}$ | $16.14^{D}$ | |
| | | | Leaf $Na^+$ content | | |
| 0 | $0.24^{h}$ | $0.18^{h}$ | $0.18^{h}$ | $0.26^{h}$ | $0.22^{D}$ |
| 100 | $4.29^{g}$ | $3.81^{g}$ | $3.50^{g}$ | $7.13^{f}$ | $4.68^{C}$ |
| 150 | $10.23^{d}$ | $8.20^{e}$ | $6.35^{f}$ | $19.41^{b}$ | $11.05^{B}$ |
| 200 | $19.25^{b}$ | $15.51^{c}$ | $8.14^{e}$ | $21.68^{a}$ | $16.14^{A}$ |
| Mean | $8.50^{B}$ | $6.93^{C}$ | $4.54^{D}$ | $12.12^{A}$ | |

†Values followed by the same letters within columns and rows are not significantly different at $P \leq 0.05$. Upper-case and lower-case letters are used for the main and interaction effects, respectively.

**Table 5.** Effects of salinity, cultivar and their interactions on $K^+$ content (mg $g^{-1}$) of root and leaf of one-year olive cuttings.

| NaCl (mM) | Cultivars | | | | Mean |
|---|---|---|---|---|---|
| | Dakal | Shiraz | Zard | Amigdalifolia | |
| | | | Root $K^+$ content | | |
| 0 | $15.28^{b\dagger}$ | $16.64^{a}$ | $15.64^{a}$ | $14.30^{b}$ | $15.93^{A}$ |
| 100 | $6.02^{f}$ | $7.88^{e}$ | $10.06^{c}$ | $9.16^{cd}$ | $8.28^{B}$ |
| 150 | $4.47^{gh}$ | $4.84^{gh}$ | $8.66^{de}$ | $4.24^{h}$ | $5.55^{C}$ |
| 200 | $5.01^{gh}$ | $5.28^{fg}$ | $5.21^{f-h}$ | $4.44^{gh}$ | $4.99^{D}$ |
| Mean | $7.70^{C}$ | $8.66^{C}$ | $10.36^{A}$ | $8.04^{C}$ | |
| | | | Leaf $K^+$ content | | |
| 0 | $13.40^{b}$ | $14.32^{ab}$ | $15.07^{a}$ | $13.63^{b}$ | $14.11^{A}$ |
| 100 | $8.85^{e-g}$ | $9.28^{ef}$ | $11.52^{c}$ | $8.31^{f-h}$ | $9.49^{B}$ |
| 150 | $9.38^{ef}$ | $9.82^{de}$ | $10.89^{cd}$ | $9.18^{ef}$ | $9.82^{B}$ |
| 200 | $9.49^{ef}$ | $7.27^{h}$ | $7.30^{h}$ | $7.84^{gh}$ | $8.35^{C}$ |
| Mean | $10.35^{AB}$ | $10.17^{BC}$ | $11.20^{A}$ | $9.74^{C}$ | |

†Values followed by the same letters within columns and rows are not significantly different at $P \leq 0.05$. Upper-case and lower-case letters are used for the main and interaction effects, respectively.

The selective accumulation of $K^+$ over $Na^+$ was estimated by the ratio $K^+/(K^+ + Na^+)$ of these ion concentrations in the olive above ground organs. Although salt treatment led to a significant drop of $K^+$ concentrations in both shoots and roots, the cultivar 'Zard' exhibited a strong selectivity for potassium uptake, as shown by Table 6. Indeed, despite decreasing with higher salt treatments, $K^+/(K^+ + Na^+)$ selectivity ratios within the plant organs remained high.

In this study, the correlation between various physiological indices (SOD, POX, EL, RLCC, $P_n$, E) and leaf and root $K^+$ and $Na^+$ and $K^+/(K^+ + Na^+)$ ratio in olive cuttings subjected to salt stress were analyzed. Surprisingly, results showed that significant correlations

existed between and among these physiological indices and ion concentrations (Table 7).

It has been proposed that physiological mechanisms underlying salt tolerance, such as ion exclusion, are more relevant criteria for improving salt tolerance in crops (Dionisio-Sese and Tobita, 1998). Both glycophytes and halophytes cannot tolerate large amounts of salt in the cytoplasm and therefore, under saline conditions they either restrict the excess salts in the vacuole or compartmentalize the ions in different tissues to facilitate their metabolic functions (Yadav et al., 2011).

**Table 6.** Effects of salinity, cultivar and their interactions on $K^+/(K^+ + Na^+)$ ratio in roots and leaves of one-year olive cuttings.

| NaCl (mM) | Cultivars | | | | Mean |
|---|---|---|---|---|---|
| | Dakal | Shiraz | Zard | Amigdalifolia | |
| $K^+/(K^+ + Na^+)$ ratio in root | | | | | |
| 0 | $0.73^{b\dagger}$ | $0.76^{b}$ | $0.73^{b}$ | $0.81^{a}$ | $0.76^{A}$ |
| 100 | $0.28^{e}$ | $0.32^{d}$ | $0.34^{d}$ | $0.38^{c}$ | $0.33^{B}$ |
| 150 | $0.16^{g}$ | $0.16^{g}$ | $0.21^{f}$ | $0.16^{g}$ | $0.17^{C}$ |
| 200 | $0.14^{g}$ | $0.13^{g}$ | $0.13^{g}$ | $0.16^{g}$ | $0.14^{D}$ |
| Mean | $0.33^{C}$ | $0.34^{BC}$ | $0.35^{B}$ | $0.38^{A}$ | |
| $K^+/(K^+ + Na^+)$ ratio in leaf | | | | | |
| 0 | $0.98^{a}$ | $0.99^{a}$ | $0.99^{a}$ | $0.98^{a}$ | $0.99^{A}$ |
| 100 | $0.68^{c}$ | $0.71^{c}$ | $0.77^{b}$ | $0.54^{e}$ | $0.67^{B}$ |
| 150 | $0.48^{f}$ | $0.55^{e}$ | $0.63^{d}$ | $0.32^{h}$ | $0.50^{C}$ |
| 200 | $0.36^{g}$ | $0.32^{h}$ | $0.47^{f}$ | $0.27^{i}$ | $0.36^{D}$ |
| Mean | $0.63^{B}$ | $0.64^{B}$ | $0.72^{A}$ | $0.53^{C}$ | |

† Values followed by the same letters within columns and rows are not significantly different at $P \leq .05$. Upper-case and lower - case letters are used for the main and interaction effects, respectively.

**Table 7.** Correlation between olive cultivars traits under salinity stress conditions.

| Traits | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | | | | | | | | | | | |
| 2 | $0.87^{**}$ | 1 | | | | | | | | | | |
| 3 | $0.72^{**}$ | $0.84^{**}$ | 1 | | | | | | | | | |
| 4 | $-0.46^{*}$ | $-0.69^{**}$ | $-0.86^{**}$ | 1 | | | | | | | | |
| 5 | $-0.68^{**}$ | $-0.86^{**}$ | $-0.89^{**}$ | $0.86^{**}$ | 1 | | | | | | | |
| 6 | $-0.54^{*}$ | $-0.79^{**}$ | $-0.89^{**}$ | $0.93^{**}$ | $0.95^{**}$ | 1 | | | | | | |
| 7 | $0.92^{**}$ | $0.96^{**}$ | $0.84^{**}$ | $-0.69^{**}$ | $-0.87^{**}$ | $-0.77^{**}$ | 1 | | | | | |
| 8 | $0.43^{*}$ | $0.68^{**}$ | $0.87^{**}$ | $-0.94^{**}$ | $-0.86^{**}$ | $-0.94^{**}$ | $0.67^{**}$ | 1 | | | | |
| 9 | $-0.61^{**}$ | $-0.83^{**}$ | $-0.83^{**}$ | $0.75^{**}$ | $0.94^{**}$ | $0.89^{**}$ | $-0.81^{**}$ | $-0.78^{**}$ | 1 | | | |
| 10 | $-0.58^{**}$ | $-0.72^{**}$ | $-0.78^{**}$ | $0.61^{**}$ | $0.81^{**}$ | $0.74^{**}$ | $-0.71^{**}$ | $-0.62^{**}$ | $0.85^{**}$ | 1 | | |
| 11 | $-0.74^{**}$ | $-0.92^{**}$ | $-0.84^{**}$ | $0.73^{**}$ | $0.92^{**}$ | $0.85^{**}$ | $-0.91^{**}$ | $-0.76^{**}$ | $0.96^{**}$ | $0.83^{**}$ | 1 | |
| 12 | $-0.61^{**}$ | $-0.82^{**}$ | $-0.91^{**}$ | $0.88^{**}$ | $0.96^{**}$ | $0.96^{**}$ | $-0.82^{**}$ | $-0.93^{**}$ | $0.92^{**}$ | $0.83^{**}$ | $0.91^{**}$ | 1 |

†Superoxide dismutase activity: (1); peroxidase activity: (2); electrolyte leakage: (3); relative chlorophyll content: (4); photosynthesis rate: (5), transpiration rate: (6); root $Na^+$ content: (7); leaf $Na^+$ content: (8); root $K^+$ content: (9); leaf $K^+$ content: (10); $K^+/(K^+ + Na^+)$ ratio in root: (11); $K^+/(K^+ + Na^+)$ ratio in leaf: (12).

$^{*, **}$ Significant at 5% and 1% probability level, respectively.

In our study, $Na^+$ concentration in leaves and roots increased significantly whereas $K^+$ decreased significantly, as has also been reported by Chartzoulakis et al. (2002). The accumulation of $Na^+$ in roots provides a mechanism for olive to cope with salinity in the root zone and/or may indicate the existence of an inhibition mechanism of $Na^+$ transport to leaves (Chartzoulakis et al., 2002). Such kind of mechanism has already been observed in salt tolerant cultivars of different crops such as radish (Noreen et al., 2012). It is known that olive cultivars have an effective salt-exclusion mechanism operating in their roots, which limited salt translocation to the leaves (Demiral et al., 2011). The effectiveness of $Na^+$ exclusion mechanism in the roots differed significantly among studied cultivars, working effectively in 'Zard' (by inhibiting translocation of $Na^+$ to the aerial part) and being much less efficient in 'Amigdalifolia'. 'Zard' could maintain growth whilst accumulating high concentrations of $Na^+$ in its leaves. Activities of antioxidant enzymes involved in oxygen metabolism during salt stress may compensate for $Na^+$ accumulation in the leaves of 'Zard'. Also the high tissue tolerance of 'Zard' is likely to involve sequestration of $Na^+$ into intracellular vacuoles and the synthesis of compatible solutes that accumulate in the cytoplasm to balance the osmotic potential of the vacuolar $Na^+$ (Widodo et al., 2009).

In our study, salt stress led to a significant decrease in $K^+$ concentration in all cultivars. The increase in $Na^+$ ion content and decrease in $K^+$ ion uptake disturbs ionic imbalance as observed in most species exposed to salt stress (Khan and Panda, 2008). 'Zard' as the more tolerant cultivar may be better adapted to cope with decreased $K^+$ levels in its leaves. Contents of certain amino acid have been observed to increase in arabidopsis leaves and roots when grown under $K^+$-deficient conditions. Increased levels of amino acids may act to balance the loss of charge due to the strongly reduced $K^+$ levels in those plants (Armengaud et al., 2009). In our study, the result shows that there is higher uptake of $Na^+$ (600%) and lower uptake of $K^+$ in 'Amigdalifolia' than other cultivars. High uptake and accumulation of $Na^+$ and antagonistically low uptake, translocation and accumulation of $K^+$ could suppress growth by decreasing the capacity of osmotic adjustment and turgor maintenance or by inhibiting metabolic activities (Khan and Panda, 2008). The highest decrease of $K^+$ which took place at the root suggests that olive is able to maintain relatively high $K^+$ levels in young leaves, and this may act as the major monovalent cationic osmoticum in the presence of external salt (Chartzoulakis et al., 2002). Compared with other three cultivars, 'Zard' had significantly higher leaf $K^+$ levels. Increasing evidence has shown that $K^+$ can involve osmotic adjustment of salt-stressed plants (Wang et al., 2013). Concerning $K^+/Na^+$ selectivity, our data (Table 3) are consistent with previous studies, showing the presence of a strong relation between potassium and sodium uptake in plant performance under salinity (Ben Amor et al., 2005; Sharbatkhari et al., 2013). Thus, 'Zard' capacity to conserve $K^+$ supply when $Na^+$ is high in the medium constitutes a key feature of its salinity tolerance, as reported by Ben Amor et al. (2005).

## CONCLUSIONS

In summary, it seems that 'Zard' is salt tolerant 'Shiraz' and 'Dakal' are intermediate while 'Amigdalifolia' is the less tolerant one. Salt-tolerant cultivar 'Zard' exhibited a moderate increase in $Na^+$ accumulation in the leaves even at high salinity level whereas the salt-sensitive cultivar, 'Amigdalifolia', exhibited high leaf $Na^+$ accumulation resulting in indicators of oxidative damage such as a small increase in SOD activity, an increase in EL, and decrease in RLCC. Our data confirmed that there are significant genotypic differences in salt tolerance among olive cultivars that seems to be partially related to the salt exclusion mechanisms at the root level, which prevent $Na^+$ and/or $Cl^-$ translocation to the above ground parts but more comprehensive studies are needed to more precisely investigate salt resistance of these olive genotypes.

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# مقدار یون‌ها و رابطه آن با برخی ویژگی‌های فیزیولوژیکی در رقم‌های زیتون در پاسخ به تنش شوری

**فرزانه علیایی، بهرام بانی نسب*، سیروس قبادی، مهدیه غلامی**

بخش علوم باغبانی، دانشکده کشاورزی، دانشگاه صنعتی اصفهان، اصفهان، ج. ا. ایران

*نویسنده مسئول

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***واژه های کلیدی:***
آنتی اکسیدان آنزیم
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زیتون
پارامترهای فتوسنتزی
شوری

**چکیده**- زیتون (*Olea europaea* L.) یکی از باارزش‌ترین و گسترده‌ترین درختان میوه در ایران است. تغییراتی که در ثبات غشا، فتوسنتز و فعالیت آنزیم‌های آنتی‌اکسیدان در چهار رقم زیتون ('دکل'، 'شیراز'، 'زرد' و 'آمیگدالیفولیا') در اثر تنش شوری ایجاد می‌شود با تأکید بر رابطه بین این ویژگی‌ها و تجمع یون‌های سدیم و پتاسیم مورد بررسی قرار گرفت. گیاهان در شرایط گلخانه‌ای در معرض چهار تیمار شوری (۰، ۱۰۰، ۱۵۰ و ۲۰۰ میلی مولار کلرید سدیم) قرار گرفتند. تیمار گیاهان زیتون با سطح‌های بالای شوری، باعث کاهش مقدار کلروفیل نسبی برگ، فتوسنتز، تعرق و مقدار پتاسیم برگ و ریشه شد. تنش کلرید سدیم فعالیت آنزیم‌های سوپراکسید دیسموتاز و پراکسیداز را در برگ‌های زیتون افزایش داد. با افزایش غلظت کلرید سدیم در خاک، غلظت یون سدیم در برگ‌ها و ریشه‌ها افزایش یافت. تفاوت در کارایی مکانیسم دفع یون سدیم در رقم‌های زیتون مورد مطالعه، سبب تفاوت در میزان تحمل آن‌ها به تنش شوری شد. رقم 'زرد' بدون هیچ آسیب مشهودی به سلول‌ها، بیشترین تحمل را به غلظت‌های بالای نمک از خود نشان داد. رابطه بین ویژگی‌های مورد مطالعه در پاسخ به تنش و تجمع یون‌ها در برگ‌ها و ریشه‌های رقم‌های زیتون به طور کامل مورد بحث قرار گرفت.