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Enzymatic antioxidant responses and mineral status in roots and leaves of olive plants subjected to fluoride stress

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ABSTRACT

Fluoride (F) as one of the most toxic pollutants affecting the environment severely restricted plant growth and development. The Fluoride toxicity was studied in young olive plants (*Olea europaea* L. cv Chemlali), which were irrigated for 5 months with increasing NaF concentration: 0 (control), 20, 40, and 80 mM NaF. The NaF application resulted in an increase of the F content in plant tissues with a higher level in roots compared to leaves. Olive plants treated with 20 or 40 mM NaF maintained their mineral status and activated their antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase). In fact, lipid peroxidation and electrolyte leakage were not affected in the plant leaves. However, olive plants treated with 80 mM NaF showed (i) the highest reduction of antioxidant enzyme activities and mineral contents, and (ii) an increase of oxidative stress markers such as hydrogen peroxide, thiobarbituric acid reactive substances and electrolyte leakage in both roots and leaves.

1. Introduction

Fluoride (F) is believed as one of the most important contaminant in the ecosphere (Koblar et al., 2011; Panda, 2015). In fact, F is released into water, air and soil through natural weathering and human activities (Yi et al., 2016). Agricultural soils rich in F are common due to long-term accumulation of F from multiple sources including industrial waste, and extensive application of phosphate fertilizers and F-containing pesticides (Wahid et al., 2014; Choubisa and Choubisa, 2016).

Many researchers demonstrated that F may directly or indirectly inhibit several physiological and biochemical processes of plants by disturbing their metabolism, which cause growth inhibition and plant death (Koblar et al., 2011; Li et al., 2011; Brougham et al., 2013). In fact, F is known to specifically disrupt cellular redox homoeostasis and to generate the overproduction of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radical (OH•) and hydrogen peroxide (H_2O_2) (Saleh and Abdel-Kader, 2003; Yang et al., 2015). High levels of ROS will trigger phytotoxic reactions by damaging proteins, DNA and lipids (Yang et al., 2015; Chu et al., 2016). Consequently, the manifestations of ROS damage in plants include lipid peroxidation and electrolyte leakage resulting in the destruction of cellular structures (Li et al., 2011; Farooq et al., 2016). Additionally, it was reported that uptake, transport, and subsequent distribution of nutrients by the plants can be affected by the presence of F ions. In particular, F is harmful for plants because it can replace some essential elements that play key roles in enzymes active sites. Indeed, it was reported that F limits micro and macro-elements uptake in tea plant (*Camellia sinensis*), such as Zn, Ca and K (Li and Ni, 2009; Panda, 2015).

To cope with ROS under such circumstances, plants provide enzymatic and non-enzymatic antioxidants defense systems, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and phenolic compounds (Wu et al., 2015; Cai et al., 2016; Farooq et al., 2016).

Recent studies on the effects of F on the growth and physiological characteristics were focused on F-sensitive plants, such as *Prunus dulcis* (Elloumi et al., 2005) and *Punica granatum* (Ben Abdallah et al., 2006). However, little is known about the toxic effects of F in F-tolerant plants, such as olive. The olive plant, *Olea europaea* L. cv Chemlali., is among the main crops characterizing arid region in the southern Tunisia not only for its tolerance to climatic conditions of the region, but also for its interest in socio-economic balance. Furthermore, this species is interesting in limiting soil erosion and preserving the green landscape in such areas

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suffering from low precipitation and/or high temperature. Recently, several areas in southern Tunisia are continuously subjected to F pollution because there is an expansion of industrial activities. The aim of this study was to elucidate the abilities of the young olive plant to overcome F-induced stress. Macro-nutrients status, oxidative stress markers and antioxidant enzyme activities in roots and leaves of the young olive plant subjected to different F treatments were investigated.

2. Materials and methods

2.1. Plant material and treatment conditions

Trials were conducted at the Olive Tree Institute (Sfax, Tunisia). 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 used in this experiment was from the experimental site of the Olive Tree Institute that was far from the polluted area (34°43′N, 10°41′E). The soil was characterized by an organic matter of 1.19%, an electrical conductivity (EC) of 2.21 mS cm^{-1}, a pH of 7.16 and a total fluoride of 20 mg kg^{-1} soil. The pots were kept under ambient environmental conditions with natural sunlight and temperature. During course of the experiment, the average temperature and relative moisture ranged from 20 ± 8 to 10 ± 5 °C and from 50 to 70%, for day to night, respectively. The average photosynthetically active radiation was 700 ± 1100 μmol m^{-2} s^{-1}. All plants were subjected to the following treatments during five months (November 2009 to April 2010). The *Cp* represented control plants irrigated with tap water. The *F1*, *F2* and *F3* treatments represented stressed olive plants irrigated with tap water containing 20, 40 and 80 mM NaF, respectively. Each treatment was conducted with 9 young olive plants divided into 3 groups, each of 3 plants. The tap water was supplied by the Sfax Water Corporation in the area. It was characterized by EC = 1.2 dS m^{-1}, pH = 7.4, Na^+ = 145 mg L^{-1}, Cl^- = 226 mg L^{-1}, K^+ = 250 mg L^{-1}, Ca^{2+} = 94 mg L^{-1} and Mg^{2+} = 57 mg L^{-1}. The amount of water used for irrigation during the experimental period was equal to that lost by evapotranspiration. In fact, at the beginning of each month during the experimental period, all plants were weighed in the early morning (W1) and in the late evening (W2). The weight difference (W1 − W2) allowed the calculation of the amount of water lost by evapotranspiration.

At the end of the experiment, harvested plants were divided into leaves and roots, washed extensively with distilled water, dried with filter paper and weighted. Samples of leaves and roots were oven-dried at 70 °C to a constant weight. Finely, the oven-dried plant materials were ground in a stainless steel electric grinder.

2.2. Determination of fluoride content

Fluoride content in tissues was determined using the potentiometric technique as described by the Association of Official Analytical Chemists (AOAC, 1975). For fluoride determination, 2 g of dry weight were mixed with 8 g of sodium carbonate and calcinated in a muffle furnace at 450 °C for 5 h. Then, the calcinated material was dissolved in 5 M HCl and the pH of the HCl extract was adjusted at 5.3 by gradual addition of glacial acetic acid. After that, 10 mL of the extraction solution were mixed with 10 mL of total ionic strength adjustment buffer solution (TISAB) and the fluoride determination was made with a specific fluoride electrode (inoLab/Model WTW). The calibration was realized by standard NaF solutions (0.1, 1.0, 10.0, 50.0 and 100 mg L^{-1}) as previously described by Zouari et al. (2014).

2.3. Determination of mineral nutrients content

Mineral nutrients content was measured following the procedure described by Bankaji et al. (2015). At the beginning, 0.5 g of dry leaves or roots samples were placed in an oven at 250 °C for 3 h and then digested with 10 mL of 1 M HNO_3. The resultant solutions were adjusted to 25 mL using distilled water. The contents of potassium (K), calcium (Ca) and magnesium (Mg) were determined using atomic absorption spectrophotometry (Perkin Elmer A Analyst 300, USA).

2.4. Determination of hydrogen peroxide (H_2O_2), thiobarbituric acid reactive substances (TBARS) and electrolyte leakage (EL)

Fresh leaf and root samples were used for H_2O_2, TBARS and EL determination. Hydrogen peroxide level was determined according to Sergiev et al. (1997) method. A 0.5 g of fresh samples was homogenized in an ice bath with 5 mL of 0.1% (*w*/*v*) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000*g* for 15 min. Then, 1 mL of the supernatant was added to 0.5 mL of 10 mM potassium phosphate buffer pH 7.0 and 1 mL of 1 M potassium iodide. The supernatant absorbance was measured at 390 nm with UV/vis spectrophotometer. Hydrogen-peroxide contents were calculated using a standard curve.

The level of lipid peroxidation in leaf and root tissues were estimated by the content of the thiobarbituric acid reactive substances (TBARS) expressed as equivalents of malondialdehyde (MDAeq), which is a product of lipoperoxidation. The TBARS content was determined by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968) with minor modifications reported by Zouari et al. (2016). A 0.5 g of fresh sample was homogenized in 5 mL of 0.1% TCA. The homogenate was centrifuged at 12,000*g* for 5 min. After that, 4 mL of 20% TCA containing 0.5% TBA were added to 1 mL aliquot of the supernatant. The mixture was heated at 95 °C for 30 min, and then quickly cooled in an ice bath. After centrifugation at 12,000*g* for 10 min, the supernatant absorbance was measured with Helios β-spectrophotometer at 532 nm and the value of the non-specific absorption at 600 nm was subtracted. The TBARS content was calculated using a molar extinction coefficient of 155 mM^{-1} cm^{-1}.

Electrolyte leakage was measured as described by Lutts et al. (1996). Fresh leaf and root samples (0.25 g) were cut in small parts, put into test tubes containing 10 mL of deionized water and incubated at room temperature on a rotary shaker for 24 h. Subsequently, the initial electrical conductivity of the medium (EC1) was assessed. The samples were placed in an oven at 90 °C for 2 h. Thereafter, they were cooled at 25 °C and a second measurement of the electrical conductivity (EC2) was determined. Electrolyte leakage (EL) was calculated using the following equation:

$$\text{ElectrolyteLeakage}\,(\%) = \frac{\text{EC1}}{\text{EC2}} \times 100$$

2.5. Enzyme activities

Antioxidant-enzyme activities of roots and leaves were determined spectrophotometrically. Fresh tissues (0.5 g) were homogenized in 0.8 mL of 100 mM ice-cold K-phosphate buffer pH 7.0. The homogenates were centrifuged at 15,000*g* for 15 min and the supernatants were used for the determination of enzyme activities. All procedures were performed at 0–4 °C.

2.5.1. Determination of superoxide-dismutase activity (SOD, EC 1.15.1.1)

SOD activity was determined according to the method of Giannopolitis and Ries (1977) following the inhibition of photochemical reduction due to nitro-blue tetrazolium (NBT). The reaction mixture contained 40 mM K-phosphate buffer pH 7.8, 10 mM methionine, 33 μM NBT, 3.3 μM riboflavin, 0.66 mM EDTA and 0.17% of enzyme extract in a final volume of 3 mL. The reaction mixture was incubated for 30 min under 15-W fluorescent lamp at 28 °C, and absorbance was measured at 560 nm using Helios β-spectrophotometer (Thermospectronic).