# Influence of Gibberellin on Increasing of Sodium Chloride Tolerance via Some Morpho-Physiological Changes in Two Olive Cultivars

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**Summary**

Cultivars of Olive (*Olea europaea* L.) have different ability for adaption to saline environments. Salinity is one of the major factors limiting plant growth and development. The aim of this research was to study the salinity status tolerance of two olive cultivars: 'Zard' and 'Shiraz' and also the possibility of using gibberellin to increase plant salt tolerance. Treatments were different concentrations of sodium chloride: 0, 50, 100 and 200 mg $L^{-1}$, which were added to the pots via irrigation water, and foliage application of gibberellin ($GA_3$) at 0, 10 and 100 mg $L^{-1}$, which were applied one month after salinity treatments on plants. The results showed that increasing salinity declined leaf area; shoots and roots length; leafs, shoots and roots fresh and dry weights, and increased the amount of leaf proline, and leaf and roots $Na^+$ and $Cl^-$. Application of gibberellin reduced sodium and chloride concentrations in plants, but increased the amount of potassium and chlorophyll. The rate of $Na^+$ accumulation in leaves and roots was lower in 'Shiraz' than in 'Zard'. In different levels of sodium chloride, gibberellin increased the synthesis and accumulation of proline in two cultivars, but this increase was more pronounced in 'Shiraz'. 'Shiraz' showed more vegetative growth than 'Zard'.

**Key words**

growth, ions accumulation, *Olea europaea*, salinity stress, tolerance mechanisms

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## Introduction

Salinity is an important factor affecting plant productivity and constitutes a problem concerning a significant portion of the planet, especially in regions with hot, dry climates (Chartzoulakis et al., 2006; Bazakos et al., 2015). The availability of fresh water is one of the major limitations for crop production. Therefore, the use of non-conventional water resources, such as saline water and reclaimed sewage effluent, has increased in recent years. The utilization of such water resources accelerates the salinization of the upper layer of the soil, where most root activity takes place, and generally decreases crop production (Demiral et al., 2011).

Plants face two basic problems in saline environments. First, excess salt in soil lowers the osmotic potential of soil solution and leads to decreased water uptake and consequently water deficit in plants. This in turn leads to perturbations in cell division and/or extension and influences the integrity of metabolic reactions in plants. Second, increased uptake and accumulation of $Na^+$ and $Cl^-$ ions decreases the absorption of essential minerals and imposes toxicity to plants (Munns, 1993; Tester and Davenport, 2003). The significance of salinity for the agronomical and physiological aspects of plants is enormous.

All salts can affect plant growth, but not all inhibit growth. Among the most common effects of salinity is growth inhibition by NaCl. For some plants, particularly fruit trees such as citruses and grapevines, accumulation of both $Na^+$ and $Cl^-$ in the roots and aerial parts is the most damaging to the plants often by inhibiting photosynthesis (Munns, 1993; Flower and Yeo, 1988; White and Broadley, 2001). $Na^+$ is the primary cause of ion-specific damage (such as reduction in $K^+$ activity) (Tabatabaei, 2006). Improving plant resistance to salinity may provide yield stability in subsistence agriculture (Flowers and Yeo, 1995; Flowers, 1999; Asghari, 2008).

Usage of plant growth regulators as an easy and rapid tool can help to ameliorate the adverse effects of abiotic stresses such as salinity. Gibberellins ($GA_3$) play a vital role in the detoxification of heavy metals and in tolerance to salt stress by improving plants growth, chlorophyll synthesis and activities of antioxidant enzymes, and by preventing lipid peroxidation (Saeidi-Sar et al., 2007; Maggio et al., 2010). Some researchers have used plant growth regulators (PGRs) for reducing or eradicating the negative effects of salinity (Angrish et al., 2001; Chakrabarti and Mukherji, 2002). For example, the exogenous application of PGRs [auxins (Khan et al., 2004), gibberellins (Shaheena et al., 2005), cytokinins (Gul et al., 2000)] produces some benefit in alleviating the adverse effects of salt stress and also improves germination, growth, development, seed yield, and yield quality (Egamberdieva, 2009). Abd-El-Samad (1998), Abd-El-Samad and El-Komy (1998) and Azooz et al. (2004) reported that indole acetic acid (IAA) and gibberellic acid stimulate growth in sorghum under stress conditions.

Olive (*Olea europaea* L.) tree cultivation is widespread throughout the Mediterranean, where it has been adapted to the dry climate of the region. However, irrigation at primary stages of plant establishment and during certain growth phases is essential for satisfactory fruit production. Nevertheless, the availability of good-quality irrigation water is limited, and in some areas the use of saline water is unavoidable. Although the olive trees are moderately tolerant to salinity (Mass and Hoffman, 1977), significant differences in salt tolerance have been reported among cultivars (Therios and Misopolinos, 1988; Tattini, 1994; Chartzoulakis et al., 2002).

The present study was therefore designed as an attempt to characterize the influence of $GA_3$ on the adverse effects of salt stress in two olive cultivars.

## Materials and methods

Experiment was conducted in the greenhouse of Shiraz University of Agricultural Sciences during 2011-2012. One year-old own rooted plants of two olive (*Olea europaea* L.) cultivars ('Zard' and 'Shiraz') were transplanted into 8-liter pots filled with sand and perlite (1/1, v/v) and irrigated with 200 ml Hoagland nutrient solution. The pots were kept in the glasshouse with natural sunlight and at temperatures 30 ± 3 during the day and 20 ± 3°C during the night. Treatments were four salinity concentrations as NaCl (0, 50, 100, 200 mg $L^{-1}$) combined with three gibberellin ($GA_3$) concentrations (0, 10, 100 mg $L^{-1}$). Treatments were established in four replications. After four weeks of transplanting, salinity treatments were started. For avoiding osmotic shock, they were added into the pots in three steps with irrigation water. After 30 days of salt application, the plants were subjected to $GA_3$ (Sigma Chemical Co., St. Louis, U.S.A.) sprays. Plants were harvested 16 weeks later for measuring growth parameters, ionic contents, proline, and chlorophyll. The shoots and roots length were measured using ruler. Leaf area surface was determined by leaf area meter (LI-300- USA). The harvested shoots and roots were weighed, dried in an oven for two days at 85°C, re-weighed and grounded for determination of ion compositions. Sodium and potassium contents were measured by flame photometry (model JENWAY, PEP-7) after digesting about 100 mg of plant material in a mixture of concentrated nitric and perchloric acid (3:1) at 175°C. Chloride content was measured at 450 nm according to the method of Diatloff and Rengel (2001) using Hg $(SCN)_2$ and FeNO3. To measure the chlorophyll content, after weighing 0.2 g fresh tissue of leaves, they were grounded with a pestle and mortar in 5 ml of distilled water in cool temperature of about 10°C and dim light condition to change into unified mass and then its volume was made up to 25 ml with distilled water. The amount of 4.5 ml of 80% acetone was added to 0.5 ml of the mixture and centrifuged at 3500 (× g) for 15 minutes. Then, the absorbance of the supernatant solution was measured at 645 and 663 nm in a BioQuest CE 2502 spectrophotometer (Arnon, 1949). To measure the proline content in the leaves, 0.1 g fresh leaves grounded well with pestle and mortar in 10 ml of 3.3% sulphosalicylic acid. After filtering, the filtrate was collected in a test tube and kept in an ice and water mixture. In the next step, 2 ml of ninhydrin solution (1.25 ninhydrin + 20 ml 6M phosphoric acid + 30 ml pure acetic acid) and 2 ml of acetic acid were added to every test tube containing the extract. The tubes were placed in a boiling water bath maintained at 100°C for one hour and then the mixtures were cooled. Under the hood, 6 ml Toluene was added to each tube and shook well for 15-20 seconds. In this condition, two different phases were made. The absorbance was measured at 520 nm by spectrophotometer for 1 ml of the upper phase containing proline (Bates et al., 1973).

The experiment was carried out in a completely randomized factorial design. The factors were four levels of salinity and three levels of $GA_3$ with four replications. Data were subjected to analysis of variance (ANOVA) using the SPSS version 15.0 statistical package (SPSS, Inc., Chicago, USA). For comparison of means the Tukey's test was applied ($P<0.05$).

## Results

The results of analysis of variance showed a high interaction between cultivar, salinity and gibberellin for all measured growth traits except for shoot and root dry weight (DW) (Table 1).

**Table. 1.** ANOVA results of the significance of the effect of cultivar (Cul), salinity (Sal) and gibberellin (GA) interactions (Cul×Sal, Cul×GA, Sal×GA and Cul×Sal×GA) on growth traits of two olive cultivars

| Source of Variation | Leaf area | Shoot FW | Root FW | Shoot DW | Root DW | Shoot L | Root L |
|---|---|---|---|---|---|---|---|
| Cul | ** | ** | ** | ** | ** | ** | ** |
| Sal | ** | ** | ** | ** | ** | ** | ** |
| GA | ** | ** | ** | ** | ** | ** | ** |
| Cul×Sal | ** | ** | ns | ** | ns | ** | ** |
| Cul×GA | ** | ** | ** | ns | ns | ** | ** |
| Sal×GA | ** | ** | * | ** | * | ** | ** |
| Cul×Sal×GA | ** | ** | ** | ns | ns | ** | ** |

Note: * significant at $P = 0.05$,** significant at $P = 0.01$, ns - not significant

### Effects of $GA_3$ on plant growth, biomass and leaf area

Growth of plants (shoot and root length indexes) was reduced when NaCl concentrations was increased to 200 mg $L^{-1}$ in the pots. The application of $GA_3$ at 100 mg $L^{-1}$ improved the growth of both olive cultivars. At 200 mg $L^{-1}$ NaCl, $GA_3$ spraying with 100 mg $L^{-1}$ significantly increased the length of roots in both cultivars and the shoot length in 'Shiraz' cultivar. Generally, gibberellin was more effective in increasing the length of shoots and roots in 'Shiraz' than in 'Zard' (Fig. 1 a and b).

In control plants (no treatments), the largest leaf area surface was obtained in 'Shiraz' cultivar, compared with the corresponding values in 'Zard' cultivar. High concentration of salinity (200 mg $L^{-1}$) decreased the leaf area by 43% and 40% in 'Zard' and 'Shiraz', respectively, compared to their controls. Interactive effect of salinity and $GA_3$ concentration on leaf area was significant in both cultivars. It was interesting that in 200 mg $L^{-1}$ of NaCl, 100 mg $L^{1}$ $GA_3$ promoted the leaf growth to the same surface as in the control plants (Fig. 1c).

The results of analysis of variance showed a high interaction between cultivar, salinity and gibberellin on all measured physiological traits except for chlorophyll content (Table 2).

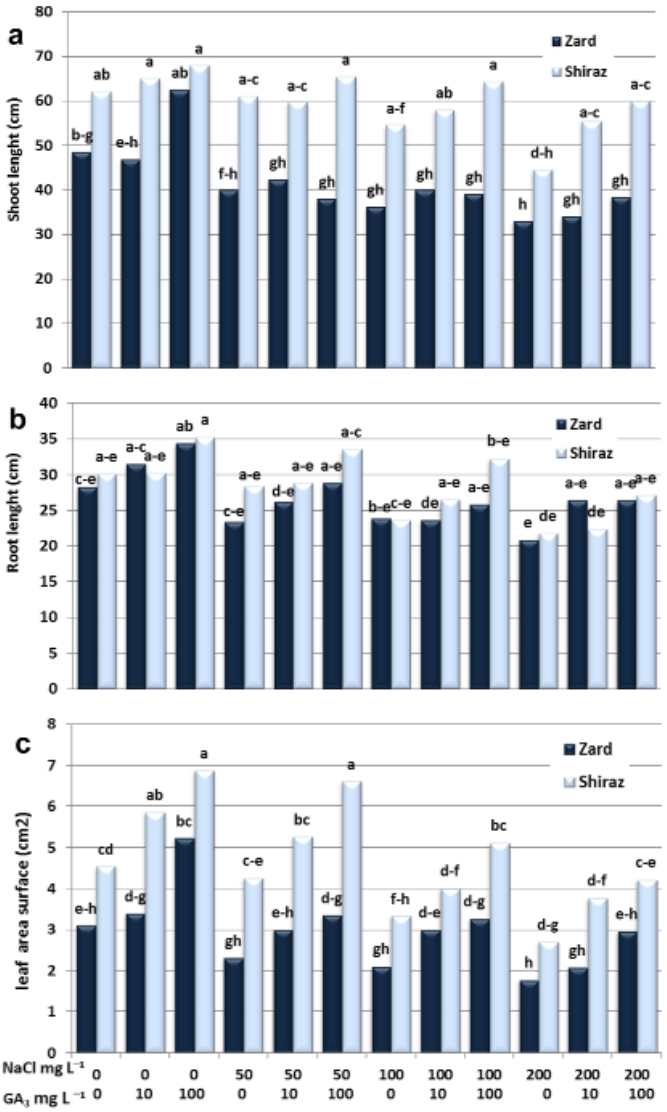


**Figure 1.** Combined effects of NaCl and $GA_3$ on shoot and root length (a and b) and leaf area (c) of two olive cultivars (Note: means with the same letters are not significantly different at $P<0.05$ using Tukey's test).

**Table. 2.** ANOVA results of significance of the effect of cultivar (Cul), salinity (Sal), and gibberellin (GA) interactions (Cul×Sal, Cul×GA, Sal×GA and Cul×Sal×GA) on physiological traits of two olive cultivars

| Source of Variation | Proline | Chlorophyll | Leaf Na+ | Root Na+ | Leaf K+ | Root K+ | Leaf $Cl^-$ | Root $Cl^-$ |
|---|---|---|---|---|---|---|---|---|
| Cul | ** | ** | ** | ** | ** | ** | ** | ** |
| Sal | ** | ** | ** | ** | ** | ** | ** | ** |
| GA | ** | ** | ** | ** | ** | ** | ** | ** |
| Cul×Sal | ** | ns | ** | ** | ** | ** | ** | ** |
| Cul×GA | ** | ns | ** | ** | ** | ** | ** | ** |
| Sal×GA | ** | * | ** | ** | ** | ** | ** | ** |
| Cul×Sal×GA | ** | ns | ** | ** | ** | ** | ** | ** |

Note: * significant at P = 0.05,** significant at P = 0.01, ns - not significant

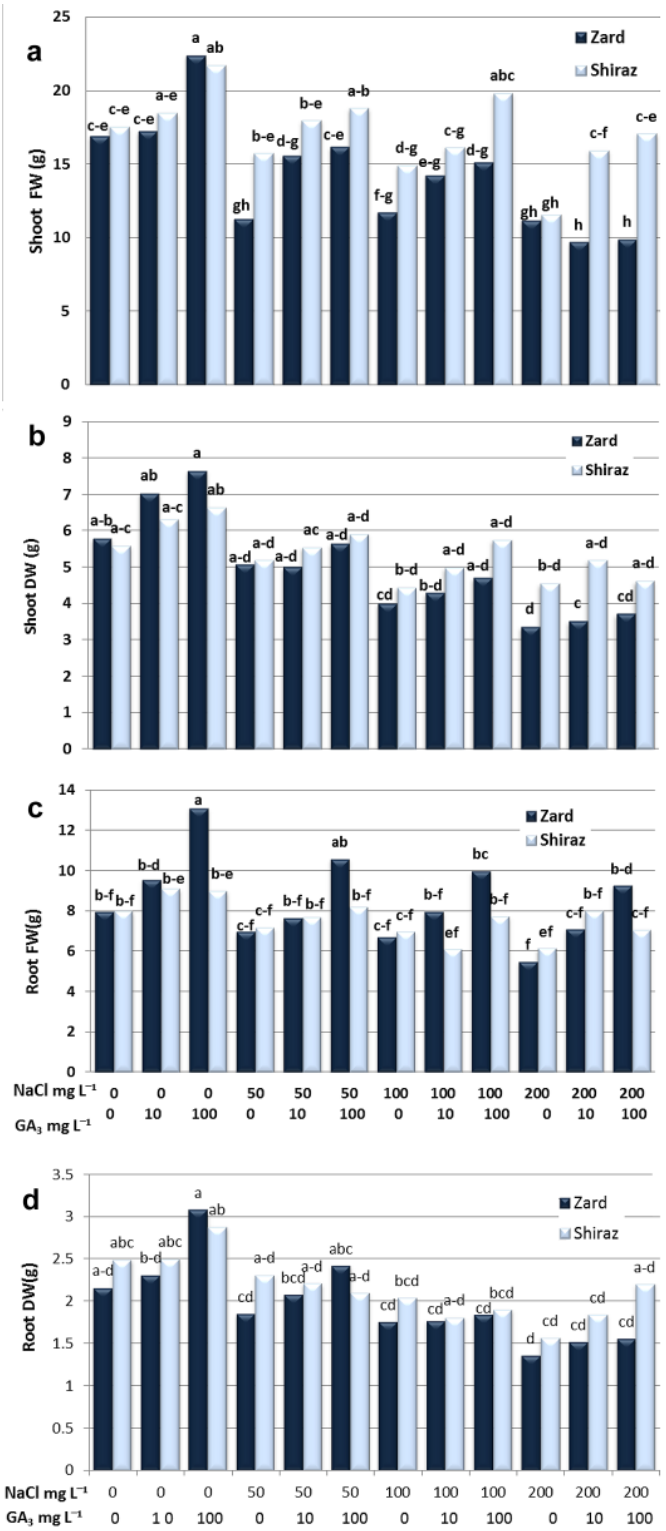


**Figure 2.** Combined effects of NaCl and $GA_3$ on shoot fresh and dry weight (a and b), root fresh and dry weight (c and d) of two olive cultivars (Note: means with the same letters are not significantly different at $P<0.05$ using Tukey's test).

### Effects of $GA_3$ on proline and chlorophyll content

Proline concentration of both cultivars significantly increased with increasing salinity. The proline accumulation was greater in the 'Shiraz' cultivar compared to the 'Zard' cultivar. In both cultivars, the highest proline concentrations were obtained in plants treated with 200 mg $L^{-1}$ NaCl. In each level of salinity, foliar application of $GA_3$, especially at 100 mg $L^{-1}$, also significantly increased proline concentrations (Fig. 3a).

The results showed that leaf chlorophyll content was significantly reduced in the salt-treated plants. The maximum of chlorophyll reduction was observed in plants under 200 mg $L^{-1}$ of NaCl. In all levels of salinity, application of $GA_3$ at 100 mg $L^{-1}$ increased the chlorophyll content of olive plants in both cultivars. However, chlorophyll content in 'Zard' was higher than in 'Shiraz' (Fig.3 b).

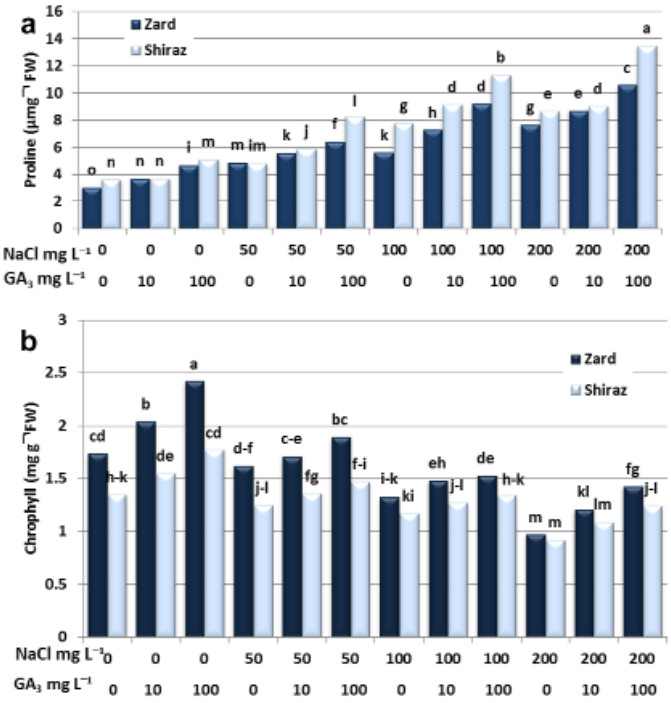


**Figure 3.** Combined effects of NaCl and $GA_3$ on proline (a) and chlorophyll (b) content of two olive cultivars (Note: means with the same letters are not significantly different at $P<0.05$ using Tukey's test).

### Effects of $GA_3$ on leaf and root $Na^+$, $K^+$ and $Cl^-$ contents

In both cultivars, the leaf $Na^+$ content was below 5 mg $g^{-1}$ DW in control plants, while the amount of $Na^+$ in roots of 'Shiraz' was considerably lower than 'Zard'. The $Na^+$ content in the leaves and roots increased as salinity concentration increased in root zone. Foliar application of $GA_3$ significantly decreased the $Na^+$ concentration in both organs (Fig. 4 a, b).

Salinity in the root zone led to a significant decrease in $K^+$ concentration in the plant tissue in both cultivars regardless of the $GA_3$ levels. The lowest concentrations of $K^+$ occurred in the treatments

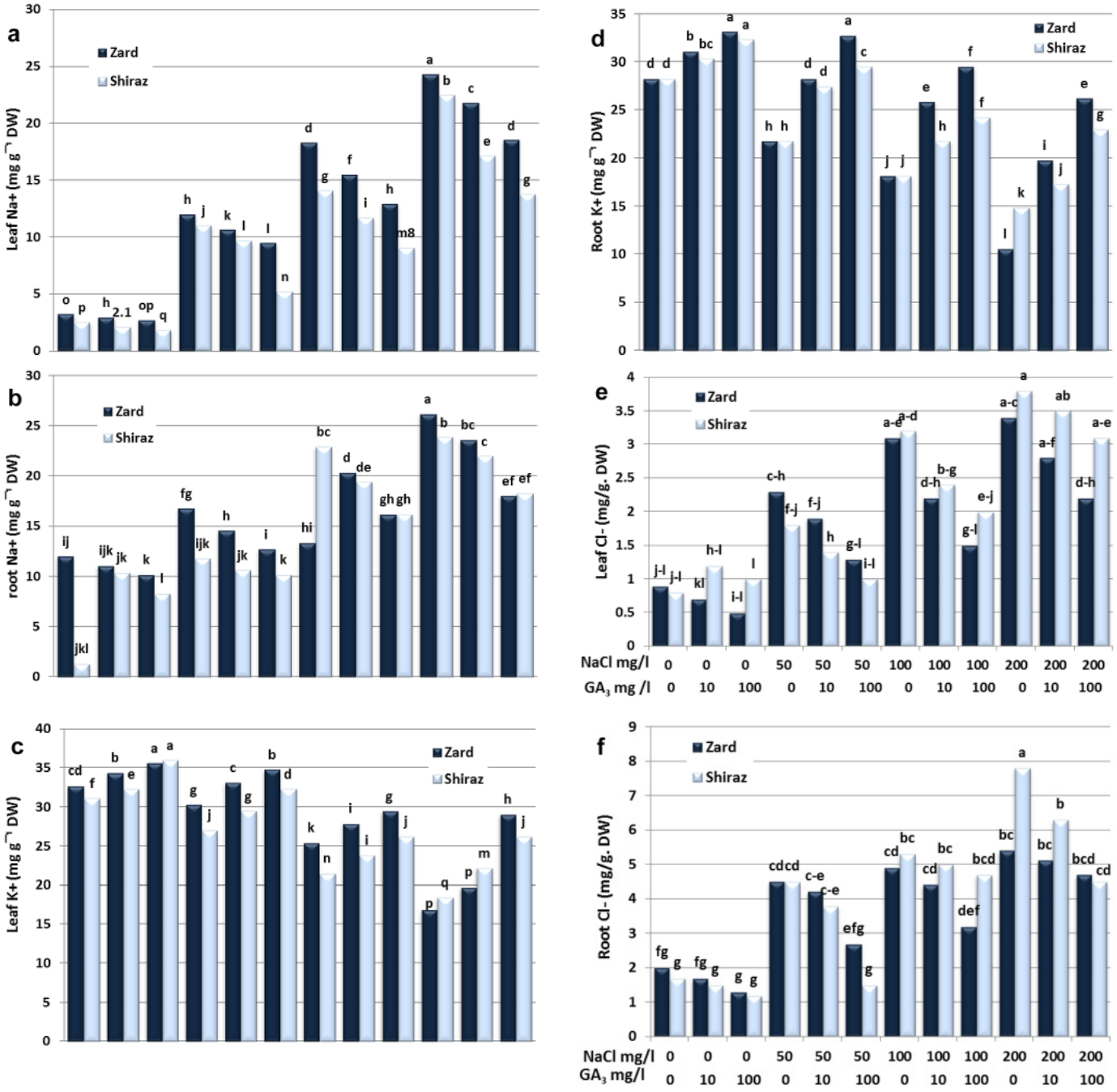


**Figure 4.** Combined effects of NaCl and $GA_3$ on leaf and root $Na^+$ (a and b), $K^+$ (c and d) and $Cl^-$ (e and f) contents of two olive cultivars (Note: means with the same letters are not significantly different at $P<0.05$ using Tukey's test).

with the highest NaCl levels (200 mg $L^{-1}$). In all levels of salinity, spraying the plants with $GA_3$ increased K content of leaves and roots (Fig. 4 c, d).

Leaf and root $Cl^-$ contents were also affected by increasing salinity levels. The application of $GA_3$ under such conditions was found to decrease $Cl^-$ concentration in both cultivars (Fig. 4 e, f).

## Discussion

Exposure to high salinity induced a general modification in all parameters studied. In addition, higher concentration of NaCl (200 mg $L^{-1}$) provoked stronger deleterious effects. Meanwhile, treatment with $GA_3$ clearly mitigated the adverse effects of salt stress based on the improved parameters studied as compared to the untreated plants. The drastic increase in the concentration of $Na^+$ and $Cl^-$ in

tissues that following plant exposure to salinity led to toxicity as it was evidenced by reduced plant growth. It is well documented that salt tolerance in glycophytes is associated with the ability to limit the uptake and/or transport of salt ions (mainly $Na^+$ and $Cl^-$) from root to shoot (Greenway and Munns, 1980; Tester and Davenport, 2003; Ben-Ahmed et al., 2006; Raza et al., 2007). The exposure of plants to salinity commonly results in a water deficit in plant cells, and maintaining osmotic homeostasis requires an adjustment in osmosis, either by the uptake of soil solutes or by the synthesis of metabolically compatible compounds (Tester and Devenport, 2003). It is known that olive cultivars have an effective salt-exclusion mechanism operating in their thin roots. The control mechanism includes limiting salt translocation to the leaves rather than salt absorption by the roots (Demiral, 2005). Additionally, the process is also supported by some other mechanisms such as stomata closure (Fernandez et al., 1997) and leaf tolerance to dehydration (Dichio et al., 2002). Leaf abscission at high salinity (200 mg $L^{-1}$) occurred in 'Shiraz' and 'Zard' and led to further reduction in total leaf area. This may be caused by ions accumulation in the leaves, particularly old leaves (Greenway and Munns, 1980). The reduction in plant growth was due to the reduced leaf growth, which agrees with finding of Cramer (2002) and Tabatabaei (2006). The reduction observed in leaf area and dry matter of the salt-treated plants (Fig1 c, Fig 2 b) can be attributed to the changes in plant water relations under salt stress, which cause a reduction in meristem activity as well as cell elongation (Shah, 2007) thereby inhibiting leaf expansion (Bernstein, 1993). Furthermore, high salinity is known to induce ionic stress, which causes premature abscission and senescence of adult leaves, thus reducing the available photosynthetic area (Munns, 2002). Thus, the observed decrease in dry matter of the salt-stressed plants can be traced to the scanty recovery of leaves following limited photosynthesis production. Aldesuquy and Ibrahim (2001) proposed that hormones used during salt stress may reduce water loss rates and cause a concomitant increase in leaf water potential and carbon gain rates. In the present study, foliar application of $GA_3$ might have de-repressed the leaf area expansion and caused increased dry weight production in the salt-treated plants. The observed chlorophyll depletion may be considered to be a result of the inhibition of chlorophyll biosynthesis following an increase in ethylene production brought about by the elevated NaCl content (Khan, 2003). Further, chlorophylase activity increases during stress conditions (Singh and Jain, 1981), suggesting that the observed low chlorophyll content could be a result of both decreased synthesis and increased degradation under salt stress. However, treatment of the salt-stressed plants with $GA_3$ restored normal chlorophyll levels. This may well be attributed to the $GA_3$–generated enhancement of ultra-structural morphogenesis of plastids coupled with retention of chlorophyll and delay of senescence caused by the hormone treatment (Khan et al., 2010). Similarly, the enhanced accumulation of proline in the plants raised from $GA_3$ may represent: a major biochemical adaptation in plants, osmotic adjustment (Siddiqui et al., 2008; Khan et al., 2010), membrane stabilizer (Bandurska, 2001), and reactive oxygen species (ROS) scavenger (Matysik et al., 2002). The reduction of $K^+$ in olive leaves found in this study (Fig 4 c, d) confirmed previous results by Tattini et al. (1994) and Loupassaki et al. (2002). Usually, $K^+$ concentration is reduced by increased salinity, although there are some tree species, like citrus, where salinity does not significantly affect leaf $K^+$ content, depending on the rootstock used (Storey and Walker, 1999). Therefore, plant metabolism is directly affected by the alteration of $K^+/Na^+$ ratio under saline conditions. It has been suggested that $K^+$ is one of the osmolytes and that its accumulation in plant cells might facilitate osmotic adjustment, lowers the internal osmotic potential, and contributes to salt tolerance (Hasegawa et al., 2000). An important mechanism to avoid the deleterious effects of salinity in olive trees is the ability to limit uptake and/or transport of saline ions (sodium and chloride) from the root zone to aerial parts; salt tolerance in olive cultivars is associated with effective mechanisms of ion exclusion and retention of sodium and chloride in the root (Kchaou et al., 2010). $GA_3$ induces $Ca^{2+}$ and other nutrients uptake that may be involved in plant tolerance to stress by regulating antioxidant metabolism and reduction of the lipid peroxidation of cell membrane (Siddiqui et al., 2008; Khan et al., 2010; Badr-uz-Zaman et al., 2010). Furthermore, these may also be involved in signal transduction (McAinsh et al., 1996) and gene expression (Braam, 1992). Tattini (1994) reported that the resistance mechanism of salt tolerant olive cultivars is probably related to the ability to maintain an appropriate $K^+/Na^+$ ratio in actively growing tissue. Therefore, it may be speculated that higher $K^+/Na^+$ and $(K^+ + Ca^{2+} + Mg^{2+})/Na$ ratios of the plant leaves can be accepted as key indicators reflecting the levels of adaptation of the cultivar to salt stress (Kasırğa and Demiral, 2016).

## Conclusion

Our results indicate that high concentrations of salt (NaCl) provoke a general modification in all morphological and physiological parameters studied. Application of $GA_3$ ameliorates the deleterious effect of $Na^+$ and $Cl^-$ ions. Cultivar 'Shiraz' has the ability to maintain higher $K^+$ and $K^+/Na^+$ ratio than 'Zard' and better growth performance. Therefore, it can be concluded that 'Shiraz' is possibly less sensitive to salinity.

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