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Foliar potassium nitrate spray induces changes in potassium-sodium balance and biochemical mechanisms in olive (*Olea europaea* L. cv Chemlali) plants submitted to salt stress

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ABSTRACT

Purpose: Nowadays with the precipitation scarcity induced by climate change, the use of non-conventional water resources in irrigation is needed such as saline water. The irrigation of salt tolerance species like olive could be adopted with potassium foliar spray. In this work we present how olive plants modulate sodium potassium balance and metabolism to mitigate salt stress. **Research method**: A pot experiment was conducted to assess how potassium nitrate modify Na/K ratio and biochemical compounds in olive plants. One-year-old olive plants (cv *Chemlali*) irrigated with a saline water (10g/L) were subjected to three treatments: K0, K1 and K2 (0, 1 and 2% of potassium nitrate foliar spray, respectively). **Findings:** Results showed differences between treatments. The mineral composition particularly the sodium and potassium content of leaves and roots revealed that the K1 and K2 treatments slightly increased K/Na in leaves and decreased in roots. Moreover, the salt stress was moderate through the osmotic adjustment. The accumulation of osmolytes (proline and soluble sugars) decreased by k1 and K2 treatments. Secondry metabolites (phenols) showed an increase by K1 and K2. Lipid peroxydation was also reduced by treatments especially in young leaves and then increased. In conclusion, potassium can be recommended in order to mitigate the harmful effects of salinity. **Research limitations:** No limitations were founded. **Originality/Value:** In the condition of current water scarcity the saline water could be used with potassium application.

INTRODUCTION

Salinity is one of the major abiotic stresses which strongly affect plant productivity. Tunisia like many countries shows important salt land areas and saline water resources. About 10% of total area of the country is affected by salinity that occurs naturally or is caused by the excessive use of fertilizers (Hachicha, 2007). Moreover, 74% of total water resources show high salinity (more than 1.5g/l). These critical conditions strongly affect agricultural production especially when climatic changes increased aridity (IPCC, 2014) as consequent the irrigation is necessary even the use of salty water. Sodium Chloride (NaCl) is considered as the most dominant soluble salt impairing dysfunction of physiological and biochemical plant mechanisms (Pessarakli & Szabolcs, 2010). These harmful elements cause salt stress which includes multiple stresses such as osmotic stress, ionic imbalance and nutrient deficiency. In fact, osmotic stress reduces the capacity for the water uptake and alters the ionic homeostasis within the plant cell (Zhu, 2002). Moreover, the salt uptake induces ion toxicity (Munns et al., 2006) due to its effects on the K+/Na+ balance in cell plant (Zörb et al., 2014). That's why we thought to exploit the characteristics of potassium as an osmoticum through the maintaining of plant cell turgor (White, 2013) and the regulation of the K^+/Na^+ ratio (Hamrouni et al, 2011; Zörb et al., 2014) to avoid salt stress.

The olive tree (*Olea europaea* L.) is one of the most important crops of the Mediterranean basin that is well-known for its tolerance to salinity (Maas & Hoffman, 1977) because it showed the toxic ions excluding ability. Similarly, it was affirmed that olive salt stress response depends on cultivar (Chartzoulakis et al., 2002; Chartzoulakis, 2005) and water salinity degree (Therios & Misopolinos, 1988; Marin et al., 1995). Irrigation with saline water having EC between 5 and 10 $mScm^{-1}$ is feasible (Bouaziz, 1990) and the majority of olive cultivars may be irrigated with saline water having EC between 3 and 6 mScm-1 without significant yield reduction (Aragüés et al., 2005).

Under current conditions (increase in olive trees irrigated areas, the high water demand, the poor quality of water resources) we are forced to find solutions such as intervention by the use of mineral elements to alleviate the salt stress. Due to great role played by the potassium element in various physiological functions (Dbara et al., 2019) and particularly in condition of salt stress (Larbi et al., 2020) it was used in this experiment.

This paper consists in the study of the response of olive plants grown under salt condition to foliar potassium supply, through the potassium sodium balance and the biochemical changes. So, we followed the sodium potassium translocation in olive plants since they have the same pathway and check how the potassium treatments can trap sodium in roots. Knowing that, due to salinity, sodium (Na+) ions increased and K+/Na+ ratio decreased in leaves leading to growth cessation (Methanni et al., 2018) and causes phytotoxicity (Hu & Schmidhalter, 2005). Also, we studied various stress indexes basing on previous researches which affirmed that under salt or drought conditions various reactions and modifications occur. Osmotic adjustment was known as adaptive mechanism to avoid stressful conditions through the accumulation of solute compounds (proline, soluble sugars) (Munns ET Tester, 2008). The over production of reactive oxygen species induces oxidative damage in membrane lipids. Consequently, lipid peroxidation take place (Smirnoff, 1993). The Malondialdehyde (MDA) content is always used as a marker of oxidative damage. Non-enzymatic antioxidants were also involved such as phenolic compounds (Grace, 2005). Regardless their marker role, these indexes protect plant structure and organs.

MATERIALS AND METHODS

Plant material and growth conditions

The study was carried out in a greenhouse at the Regional Research Centre of Horticulture and Organic Agriculture Chott Mariem Tunisia (35°.92, 10°.56). 'Chemlali', the most oil olive cultivar cultivated in Tunisia, was used for the experiment. One-year-old homogenous plants from a commercial nursery were potted in polyethylene pots (10 L) containing a mixture of sand and perlite (2:1). The microclimatic controlled conditions inside the greenhouse showed a daily temperature between 30 and 35°C and an average relative humidity of 65%. The photosynthetically active radiation ranged between 650 and 1000 $molm^{-2}s^{-1}$. The experiment was performed during three months.

Experimental design and treatments

A randomized complete block design was adopted for the experiment with five repetitions. We created the saline conditions through the irrigation of olive plants by saline water. The sodium concentration was chosen basing on previous studies which affirmed that olive plants tolerate salt up to 4.5 g/L. In fact, it was affirmed that olive response was strongly related to the cultivar. It was recorded a significant reduction of shoot length above 25 mM for some cultivars and above 50 mM NaCl for the others. Total plant leaf area was reduced significantly above 25 mM NaCl (Chartzoulakis et al., 2002). In the experiment the salt treatment was applied as 10 g/L. Each plant was irrigated with two L twice per week. During three weeks, plants received saline irrigation after that they were subjected to three treatments such as: K0, K1 and K2 respectively untreated, potassium foliar spray at 1% and potassium foliar spray at 2%. We used KNO_3 as a treatment product which contain 46% of K_2O.

Plant sampling and analysis

Analysis of mineral elements

The analysis of mineral elements of young and mature leaves and roots were done at the end of the experiment using the total uprooted plants of each treatment. Samples were separated, washed with ionized water and then dried in an incubator (Binder) at 70°C until they reached a constant weight. Once crushed, we proceed to calcinations in an electrical oven followed by the extraction by nitric acid and perchloric acid. The extracts were used for measure of K+ and Na+ contents with Flame photometer (Jenway PFP7, UK.).

Biochemical analysis

The following of biochemical changes started three weeks after salt stress induction which coincident with the foliar potassium spray time. Sampling was done every 15 days. From each plant a number of three fully expanded leaves and three young ones were collected separately, washed and immediately stored in freezer at -40°C.

Leaf proline content

The determination of proline content was determined following the method given by Troll and Lindsley (1955) with some modifications. In detail, 0.2g of frozen leaf powder was mixed with a 4 ml of methanol 40% in glass tubes and boiled in a water bath at 100 °C for 30 min. A 1 ml aliquot of the extract was mixed with 2 ml glacial acetic acid, 2ml of the reagent mixture (120 ml distilled water, 300 ml glacial acetic acid, 80 ml for 1 h. After cooling the mixture, 4 mL of toluene was added and it was mixed using a vortex. The solution containing toluene was separated and the absorption at 528nm was read using a spectrophotometer (Biobase) and

using toluene as a blank. Proline concentration was determined according to standard curve obtained with proline standard.

Total soluble sugars

The total soluble sugars were determined with the anthrone method (Yemn & Wills, 1954). In detail, 0.1g of leaf powder was homogenised in 80% ethanol (5ml) for 24h, then 1ml of 0.2% ice-cold anthrone reagent was added to the extract. The reaction mixture was heated in a boiling water bath for 8 min and rapidly cooled to 0 °C. Absorbance was measured at 628 nm using spectrophotometer (Biobase).

Total phenol content

The total phenol content was determined spectrophotometrically at 760 nm, using the Folin-Ciocalteu reagent (Skerget et al., 2005). A portion of 125 µL leaves extract was mixed with 2.5 mL of Folin-Ciocalteu reagent, 375 µL of distilled water and 2 mL of sodium carbonate. The mixture was incubated in a water-bath for 5 min at 50°C and after cooling at room temperature, the optical density was measured with a spectrophotometer (Biobase). Gallic acid (at concentrations of 12, 28, 40 and 56 µg $mL^{-1}$was used to prepare standard curves. Total phenol content was reported as gallic acid equivalents on FW.

Lipid peroxidation

Lipid peroxidation was evaluated through the determination of the malondialehyde (MDA) content. It was estimated using the procedure described by Heath and Packer (1968). Fresh leaf was homogenized in 1% trichloroacetic acid and then centrifuged at 10,000 rpm for 15 min. Supernatant was heated with 0.05 thiobarbituric acid for 30 min at 95 °C. The supernatant was centrifuged at 5000 rpm for 5 min and the absorbance was measured at 532 and 600 nm on UV Spectrometer (Biobase). The MDA concentration was determined by molar extinction coefficient (155 mM^{-1} cm^{-1}) and the results expressed as µmol MDA g^{-1} DW

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The antioxidants present in the sample induced discoloration that was measured by spectrophotometry at 517 nm (Biobase).

Statistical analysis

The obtained data were subjected to two-way analysis of variance (ANOVA) using SPSS 20.0 statistical software for Windows. Analyses consider the different type of leaves and treatments. Means were compared by least significant differences (LSD) test at 5%. Significant differences at levels of significance are represented by different low case letters. Data are given as means values ± standard deviation.

RESULTS

Sodium Potassium Distribution

Results showed significant effect of potassium treatments in sodium potassium balance of olive plants (Fig. 1). The repartition of sodium in roots, young and mature leaves differs with treatments. Whereas the sodium content of mature leaves did not show significant differences between treatments. Young leaves which are more sensitive to salt stress accumulated 34.19% of Na in untreated plants however potassium supply trends to reduce its concentration especially in K1 treatment with an average level of 25.57%. For K2 treatment it is showed

that Na ions were more accumulated in roots (37.95%) comparatively to untreated plants (34.87%). Also, for K1 the Na concentration was about 41.01%. Results showed that potassium foliar spray treatments tended to reduce sodium accumulation particularly in young leaves.

No significant difference in K concentration was recorded in young leaves (Fig. 2). In contrast, a slight difference was found in mature leaves. Due to potassium pulverization (K1 and K2) high K content in mature leaves was found. A slight decrease of potassium concentration was observed in root content (Fig. 2).

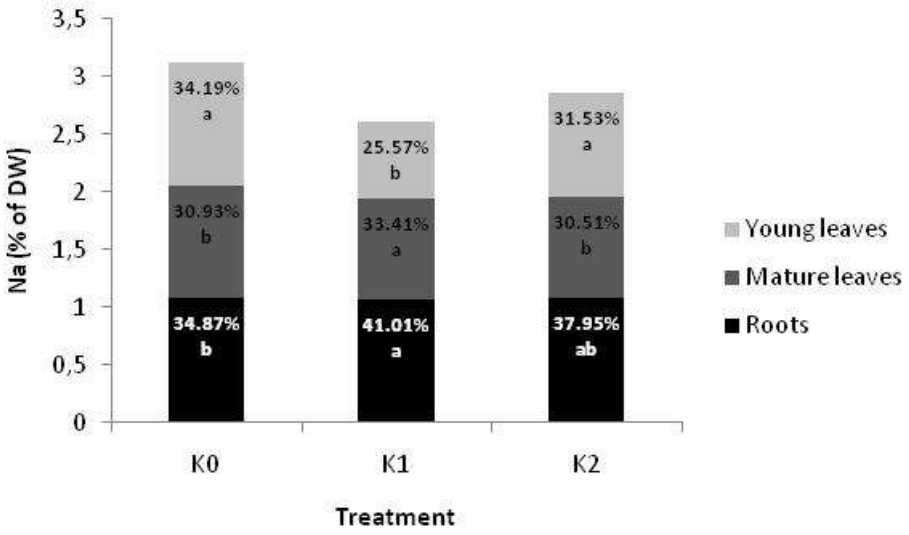

Fig. 1. Sodium distribution between roots and leaves. Significant differences between treatments are indicated with different letters ($P \leq 0.05$). K0 = untreated plants; K1 = treated plants at 1% of KNO_3; K2 = treated plants at 2% of KNO_3.

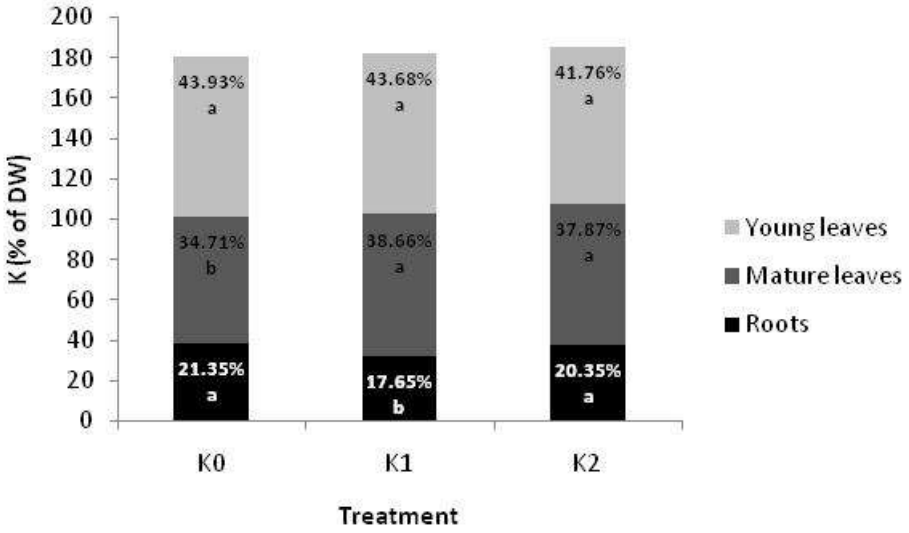

Fig. 2. Potassium distribution in roots and leaves. Significant differences between treatments are indicated with different letters ($P \leq 0.05$,). K0 = untreated plants; K1 = treated plants at 1% of KNO_3; K2 = treated plants at 2% of KNO_3.

Table 1. Potassium Sodium ratio (K/Na) in roots, young and mature leaves

	K0	K1	K2	Degree of significance
Young leaves	1.28	1.70	1.32	*
Mature leaves	1.12	1.15	1.24	ns
Roots	0.61	0.43	0.53	*

Values are means (n = 6) ± standard deviation; significant differences between the means (at least $P \leq 0.05$, according to ANOVA) appear with different letters. K0 = untreated plants; K1 = treated plants at 1% of PN; K2 = treated plants at 2% of KNO_3.

Regarding the potassium sodium ratio data is presented in the Table 1. Little differences were noted between treatments in particular between control (K0) and potassium treatment (K1 and K2) for young leaves and roots.

For all treatments, it was found that K/Na ratio increase from roots to leaves. The comparison between two types of leaves showed higher values in young leaves compared to old ones Values of K/Na reached 1.7 in young leaves of K1 treatment compared to 1.28 in control young leaves. Also a decrease of K/Na ratio in roots was observed in potassium treated plants.

Biochemical changes

Proline content

Over the experimental period, proline content of leaves followed an increasing curve (Fig. 3 and Fig. 4). From treatments application date, differences between control and potassium treatments (K1 and K2) cleared up and they are statistically significant.

In both types of leaves, it was noted that potassium pulverization led to alleviate salt stress effects by the decrease of proline accumulation (Fig. 3). During the experiment, leaves of untreated plants showed the highest level which ranged between 0.6 and 0.8 µmolmg^{-1} DW in young leaves and between 1 and 1.1 µmol/mg^{-1} DW in mature leaves. K1 and K2 reduced the proline content at about 20% in mature leaves and at 10% in young ones. No significant differences between K1 and K2 were recorded. Moreover, the mature leaves accumulated more proline than young ones (Fig. 3 and Fig. 4).

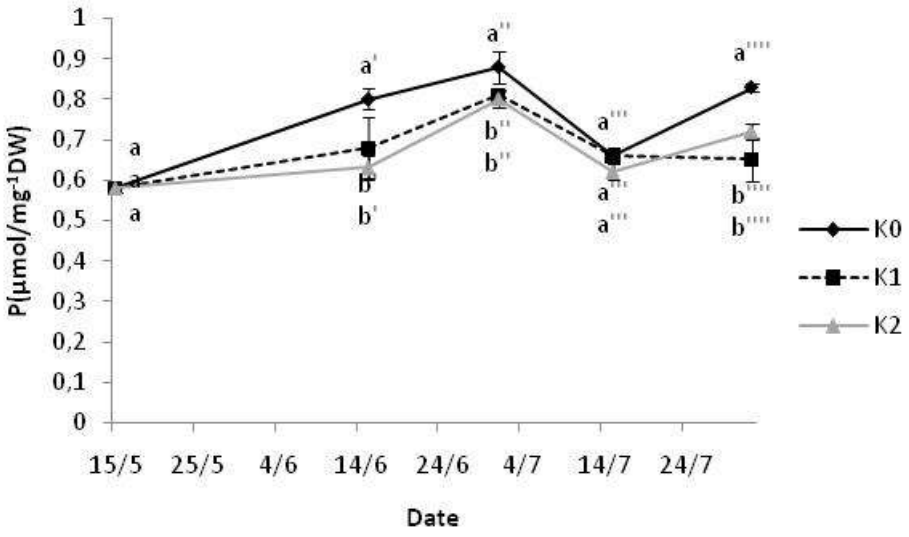

Fig. 3. Proline content in young leaves. Values are means (n = 6) ± standard deviation; significant differences between the means (at least $P \leq 0.05$, according to ANOVA) are indicated with different letters. K0 = untreated plants; K1 = treated plants at 1% of PN; K2 = treated plants at 2% of KNO_3.

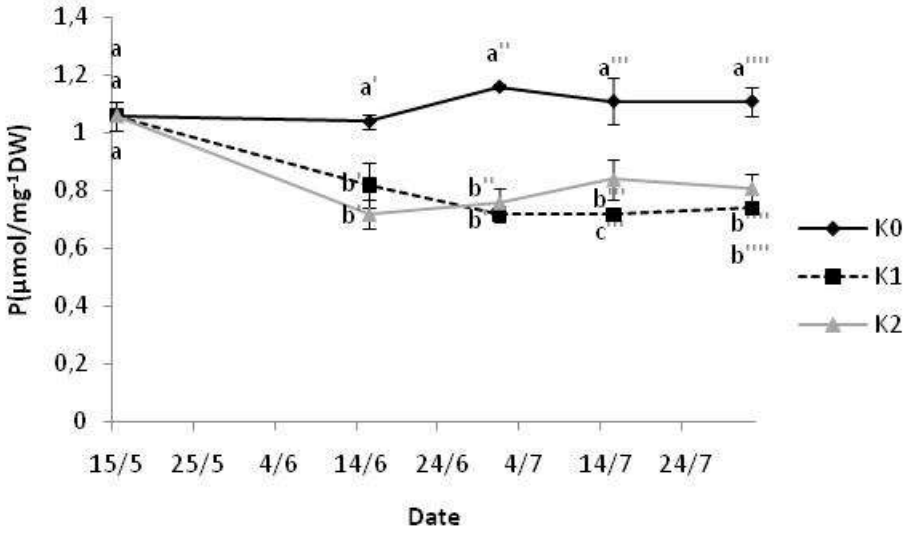

Fig. 4. Proline content in mature leaves. Values are means (n = 6) ± standard deviation; significant differences between the means (at least $P \leq 0.05$, according to ANOVA) are indicated with different letters. K0 = untreated plants; K1 = treated plants at 1% of PN; K2 = treated plants at 2% of KNO_3.

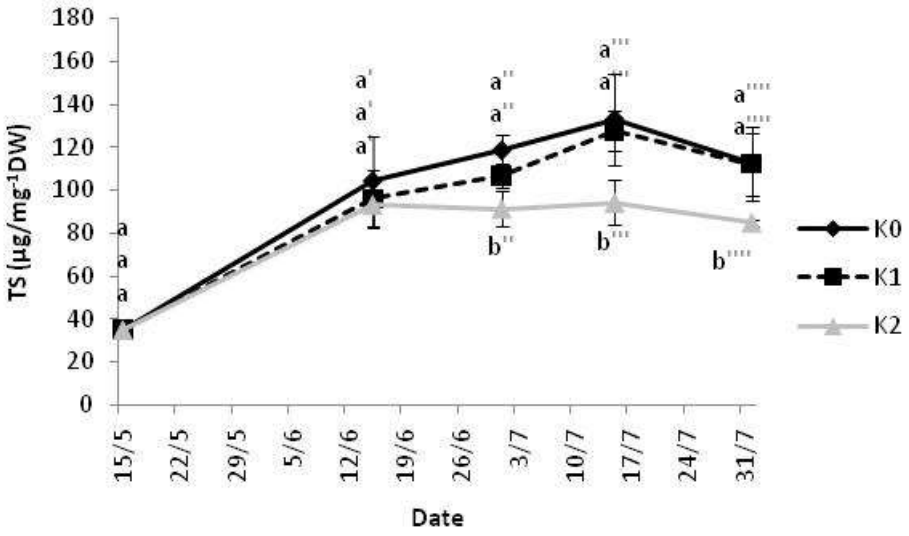

Fig. 5. Total sugars content in young leaves. Values are means (n = 6) ± standard deviation; significant differences between the means (at least $P \leq 0.05$, according to ANOVA) are indicated with different letters. K0 = untreated plants; K1 = treated plants at 1% of PN; K2 = treated plants at 2% of KNO_3.

Total sugars content

The follow up of total sugars content in leaves during the experiment period showed an increase in both leaf types (Fig. 5 and Fig. 6). In fact, the highest concentration was found in untreated stressed plants mostly in mature leaves. So, total sugars decreased with the increase of potassium rate especially differences were noted in mature leaves.

The same trend was found in mature leaves, except for the K1 treatment which approached K2 (Fig. 6). Hence, potassium treatments s reduced the total sugars accumulation in mature leaves. Except in mid-July, K1 and control treatments showed the same total sugars contents.

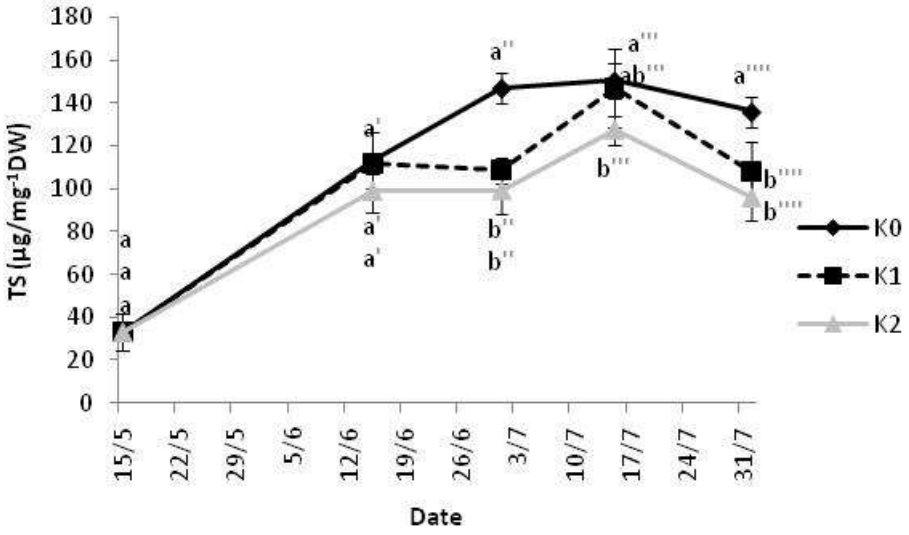

Fig. 6. Total sugars content in mature leaves. Values are means (n = 6) ± standard deviation; significant differences between the means (at least $P \leq 0.05$, according to ANOVA) are indicated with different letters. K0 = untreated plants; K1 = treated plants at 1% of PN; K2 = treated plants at 2% of KNO_3.

The comparison of total sugars accumulation between two leaf types presented small differences. The most stressed treatment reached 125µg/g FM in young leaves and 150µg/g FM in mature ones. The reduction by potassium treatments was about 30% in young leaves and ranged between 33 and 15% in mature ones.

Polyphenols

Results showed significant differences between treatments (Fig. 7 and Fig. 8). Controversy to proline and sugars, potassium nitrate application increased total phenols and flavonoids in comparison to salt stressed untreated plants. For both young and mature leaves, total phenols increased in K1 and K2 treatments. In mature leaves phenol content was higher than in young leaves, except for K1 treatment (Fig. 7).

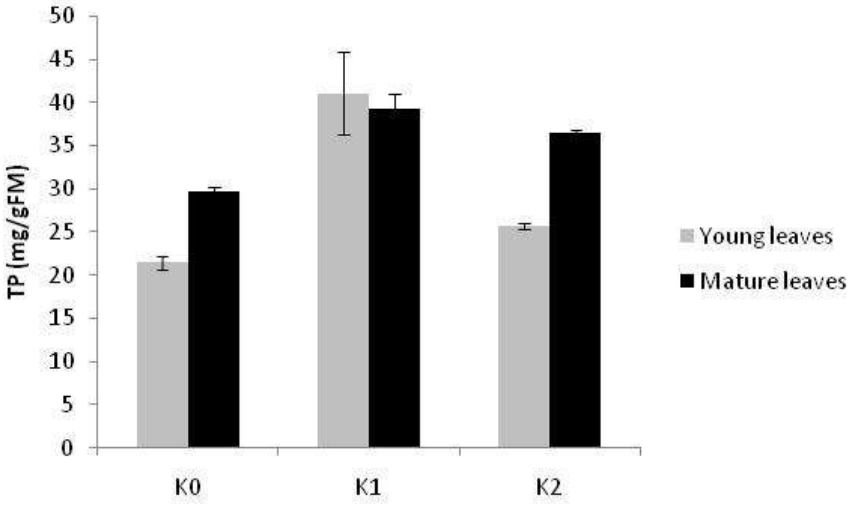

Fig. 7. Total phenols content expressed as gallic acid equivalents in young and mature leaves. Values are means (n = 6) ± standard deviation; significant differences between the means (at least $P \leq 0.05$, according to ANOVA) are indicated with different letters. K0 = untreated plants; K1 = treated plants at 1% of PN; K2 = treated plants at 2% of KNO_3.

Lipid peroxidation

The analysis of leaf oxidative damage revealed that lipid peroxidation significantly increased only in in young leaves of K2 treatment (Fig. 8) and differences between treatments decreased throughout the experimental period. In the last date of measurement, the untreated leaves presented the lowest level of MDA. However, the mature leaves content of MDA was lower in control treatment in comparison with K1 and K2 treatments (Fig. 9). Differences between untreated leaves and K1 treated leaves (K1) were insignificant.

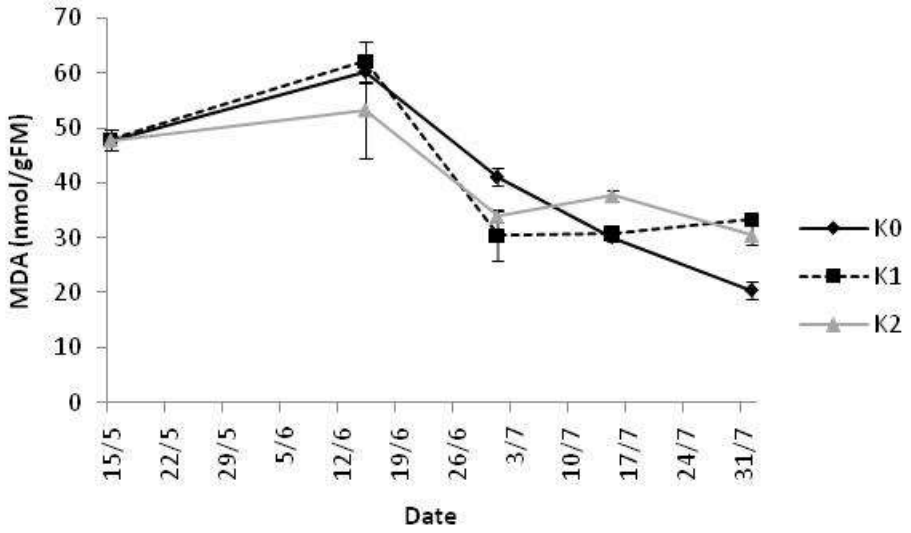

Fig. 8. MDA content in young leaves. Values are means (n = 6) ± standard deviation; significant differences between the means (at least $P \leq 0.05$, according to ANOVA) are indicated with different letters. K0 = untreated plants; K1 = treated plants at 1% of PN; K2 = treated plants at 2% of KNO_3.

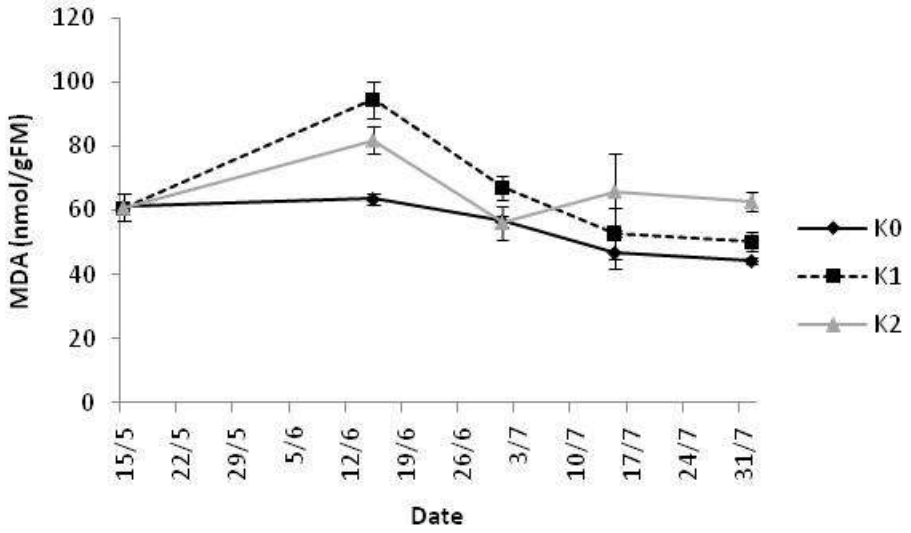

Fig. 9. MDA content in mature leaves. Values are means (n = 6) ± standard deviation; significant differences between the means (at least $P \leq 0.05$, according to ANOVA) are indicated with different letters. K0 = untreated plants; K1 = treated plants at 1% of PN; K2 = treated plants at 2% of KNO_3.

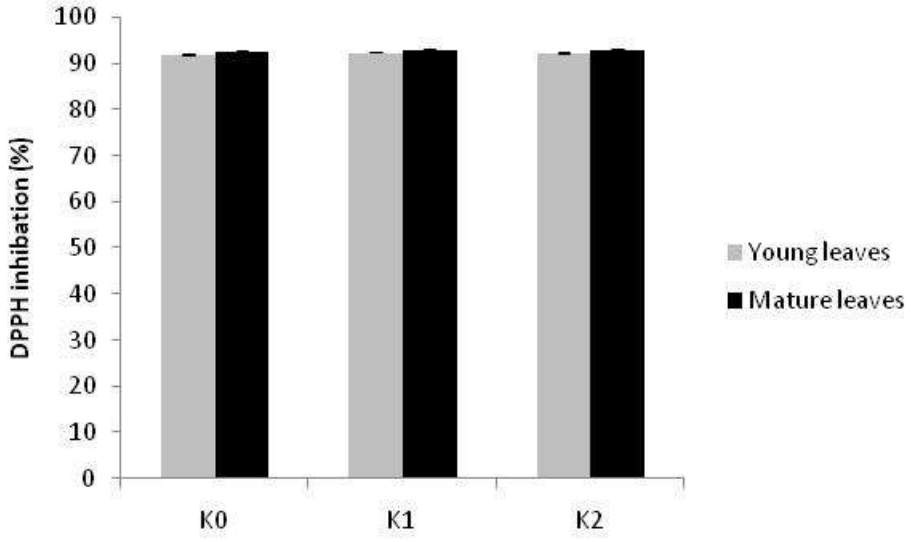

Fig. 10. Antioxidant activity in young and mature leaves. Values are means (n = 6) ± standard deviation; significant differences between the means (at least $P \leq 0.05$, according to ANOVA) are indicated with different letters. K0 = untreated plants; K1 = treated plants at 1% of PN; K2 = treated plants at 2% of KNO_3.

Antioxidant activity

Results showed that the DPPH inhibition did not affected by treatments equally for both leaf types (Fig. 10). All treatments did not change the antioxydant activity in young and mature leaves.

DISCUSSION

Several studies affirmed that the mineral nutrient uptake of plant is strongly affected by salt stress. In the present work, results showed that nitrate potassium pulverization of olive plants grown under salt stress conditions caused a change in Na and K concentrations in different plant part (roots, young and mature leaves). The existence of competition effects between these two ions was proved previously since they share the same transport system (Parida & Das, 2005). Potassium treatments (K1 and K2) reduced Na accumulation in leaves especially in young ones. This is clearly noted in potassium treatments where sodium ion transport from roots to leaves was decreased. Consequently, the toxicity was limited in sensitive organs such as young leaves. Also, the sodium exclusion with restriction to its entry and uptake in leaves was observed in potassium treatments and K1 appears more efficient than K2. It is clear that potassium pulverization traps Na ion in roots in parallel with a reduction of its accumulation in young leaves through the increase of K/Na ratio in young leaves which are the most sensitive organs.

These funding are in accordance with some previous findings. AbdulWakeel (2013) affirmed that K fertilization improved crop performance on sodium affected soils. Similarly, Demidchik and Maathuis (2007) presented that high external K reduced Na influx. Moreover, Kronzucker et al. (2008) indicated that in barley Na influx and accumulation was dependant of K supply. The lower K content recorded in leaves of untreated plants was previously indicated a high accumulation of sodium ions. Indeed, the leaf K decreased significantly with the increment in water electric conductivity (García-Caparrós, 2016). Equally, Cassaniti et al. (2009) noted a lower K^+ concentration in some ornamental plants grown under salt conditions. Moreover, Zorb et al. (2014) explained the role of potassium supply in confronting salt stress.

In fact, plant resistance can be realized through various Na flux but both mechanisms Na including and Na excluding crops, high K/Na ratio needs to be maintained in the cytosol for keeping enzymatic functions. In addition, to potassium intervention as an osmoticum under salt stress, some other organic solutes such as proline are also accumulated in response to stress. Therefore, it was published that accumulation of proline took place in the cytoplasm including cytosolic osmotic adaptation under salinity (Munns & Tester, 2008) or drought conditions (Abboud et al., 2021). Results are in accordance with this finding. Leaves of untreated plants showed the most important proline content as a response to salt stress. However, treated plants with potassium foliar spray alleviated the negative effects of salinity. This is confirming results shown in strawberry which indicate the supplementation of potassium silicate in nutrient solution decreased the proline content (Yaghubi et al., 2016). So, the potassium reduced the salt stress degree by the control of Na uptake by enhancing osmotic adjustment. Similarly, to proline, total sugars content was reduced in leaves by potassium treatments. Total sugars content was inversely proportional to potassium concentration used in foliar sprayer particularly in mature leaves. In general, the leaf sugar increased significantly with high electric conductivity of irrigation water. In addition, Gimeno et al. (2014) illustrated the role of potassium nitrate in limiting the negative effects of water stress by regulation of TS in citrus leaves. Also, Yaghubi et al. (2016) highlighted the drastic role of potassium silicate through the moderation of carbohydrates and results depend on cultivar and growing season. The decrease of TS may be due to impaired transportation through phloem system or increase in carbohydrates resources usage in order to exit sodium ions from the cells (Irrigoyen et al., 1992). Many researches illustrated that under salt stress condition the main sugar alcohol which play the osmoprotectant role is the mannitol in olive (Conde et al., 2007). The accumulation of mannitol in leaf tissue preceded any reduction in leaf area rate or net assimilation rate. The increase in leaf mannitol or glucose concentration was positively correlated with the increasing level of salinity at the root zone, but not with the accumulation of Na+ in the shoot. The mannitol is a potential osmoregulator in leaf mesophyll during salinity stress and it is in relation to the complex carbohydrate composition of olive leaves (Tattini et al., 2006). Besides, the determination of total phenols showed an increase with potassium treatments. The comparison of leaves type showed important values in mature leaves except in K1 differences were not significant. These results particularly for mature leaves, differences observed in polyphenols may be due to the accumulation of another compound. In this way, several studies demonstrate that external treatment of olive plants enhances the non-enzymatic antioxidant activity i.e total polyphenol (Methanni et al., 2018). Likewise, Petridis et al. (2012) indicated that salinity stimulated the biosynthesis of phenols and oleuropein in leaves, whereas the hydroxytyrosol concentration was either negatively or not affected by the salt stress. Oleuropein was the main phenolic compound regardless of NaCl treatments. Also, they indicated a mainly negative correlation with glucose accumulation. Moreover, a significant correlation was found between total phenols and antioxidant activity. The antioxidant capacity is well correlated with the content of total polyphenols.

MDA content increased in salt-stressed olive leaves. The addition of potassium leads to reduce it for avoiding lipid peroxydation and cell damage. Salt stress plants have been reported to present a K+ efflux that is rapidly induced favoring a significant decrease of the cytosolic K+ (Shabala et al., 2006).

CONCLUSION

In overall, results suggest that potassium treatments can be useful methods to improve salt tolerance in olive plants via the modification of Na/K balance and the took place of biochemical changes. The potassium foliar spray led to decrease the oxidative stress caused by salt stress particularly with the reduction of proline and soluble sugars. We concluded that the use of saline waters is feasible for olive cultivation. Olive plants showed osmo-regulation and various compounds were involved leading to alleviate salt stress. The presence of potassium regulated sodium flux and osmoregulators accumulation leading cells protection and compensation of the decrease of external water activity.

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Conflict of interest
All authors of the research article have no conflicts of interest to disclose.

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