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Enzymatic and non-enzymatic antioxidant responses of two *Mentha pulegium* provenances to salt stress

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The aim of the present investigation was to evaluate the impact of moderate salinity in two Tunisian *Mentha pulegium* L. provenances differing in their salt sensitivity and to identify the antioxidant system responsible for the maintenance of plant vigor in the less sensitive provenance. Seedlings of the two provenances (Tekelsa and Soliman) were hydroponically grown at 0 and 35 mM NaCl over 15 and 45 days. Both the less (Soliman) and the more sensitive (Tekelsa) provenances behaved as a sensitive glycophyte not able to tolerate such a moderate salinity. But, Soliman showed less lipid peroxidation and electrolyte leakage since it restricted sodium uptake and accumulation into its tissues. In addition, lipid peroxidation and electrolyte leakage drastically increased with plant age. Interestingly, polyphenol production and antiradical activity increased with plant age, too. Salt stress stimulated these two parameters, especially in the less sensitive provenance, Soliman. During the first weeks of salt treatment, *M. pulegium* responded to the salt-induced oxidative stress by an activation of its enzymatic system then the non-enzymatic system took place.

Key words: Antioxidant enzymes, antiradical activity, electrolyte leakage, lipid peroxidation, polyphenols.

INTRODUCTION

Salt-affected soils cover over 800 million ha throughout the world (Munns, 2005). It was postulated that 6% (more

than 800 million ha) of the world lands are affected by salinity, which is mainly due to natural causes (salt accumulation over long periods of time in arid and semi-arid regions) or to secondary salinity that affected in 2008 about 2% (32 million ha) of the dry land-farmed areas and 20% (45 million ha) of the irrigated lands in the world (Munns and Tester, 2008). Salt effects on plants can be due to a low osmotic potential of the soil solution (osmotic stress), specific ion effects (salt stress), imbalances in nutrient acquisition (nutritional stress), or a combined effect of the three factors (Ashraf, 2004; Koyro et al., 2008). According to Munns (2005), the first factor that appears after salt treatment application is the osmotic stress (first days or weeks) having no effect on shoot growth rate, followed by the salt stress (the salt specific effect) reducing shoot biomass production in sensitive plants. Salt specific effect appears after an excessive salt accumulation within plant cells (Munns, 2005).

In chloroplasts of salt-stressed plants, a salt-induced imbalance between O₂ production during the photo-

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Abbreviations: AMPs, aromatic and medicinal plants; CAT, catalases, DNA: deoxyribonucleic acid; DPPH, diphenylpicrylhydrazyl radical; DTT, dithiothreitol, DW, dry weight; EC, electrical conductivity; EDTA, ethylenediaminetetraacetic acid; EL, electrolyte leakage; FW, fresh weight; GSH, glutathione; IC₅₀, the extract concentration required to cause a 50% inhibition; MDA, malonyldialdehyde; NADP⁺, nicotinamide adenine dinucleotid phosphate; PAR, photosynthetic active radiations; PMSF, phenylmethylsulfonyl fluoride, POD, peroxidases; PVP, poly vinyl pyrrolidone, ROS, reactive oxygen species; SOD, superoxide dismutases; TBA, thiobarbituric acid, TCA, trichloroacetic acid.

chemical phase of photosynthesis and the low rates of NADP⁺ generated by Calvin cycle reactions due to CO₂ limitation often results in generated reactive oxygen species (ROS) (Cavalcanti et al., 2007; Ashraf, 2009). These reactive oxygen species can cause damage to membranes and other essential macromolecules such as photosynthetic pigments, proteins, DNA and lipids (Hernandez et al., 2000). One of their prominent effects is lipid peroxidation and loss of membrane integrity (Hernandez et al., 2000; Sairam et al., 2002). As a response, activities of some antioxidant enzymes such as superoxide dismutases (SOD), peroxidases (POD) and catalases (CAT) are generally found to be induced (Noctor and Foyer, 1998; Ashraf, 2009). In addition, salt stress often induces biosynthesis of glutathione (GSH), ascorbic acid, carotenoids, tocopherols, essential oils, and polyphenols (Navarro et al., 2006; Hernandez et al., 2010). Phenolic compounds and ascorbic acid constitute the main powerful natural antioxidants (Balasundram et al., 2006). They retard or inhibit lipid autoxidation by acting as radical scavengers (Namiki, 1990). They are also highly applied in food industry, cosmetic, pharmaceutical and medicinal materials (Maisuthiaskul et al., 2007). Hence, an enhancement in polyphenol production in plants is of great importance for all these uses especially in aromatic and medicinal plants (AMPs) (Karray-Bouraoui et al., 2010).

Currently, salt-affected soils in Tunisia cover 10% of the total area of the country (Hachicha, 2007). In these regions, AMPs are often irrigated with relatively saline waters. *Mentha pulegium* L. is one of these AMPs and was described as a potential source of phenolic compounds (Stycharz and Shetty, 2002; Karray-Bouraoui et al., 2010). Under salt treatment, it showed an increase in polyphenol concentration and an amelioration of enzymatic and non-enzymatic antioxidant properties (Oueslati et al., 2010). The aim of the present work was to study the effects of salinity on antioxidant properties in two Tunisian provenances of *M. pulegium* differing in their salt tolerance.

MATERIALS AND METHODS

Plant material and growth conditions

M. pulegium (Lamiaceae) seeds were collected in two Tunisian sites: Tekelsa (mountainous region at 50 km North Tunis; sub-humid bioclimatic stage; MAR 490 mm) and Soliman (a sabkha edge at 30 km north Tunis; superior semi-arid bioclimatic stage; MAR 370 mm). Seeds were sterilized and germinated on filter paper in Petri dishes in a growth chamber under controlled conditions (16/8h light/dark, 18 - 22°C day/night temperature, 60 - 80% relative humidity regimes ranged and 300 μmol/m²/s photosynthetic active radiations (PAR)). Two-leaf seedlings were watered with half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) then at the age of 36 days, plants were separated into two groups irrigated with a nutrient solution added or not with 35 mM NaCl. Two harvests were performed: the first after 2 weeks and the second after 6 weeks of salt treatment. For dry weight (DW) and tissue hydration determination, plants were separated into

leaves, stems and roots, weighed, oven-dried at 60°C then weighed again. Fresh samples were also taken from each plant were immediately frozen in liquid nitrogen and stored at -80°C until performing biochemical analysis.

Determination of Na⁺ concentrations

Aliquots (0.2 g) of oven-dried ground material of leaves, stems and roots were digested with 25 mL nitric acid (HNO₃) 0.5%. Sodium (Na⁺) concentrations were measured in the digests with a flame emission photometer (Jenway PFP7).

Estimation of electrolyte leakage

Electrolyte leakage was determined as described by Dionisio-Sese and Tobita (1998). Leaf and root samples (approximately 200 mg FW) were submerged into 10 ml distilled water and kept at 32°C over 2 h. Then, the initial electrical conductivity of the medium (EC1) was measured. After that, the leaf tissues were killed by autoclaving at 121°C for 20 min to release all electrolytes. Then, samples were cooled to 25°C for a final electrical conductivity (EC2) was measurement. The electrolyte leakage (EL) was calculated as EL = 100 EC1/EC2.

Estimation of lipid peroxidation

Salt-induced oxidative damage (membrane lipid peroxidation) was assessed by measuring the amount of malonyldialdehyde in 0.5 g leaf or root fresh weight according to Buege and Aust (1972). For MDA extraction, 0.5 g of leaf or root samples was homogenized with 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged for 5 min at 10,000 g. Then, 4 ml TCA (20%) containing thiobarbituric acid (TBA, 0.5%) was added per ml of the aliquot. The mixture was heated at 95°C for 30 min then cooled quickly in an ice bath. Subsequently, the mixture was centrifuged for 10 min at 10,000 g and the absorbance of the supernatant was measured at 532 nm. Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM/cm.

Protein extraction

Aliquots of frozen leaf, stem and root fresh matter were ground to a fine powder with liquid nitrogen and extracted at 4°C in 100 mM tris-HCl buffer (pH 8.0) containing 10 mM EDTA, 50 mM KCl, 20 mM MgCl₂, 0.5 mM PMSF, 1 mM DTT, 0.1% (v/v) Triton X-100 and 10% (w/w) PVP. The homogenate was centrifuged at 14,000 g for 30 min at 4°C, and supernatant was used for the determination of protein concentration and of antioxidant enzyme activities. Protein concentration was determined according to Bradford (1979) using bovine serum albumin as a standard.

Enzyme assays

Fresh leaf, stem and root samples were homogenized in 4 ml potassium phosphate buffer (20 mM; pH 7.4) containing 50 mM β-mercaptoethanol. The homogenate was filtered then centrifuged at 15,000 g for 15 min. The supernatant was used for enzyme analysis. Catalase (CAT) activity was measured spectrophotometrically according to the method of Aebi (1984), by monitoring the decline in the absorbance at 240 nm as H₂O₂ was consumed. The final volume (3 ml) of the reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), to which 30% H₂O₂ was added

(Absorbance = 0.52–0.55 at 240 nm). The reaction was activated by adding 100 ml leaf crude extract to this solution. CAT activity was expressed as units (μmol decomposed H_2O_2 per min) per mg of protein. For the measurement of peroxidase (POD) activity, the reaction mixture contained 25 mM phosphate buffer (pH 7.0), 0.05% guaiacol, 10 mM H_2O_2 and enzyme. POD activity was determined by the increase in absorbance at 470 nm due to guaiacol oxidation (Nakano and Asada, 1981).

Determination of polyphenol concentration

Harvested organs (leaves, stems and roots) were dried at room temperature for 1 week. Plant extracts were obtained by magnetic stirring for 30 min of 2.5 g dry powder with 25 ml acetone (80%). The extracts were then kept for 24 h at 4°C, filtered through a Whatman 4°N filter paper, evaporated under vacuum to dryness and stored at 4°C until it is analyzed. Total phenolics were assayed using the Folin–Ciocalteu reagent, following Singleton and Rosi (1965) method, based on the reduction of a phosphotungstate–phosphomolybdate complex by phenolics to blue reaction products and slightly modified by Dewanto et al. (2002). An aliquot of diluted sample extract was added to 0.5 ml distilled water and 0.125 ml Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before addition of 1.25 ml Na_2CO_3 (7%). The solution was then adjusted with distilled water to a final volume of 3 ml and mixed thoroughly. After incubation in the dark, the absorbance was read at 760 nm versus a blank. Leaf total phenolic concentration was expressed as milligrams of gallic acid equivalents per gram dry weight (mg GAE/g DW) through a calibration curve with gallic acid. All samples were analyzed in triplicate.

DPPH radical-scavenging activity

The diphenylpicrylhydrazyl radical (DPPH) scavenging activity was estimated according to Hanato et al. (1988). Dried plant extracts were diluted in absolute methanol at different concentrations and then 2 ml were added to 0.5 ml DPPH methanolic solution (0.2 mmol^{-1}). The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark and then the absorbance was measured at 517 nm. For each dilution of the extract, the DPPH scavenging activity was calculated as $100 (A_0 - A_1)/A_0$, where A_0 is the absorbance of the control at 30 min and A_1 is the absorbance of the sample at 30 min. The antiradical activity was finally expressed as IC_{50} ($\mu\text{g}/\text{ml}$), the extract concentration required to cause a 50% inhibition. A lower IC_{50} value corresponds to a higher antioxidant activity of the plant extract. All samples were analyzed in three replicates.

Statistical analysis

Data were subjected to a one-way ANOVA test using SPSS 11.0 for Windows and means were compared according to Duncan's multiple-range test at 5% level of significance.

RESULTS

Plant growth and tissue hydration

Under non-saline conditions, dry weights of all organs were slightly higher in Tekelsa than in Soliman over 2 as well as over 6 weeks of treatment (Table 1). At the first

harvest, salt treatment resulted in a decrease in the whole plant DW, the salt effect being more pronounced in Tekelsa (-25%) than in Soliman (-16%). Considering each organ apart, stems seemed the most affected ones in both provenances. Less marked diminution in biomass production was also observed in leaves and roots, mainly in Tekelsa. At the second harvest, salt treatment resulted in a further growth reduction in the two provenances. The whole plant DW exhibited a decline of 25 and 55%, respectively in Soliman and Tekelsa (Table 1). In Tekelsa, stems were the most affected organs over the 45 days of salt treatment (-64%) whereas in Soliman, the most pronounced growth reduction was observed in leaves (-30%). Root water content was not affected by salinity at the first harvest, whereas those of above-ground organs were significantly reduced in the two provenances (Table 1). Values ranged from 19.9 to 22.8 ml/g in roots, from 9.2 to 12.9 ml/g in stems, and from 5.3 to 9.5 ml/g in leaves. After 6 weeks of salt treatment, water contents of all organs were significantly decreased. From these results, it seems that Soliman is less salt-sensitive than Tekelsa.

Sodium accumulation

In plants grown over 2 weeks at 35 mM NaCl, leaves of the two provenances showed the highest Na^+ concentrations (3.0 and 2.0 mmol/g DW, respectively in Tekelsa and Soliman), followed by stems (2.0 and 1.3 mmol/g DW, respectively in Tekelsa and Soliman; Table 1). After 6 weeks of treatment, an overall increase in Na^+ concentrations was observed in all organs with the highest value in leaves of Tekelsa (4.9 mmol/g DW). As a whole, sodium accumulation discriminated the two provenances, the more sensitive provenance (Tekelsa) experiencing higher Na^+ concentrations in all organs at both harvests.

Lipid peroxidation

Lipid peroxidation was estimated by MDA concentration in roots and leaves of the two provenances. Results showed that under non-saline conditions, Tekelsa exhibited much higher root and shoot MDA concentrations than Soliman (Table 2). After two weeks of salt treatment, a significant increase in lipid peroxidation was observed mainly in roots in which MDA concentrations were improved from 26.4 to 50.7 and from 15.3 to 26.9 $\mu\text{mol}/\text{g}$ FW (fresh weight), respectively in Tekelsa and Soliman. Leaves of Soliman showed no significant induction of MDA accumulation after 2 weeks of treatment. After 6 weeks of treatment, an overall enhancement in MDA concentrations was observed in roots as well as in leaves of the two provenances of both control and salt-treated plants. But, no difference was found between the two provenances in MDA concentration of the same organ and the same treatment.

Table 1. Dry weights (DW) per organ and per plant, water contents, and sodium concentrations in roots, stems, and leaves of two Tunisian provenances of *M. pulegium* grown over 2 and 6 weeks at 0 or 35 mM NaCl. Values are means of 6 replicates. In each line, values followed by different letters are significantly different according to Duncan's multiple range test.

| NaCl (mM) | Tekelsa | | Soliman | |
|--|---------|---------|---------|---------|
| | 0 | 35 | 0 | 35 |
| 2 weeks of treatment | | | | |
| Root DW (mg) | 250.9d | 195.4a | 225.0c | 202.9b |
| Stem DW (mg) | 488.9c | 281.3a | 390.2b | 282.5a |
| Leaf DW (mg) | 574.7d | 415.5a | 480.4c | 461.0b |
| Whole plant DW (mg) | 1314.5d | 1032.5b | 1095.6c | 946.4a |
| Root water content (ml/g DW) | 22.8b | 21.8b | 21.3ab | 19.9a |
| Stem water content (ml/g DW) | 12.9b | 9.2a | 12.5b | 9.4a |
| Leaf water content (ml/g DW) | 9.5b | 5.4a | 9.1b | 5.3a |
| Root Na ⁺ concentration (mmol/g DW) | 0.04a | 1.29b | 0.04a | 1.22b |
| Stem Na ⁺ concentration (mmol/g DW) | 0.04a | 1.99c | 0.04a | 1.33b |
| Leaf Na ⁺ concentration (mmol/g DW) | 0.04a | 3.00c | 0.04a | 2.01b |
| 6 weeks of treatment | | | | |
| Root DW (mg) | 536.7c | 221.6a | 532.9c | 421.6b |
| Stem DW (mg) | 2425.3d | 980.6a | 2097.3c | 1615.3b |
| Leaf DW (mg) | 1525.2d | 934.2b | 1439.6c | 695.3a |
| Whole plant DW (mg) | 4487.2d | 2136.3a | 4069.8c | 2732.1b |
| Root water content (ml/g DW) | 22.7d | 19.0c | 16.0b | 11.6a |
| Stem water content (ml/g DW) | 5.8b | 5.2b | 5.5b | 3.6a |
| Leaf water content (ml/g DW) | 5.3c | 4.8b | 5.5c | 4.6a |
| Root Na ⁺ concentration (mmol/g DW) | 0.02a | 3.40c | 0.05a | 2.46b |
| Stem Na ⁺ concentration (mmol/g DW) | 0.04a | 3.40c | 0.05a | 2.72b |
| Leaf Na ⁺ concentration (mmol/g DW) | 0.05a | 4.94c | 0.05a | 3.82b |

Table 2. Root and leaf MDA concentrations and electrolyte leakages in two Tunisian provenances of *M. pulegium* grown over 2 and 6 weeks at 0 or 35 mM NaCl. Values are means of 4 replicates. In each line, values followed by different letters are significantly different according to Duncan's multiple range test.

| NaCl (mM) | Tekelsa | | Soliman | |
|------------------------------------|---------|--------|---------|--------|
| | 0 | 35 | 0 | 35 |
| 2 weeks of treatment | | | | |
| Root MDA concentration (μmol/g FW) | 26.45b | 50.70c | 15.31a | 26.94b |
| Leaf MDA concentration (μmol/g FW) | 17.71b | 28.44c | 5.53a | 7.93a |
| Root electrolyte leakage (%) | 2.90a | 29.65c | 5.54a | 19.74b |
| Leaf electrolyte leakage (%) | 19.96a | 49.60c | 18.68a | 31.83b |
| 6 weeks of treatment | | | | |
| Root MDA concentration (μmol/g FW) | 55.81b | 64.37c | 49.71a | 64.57c |
| Leaf MDA concentration (μmol/g FW) | 20.77a | 34.57b | 21.54a | 32.06b |
| Root electrolyte leakage (%) | 82.13c | 88.75d | 40.52a | 68.27b |
| Leaf electrolyte leakage (%) | 89.15c | 91.20c | 50.63a | 78.48b |

Electrolyte leakage

Membrane integrity in roots and shoots was estimated as electrolyte leakage (EL). In control plants, leaves experienced much higher EL values (around 19%) than

roots (less than 6%). At the first harvest, salt stress increased this parameter, mainly in roots in which it approached 20 and 30%, respectively in Soliman and (49.6%) was found in leaves of Tekelsa. After 6 weeks of salt treatment, EL values were drastically increased in

Table 3. Root, stem, and leaf catalase (CAT) and peroxidase (POD) activities in two Tunisian provenances of *M. pulegium* grown over 2 and 6 weeks at 0 or 35 mM NaCl. Values are means of 4 replicates. In each line, values followed by different letters are significantly different according to Duncan's multiple range test.

| NaCl (mM) | Tekelsa | | Soliman | |
|--|---------|--------|---------|--------|
| | 0 | 35 | 0 | 35 |
| 2 weeks of treatment (Unit/μg protein) | | | | |
| Root CAT activity | 60.9a | 182.2b | 50.1a | 278.5c |
| Stem CAT activity | 161.0b | 176.3c | 149.3a | 163.0b |
| Leaf CAT activity | 84.4b | 65.9ab | 59.2a | 107.5c |
| Root POD activity | 338.5d | 283.4c | 191.7a | 244.6b |
| Stem POD activity | 29.9a | 50.4b | 33.5a | 223.9c |
| Leaf POD activity | 32.4a | 32.7a | 29.6a | 21.8a |
| 6 weeks of treatment (Unit/μg protein) | | | | |
| Root CAT activity | 50.3a | 242.8d | 80.4b | 168.5c |
| Stem CAT activity | 94.0a | 379.0d | 106.5b | 218.0c |
| Leaf CAT activity | 101.0c | 44.7b | 30.6a | 218.6d |
| Root POD activity | 13.9c | 10.9b | 7.7a | 33.2d |
| Stem POD activity | 4.7b | 3.4a | 4.9b | 7.6c |
| Leaf POD activity | 5.2d | 1.4a | 3.4c | 2.6b |

both organs and in the two provenances, reaching the highest level in leaves of Tekelsa (91.2%).

Antioxidant enzyme activities

Catalase activity

CAT activity was substantially improved after 2 weeks of salt treatment in roots and at a lower magnitude in stems in both provenances (Table 3). Root CAT activity increased from 61 to 182 Unit/ μ g protein in Tekelsa and from 50 to 279 Unit/ μ g protein in Soliman. Leaf CAT activity showed a huge discrimination between the two provenances; while it doubled in Soliman, it was decreased by 22% in Tekelsa.

After 6 weeks of treatment, salt induced a higher stimulating effect on the activity of this enzyme in roots and stems of the two provenances than after 2 weeks of treatment. In leaves, it maintained the same tendency in each provenance but with a higher magnitude; it decreased CAT activity from 101 to 45 Unit/ μ g protein in Tekelsa and increased it from 31 to 219 Unit/ μ g protein in Soliman.

Peroxydase activity

The effects of two-week salt treatment on POD activity showed several variations with organ as well as with provenance (Table 3). Root POD activity was decreased from 339 to 283 Unit/ μ g protein in Tekelsa and enhanced from 192 to 245 Unit/ μ g protein in Soliman. In stems, the

activity of this enzyme was substantially improved especially in the less sensitive provenance, Soliman. In leaves, however, no significant differences were detected between treatments and/or provenances. After 6 weeks of treatment, POD activity drastically decreased in all analyzed samples, regardless of the treatment, the provenance and the organ. The highest value was observed in roots of Soliman salt-treated plants.

Leaf total polyphenol concentration

Both two-week and six-week salt treatments showed a significant positive effect on leaf polyphenol concentration in the two provenances (Table 4). However, plant age showed a much higher effect on the accumulation of these molecules. Actually, after 6 weeks of treatment, all samples contained 4 to 6 times more phenolic compounds than after 2 weeks of treatment. On the former date, polyphenol concentrations ranged from 20.2 to 23.8 mg GAE/g DW.

Leaf anti-radical activity

In the more sensitive provenance, Tekelsa, two-week salt treatment showed no significant on leaf anti-radical activity (Table 4). Six-week salt treatment, however, significantly decreased this antioxidant response. In the less sensitive provenance, Soliman, both two- and six-week salt treatments significantly enhanced anti-radical activity. Nevertheless, plant age experienced a much higher influence on DPPH-scavenging capacity. Hence,

Table 4. Leaf polyphenol concentrations and antiradical activities in two Tunisian provenances of *M. pulegium* grown over 2 and 6 weeks at 0 or 35 mM NaCl. Values are means of 4 replicates. In each line, values followed by different letters are significantly different according to Duncan's multiple range test.

| NaCl (mM) | Tekelsa | | Soliman | |
|--|---------|--------|---------|--------|
| | 0 | 35 | 0 | 35 |
| 2 weeks of treatment | | | | |
| Polyphenol concentration (mg GAE/g DW) | 3.32a | 4.65b | 3.44a | 5.00c |
| IC ₅₀ (µg/mL) | 70b | 72b | 82c | 58a |
| 6 weeks of treatment | | | | |
| Polyphenol concentration (mg GAE/g DW) | 20.22a | 23.81d | 20.49b | 21.73c |
| IC ₅₀ (µg/mL) | 33a | 37c | 39d | 36b |

DISCUSSION

Our results showed that *M. pulegium* behaved as a sensitive glycophyte not able to tolerate 35 mM NaCl. These results are in agreement with those of Oueslati et al. (2010) who found that the whole plant dry weight of this species was reduced by 46, 59, and 58%, respectively at 25, 50 and 75 mM NaCl. Nevertheless, intra-specific variability in salt-induced responses was revealed in our study. Actually, Soliman provenance was found less sensitive to the stress than Tekelsa provenance and this was clearer after 6-week salt treatment. This difference between the two provenances could be attributed to the higher capacity of Soliman to restrict sodium accumulation in above-ground organs, mainly leaves (Table 1). Sodium accumulation within tissues induced an oxidative stress, which is in agreement with literature (Chaparzadeh et al., 2004; Ben Amor et al., 2005; Ashraf, 2009). The damages resulting from the oxidative stress were estimated by MDA concentration and electrolyte leakage (EL). The results revealed a clear discrimination between the two provenances with Tekelsa being more affected and showing more oxidative damages than Soliman. Similar results were found in barley (Liang et al., 2003) and in tobacco (Ruiz et al., 2005) in which salt-tolerant cultivars showed lower levels of lipid peroxidation. By contrast, salt-sensitive rice varieties exhibited high MDA concentrations and EL values in response to salinity (Dionisio-Sese and Tobita, 1998). However, plant age was also found to be the principal factor that induced oxidative stress.

Several enzymatic systems are often involved in antioxidant responses of plants. Our results showed that POD activity was enhanced under salinity in roots and stems of the two provenances of *M. pulegium* (Table 3). But, considering the results of growth, electrolyte leakage and lipid peroxidation, one can speculate that this stimulation in POD activity did not confer to the two provenances a real protection against salt-induced oxidative stress. Gossett et al. (1994) found in cotton cultivars that an increase in POD activity could be due to an increase in membrane damages and in Ca²⁺ concentrations

induced by salt stress. Similar results were obtained in two sesame cultivars differing in salt tolerance, the more sensitive exhibiting higher POD activity (Koca et al., 2005). On the contrary, Hernandez et al. (2001) found no significant difference in POD responses to salinity between a salt-sensitive and a salt-tolerant pea cultivars. In our study, it was evident that POD activity was age-dependent, decreasing with plant age.

CAT is also one of the principal antioxidant enzymes; it eliminates H₂O₂ by transforming it into H₂O and O₂. In our study, root CAT activity was stimulated in the two provenances of *M. pulegium*, especially in the less sensitive provenance, Soliman (Table 3). In leaves, the activity of this enzyme was reduced in the more sensitive provenance (Tekelsa) and enhanced in the less sensitive provenance (Soliman). This suggests that Soliman presented a higher capacity to eliminate reactive oxygen species than Tekelsa. These results disagree with those of Ben Amor et al. (2005), Lee et al. (2001) who obtained an inhibition of CAT activity under saline conditions.

The maintenance of plant vigor at 35 mM NaCl after 6-week salt treatment could not be explained by enzymatic antioxidant responses. This suggests that other antioxidant systems were involved. Actually, leaves of salt-treated plants accumulated more phenolic compounds mainly at the second harvest, and a clear discrimination between the two provenances was found when examining leaf anti-radical capacity. Hence, the salt stimulating effect in Soliman could explain the maintenance of plant vigor up to 6 weeks of salt treatment. Our results are in agreement with those of Oueslati et al. (2010) who found an increase in total polyphenols and antiradical activities in a different Tunisian provenance (Nefza) of the same species. Navaro et al. (2006) also demonstrated that total phenolic concentration increased with salinity level in red pepper fruits. Actually, phenolic compounds constitute together with ascorbic acid the main powerful natural antioxidants (Balasundram et al., 2006; Hernandez et al., 2010).

From this study, it can be concluded (i) that polyphenol production and antioxidant activity increased with plant age, (ii) that salt stress stimulated these two parameters

in the less sensitive provenance, Soliman, and only polyphenol production in the more sensitive provenance, Tekelsa, and (iii) that after a two-week salt treatment, *M. pulegium* responded to the salt-induced oxidative stress by an activation of antioxidant enzymes, whereas after a six-week salt treatment, the less sensitive provenance increased its polyphenol production and its antiradical activity.

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