EFFECTS OF IRRIGATION WATER FLUORIDE ON RELATIVE WATER CONTENT, PHOTOSYNTHETIC ACTIVITY, AND PROLINE ACCUMULATION IN YOUNG OLIVE TREES (*OLEA EUROPAEA* L. CV CHEMLALI) IN ARID ZONES

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ABSTRACT: The effects of increasing the NaF concentration supplied through the irrigation water on some photosynthetic and physiological features of young olive trees grown under the ambient environmental conditions of an arid climate in Sfax, Tunisia, were investigated. Thirty-six uniform one-year-old self-rooted olive trees (*Olea europaea* L. cv Chemlali) were transplanted into 5 L pots and irrigated for 5 months with NaF concentrations of 0 (control), 20, 40, and 80 mM NaF. The responses of the young olive trees grown under fluoride stress were slight reductions of leaf relative water content, net photosynthesis, stomatal conductance, transpiration rate, photosynthetic pigment content, and growth traits. Interestingly, the Chemlali olive tree was relatively resistant to the fluoride stress under the experimental conditions since it maintained some photosynthetic activity and a suitable leaf relative water content. Moreover, the positive evolution in the content of proline and soluble sugars with increasing NaF stress was further evidence of the effect of these osmoticums for improving the tolerance of the Chemlali olive trees to fluoride stress.

Keywords: Chemlali olive trees; Chlorophyll; Growth trait; NaF stress; Net photosynthesis; Olive tree; Proline accumulation; Soluble sugars; Leaf relative water content.

INTRODUCTION

Increasingly, soil pollution by the fluoride ion (F), due to agricultural and industrial activities, is becoming a serious environmental problem in the world.[1,2] F is an important phytotoxic environmental contaminant, which can enter into a plant very rapidly through the roots and subsequently it is translocated to the transpiratory organs of the plant.[3,4] The phytotoxic effects of F are probably a consequence of its interference with a number of metabolic processes.[3,5] F stress induced growth alterations can be explained by a reduction of cell water content,[6] and photosynthetic activity.[7,8] In addition, under F stress plant cells exhibit a number of physiological changes including considerable osmotic adjustment, which involves the accumulation of organic solutes.[5,6,8] Furthermore, a strong correlation between higher cellular levels of proline and soluble sugars, and the fluoride stress tolerance of some plants has been shown.[8,9]

In Sfax, in the southern arid area of Tunisia, agricultural soils showed a high fluoride content due to the long term accumulation of fluoride released from a phosphate fertilizer producing factory. In this fluoride polluted habitat, olive plants

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constituted the main cultivated crops which were able to resist the fluoride stress and grow. The present work studied the effect of fluoride stress on some photosynthetic and physiological responses of young olive trees grown under the ambient environmental conditions of the arid climate in southern Tunisia.

MATERIALS AND METHODS

The trials were conducted at the Olive Tree Institute of Sfax, Tunisia. Thirty-six uniform one-year-old olive trees (*Olea europaea* L. cv Chemlali) were transplanted into 5 L pots filled with 5 kg soil (86.5% sand, 0.15% clay, and 13.35% silt). The soil was characterized by an organic matter content of 1.19%, electrical conductivity of 2.21 mS/cm, pH of 7.16, and total fluoride of 20 mg/kg soil. The basic soil characteristics were determined as described by Sparks et al.[10] The experiments were carried under ambient environmental conditions (from November 2009 to April 2010) with natural sunlight and temperature. The averages for the temperature and the relative humidity ranged between day and night from 20 ± 8 to 10 ± 5ºC and from 50 and 70%, respectively. The average of photosynthetically active radiation (PAR) was 700 ± 1100 µmol/m^2/sec during the course of the experiment. The plants were divided into four groups and subjected to the following treatments for five months: *Cp*: control plants irrigated with tap water; *Sp1*, *Sp2*, and *Sp3*: stressed plants irrigated with tap water containing 20, 40, and 80 mM NaF, respectively. Each treatment was conducted with 9 young olive plants, which were divided into 3 groups, each of 3 plants. The tap water was characterized by EC (1.2 dS/m), pH (7.4), Na^+ (145 mg/L), Cl^- (226 mg/L), K^+ (250 mg/L), Ca^{2+} (94 mg/L) and Mg^{2+} (57 mg/L). For each treatment, the daily amount of water used for irrigation during the experimental period was equal to the amount lost by evapotranspiration. After harvesting, the plants were washed extensively with distilled water, and samples of leaves and roots were then collected and frozen in liquid nitrogen at –80ºC. Other samples of leaves and roots were oven-dried at 70ºC to a constant mass and ground.

Growth traits measurements: At harvest, the growth parameters (trunk diameter, shoot elongation, and leaf area) were determined. The leaf area was estimated by a leaf-area-meter (LI-2000, LI-COR, USA).

Fluoride content: The fluoride content was determined using the potentiometric technique as previously described. [4]

Leaf relative water content and gas exchange measurements: The leaf relative water content (LRWC), net photosynthesis (Pn), stomatal conductance (Gs), and transpiration rate (E) were determined as previously described. [11]

Photosynthetic pigments, proline, and soluble sugars contents: The chlorophyll, carotenoid, proline, and soluble sugars concentrations were determined as previously described.[11]

Statistical analysis: The different measurements and analyses were made at the end of the experimental period. A one way analysis of variance (SPSS software, 17.0) was performed. Tukey's test ($p \leq 0.05$) was used to compare the averages of all the measured parameters. At least, three replicates were performed for each measurement.

RESULTS

Figure 1 shows that the F content in the leaves and roots of the young olive plants increased significantly (p≤0.05) with increasing NaF concentrations through the irrigation water. With all three of the NaF treatments, the roots accumulated a higher F content than the leaves. The highest F content was measured in the roots (220 µg/g dry weight) and the leaves (1000 µg/g dry weight) of the *SP3*-treated plants (80 mM NaF).

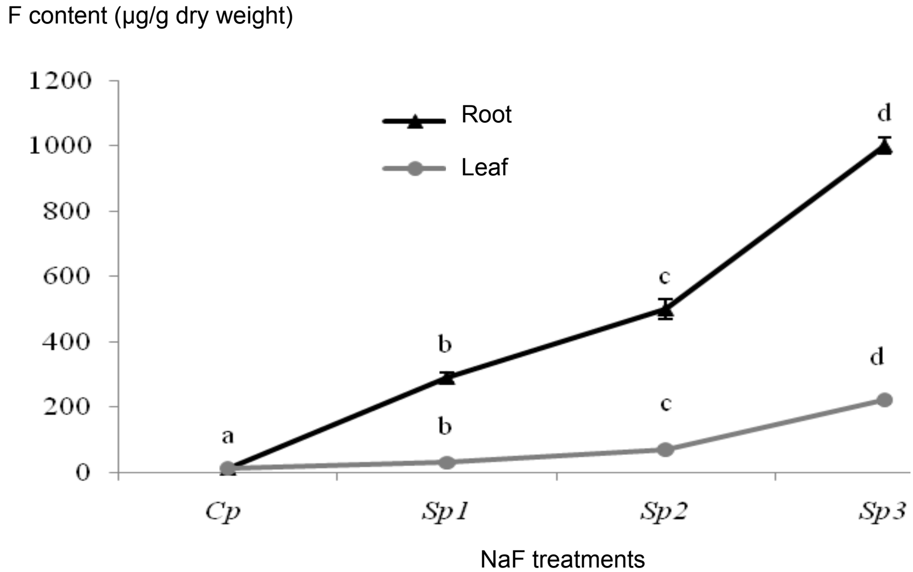

Figure 1. Fluoride (F) contents in the leaf and root of young olive plants subjected to the different NaF treatments. Values represent the means of 3 replications per treatment ± SD. Different letters indicate significant differences between treatments (p≤0.05, Tukey's test).

The results showed that the leaf relative water content (LRWC) decreased significantly (p≤0.05) in the *Sp2*- and *Sp3*-treated plants compared to the control plants, and in the *Sp3*-treated plants compared to the *Sp2*-treated plants (p≤0.05, Table). In comparison to the control plants, the LRWC decreased by about 5% and 13% in *Sp2*- and *Sp3*-treated plants, respectively. The Table also shows the gas exchange parameters (net photosynthesis Pn, transpiration rate E, and stomatal conductance Gs) in the leaves of the olive plants subjected to the different NaF treatments. As compared to the control plants, the *Sp1*-treated plants (20 mM NaF) did not show a significant change in their Pn and Gs parameters but the *Sp2*- and *Sp3*-treated plants did show a significant (p≤0.05) decrease in these parameters. The E parameter was significantly reduced (p≤0.05) in the *Sp1*- and *Sp2*-treated plants compared to the control plants and in the *Sp3*-treated plants compared to the *Sp1*- and *Sp2*-treated plants (p≤0.05). Moreover, the decreases in the Pn, Gs, and E gas exchange parameters in the fluoride-treated plants was accompanied by a reduction in the photosynthetic pigment (Table). The highest reduction of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids was recorded in the *Sp3*-treated plants. Furthermore, significant reductions (p≤0.05) occurred in the growth characteristics (trunk diameter, shoot elongation, and leaf area) in the *Sp3*-treated plants compared to the control and *Sp1*- and *Sp2*-treated plants (Table).

Table. Leaf relative water content, chlorophyll, and carotenoids contents, gas exchanges, and growth traits from young olive plants subjected to different treatments

Parameters	NaF treatments			
	Cp	*Sp1*	*Sp2*	*Sp3*
Leaf relative water content (%)	86.30 ± 1.26[a]	84.90 ± 1.03[ab]	82.17 ± 1.14[b]	74.83 ± 1.75[c]
Chlorophyll a (mg/g fresh weight)	0.45 ± 0.03[a]	0.43 ± 0.03[a]	0.38 ± 0.02[b]	0.31 ± 0.02[c]
Chlorophyll b (mg/g fresh weight)	0.23 ± 0.01[a]	0.22 ± 0.01[ab]	0.19 ± 0.01[b]	0.17 ± 0.02[c]
Total chlorophyll (mg/g fresh weight)	0.68 ± 0.02[a]	0.65 ± 0,02[a]	0.58 ± 0.02[b]	0.48 ± 0,01[c]
Carotenoids (mg/g fresh weight)	0.20 ± 0.01[a]	0.20 ± 0.01[a]	0.21 ± 0.01[a]	0.19 ± 0.01[b]
Net photosynthesis (µmol/m^2/sec)	13.40 ± 1.28[a]	12.59 ± 0.76[ab]	11.32 ± 1.02[b]	8.34 ± 0.60 [c]
Stomatal conductance (mmol/m^2/sec)	79.16 ± 4.30[a]	73.28 ± 4.98[a]	61.32 ± 3.82[b]	33.16 ± 2.74[c]
Transpiration rate (mmol/m^2/sec)	2.84 ± 0.27[a]	2.22 ± 0.25[b]	1.94 ± 0.22[b]	1.30 ± 0.13[c]
Trunk diameter (cm)	0.58 ± 0.03[a]	0.59 ± 0.04[a]	0.55 ± 0.04[a]	0.41 ± 0.03[b]
Shoot elongation (cm)	8.50 ± 0.67[a]	8.53 ± 0.94[a]	8.10 ± 0.82[a]	5.60 ± 0.79[b]
Leaf area (mm^2)	685.22 ± 60.69	682.4 ± 63.15 [a]	658.02 ± 54.93[a]	534.16 ± 46.08[b]

Values represent the means of 3 replications per treatment ± SD. Different letters indicate significant differences between treatments ($p \leq 0.05$, Tukey's test).

Under fluoride stress, proline accumulation in both plant tissues (leaves and roots) increased significantly with increasing NaF concentrations (Figure 2A). In the *Sp3*-treated plants, the proline content increased by 133% in the leaves and by 83% in the roots. In parallel to the increase of proline, the fluoride-stressed olive trees accumulated more soluble sugars. Compared to the control plants, the soluble sugar accumulation was higher in the leaves and roots of the *Sp3*-treated plants by factors of 1.4 and 1.3, respectively (Figure 2B).

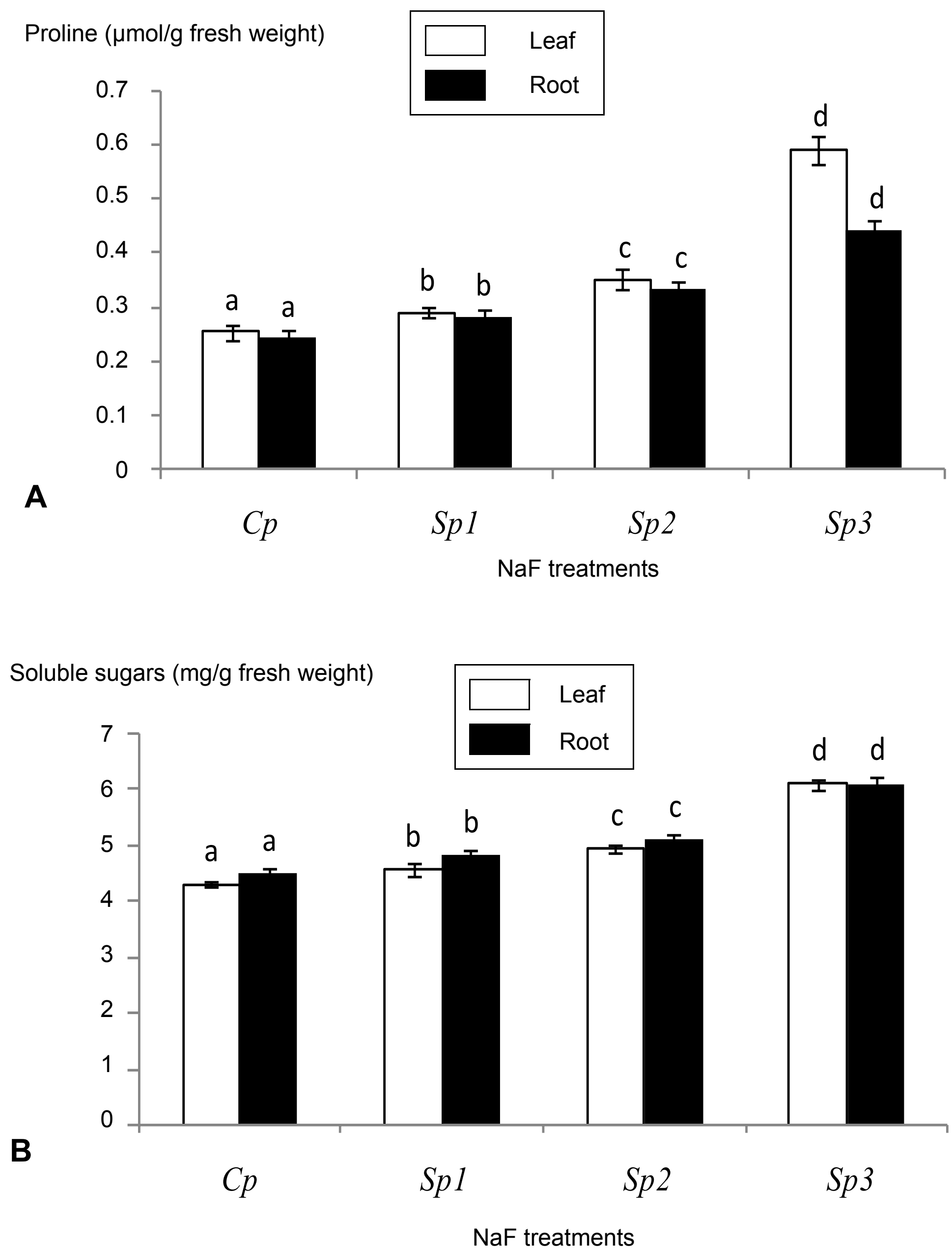

Figure 2. (A) Proline and (B) soluble sugars contents in leaf and root of young olive plants subjected to the different NaF treatments. Values represent the means of 3 replications per treatment ± SD. Different letters indicate significant differences between treatments ($p \leq 0.05$, Tukey's test).

DISCUSSION

The results obtained showed that the LRWC, which decreased slightly with increasing NaF concentrations, could be a useful indicator of plant tolerance to abiotic stress.[11] Similar results were reported in several crops, such as mung beans,[6] and jack pine seedlings,[12] grown with exposure to fluoride. This decrease in the LRWC in the fluoride-stressed olive trees could be explained by the high F concentration in the plant tissues causing osmotic stress and partial dehydration at the cellular level. The Chemlali olive tree remained tolerant to fluoride and had limited water loss, since a relatively high value of the LRWC (>70%) was recorded in the *Sp3*-treated olive plants.The reduction in chlorophyll content in the fluoride-stressed olive trees could be due to structural alterations in the chloroplasts such as disorganization of the thylakoid system, damage to the stroma chloroplasts,[13,14] inhibition of chlorophyll biosynthesis, and/or increased chlorophyll degradation.[5,8] The observed deleterious effect of fluoride accumulation on leaf chlorophyll content was also reported in several plants, such as paddy saplings,[8] and tea plants,[13] grown under fluoride stress. In addition, the decrease in the photosynthetic pigments observed in the fluoride-stressed olive tree could be considered to be a result of the Pn decrease (Table). Moreover, according to Elloumi et al.,[15] the decrease of photosynthesis, stomatal conductance, and transpiration rates observed in almond plants grown in a fluoride polluted zone could be attributed to abnormalities of the stomata, such as less stomal density and stomatal closure. The reduction of the photosynthetic performance in the F-stressed Chemlali olive trees could be considered as a dehydration avoidance mechanism, and to constitute an adaptive mechanism of olive plants rather than as a merely negative consequence of the fluoride stress[11].

The present data show a reduction in the different growth traits (trunk diameter, shoot elongation, and leaf area) under high fluoride stress, which could be explained by the reduction of photosynthetic activities and the impairment of the uptake and translocation of nutrients and water into the plant organs.[16,17] Similar findings were reported in watermelon[5] and gram seed [18] developed in a fluoride polluted environment.

The results obtained also show that the higher the fluoride treatment was, the more important the accumulation was of proline and soluble sugars in the leaf and root tissues of the young olive plants. The accumulation of proline and soluble sugars in stressed olive plant tissues can be used as endpoints to assess fluoride tolerance.[5, 6, 8] The capacity for osmotic adjustment observed in the fluoride-stressed olive trees via the accumulation of proline and soluble sugars was also recorded in the case of salt-stressed young olive trees.[11] In the present study, the high accumulation of proline and soluble sugars justified the important role of these osmoticums in the activation of water uptake to maintain cellular tissue turgor.[6,19]

CONCLUSION

The distribution of fluoride in F-treated olive plants displayed a differential pattern among the root and leaf tissues with a higher fluoride concentration in roots. The leaves of the young olive plants showed less fluoride concentration than the roots in such a way as to protect the photosynthetic apparatus of stressed plants. The relatively high value of the leaf relative water content and the maintenance of photosynthetic activity in the plants treated with the highest NaF level (*Sp3*) revealed the effectiveness of the Chemlali olive tree in resisting the stress of exposure to high levels of fluoride (80 mM NaF in the irrigation water).

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