African Journal of Biotechnology Vol. 11(27), pp. 7109-7118, 3 April, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3493
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Salt effect on physiological, biochemical and anatomical structures of two *Origanum majorana* varieties (Tunisian and Canadian)

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Accepted 2 March, 2012

In this study, we evaluated the salt concentration effect on plant growth, mineral composition, antioxidant responses and anatomical structure of two varieties of *Origanum majorana* after exposure to NaCl treatment. Our results show an inclusive behaviour of the two varieties, since the majority of sodium was exported and accumulated in their aerial parts. The Canadian variety (CV) appeared relatively more tolerant to salt than the Tunisian one (TV). Transversal section of leaves showed a thickening of dorsal and ventral cuticle, more importantly in CV than in TV, in the presence and in absence of salt. This was accompanied by an increase in the length of palisade cells, and the width of spongy collenchyma lacuna. The stem had a subquadrangular shape in TV and quadrangular in the Canadian variety. At mature stage, the stem pit was reabsorbed in the TV and replaced by a large cavity, whereas it remained unchanged in CV. The relative salt tolerance of the CV was related to: (1) a good selectivity in favour of K^+: (2) a strong peroxidase activity and (3) an increase in the lengthening of palisade cell accompanied with an increase of lacunae in spongy parenchyma in CV.

Key words: ***Origanum majorana***, salinity, growth, mineral nutrition, leaves, stems, anatomical, antioxidant.

INTRODUCTION

In modern agriculture and trade, importance accorded to plants is not restricted to food, forage, and fibre, but to secondary metabolites having desired aromatic or therapeutic qualities, or providing source of material for the perfume and chemical industries (Banchio et al., 2008). Sweet marjoram (***Origanum majorana*** L.), a member of the Lamiaceae family, is an aromatic plant; of great economic and industrial importance. It is known since antiquity for its therapeutic properties (Baatour et al., 2011). Notably among all Lamiaceae species, it is used in gastronomy for its spicy herbaceous notes (Circella et al., 1995), especially in the Mediterranean culinary delights. Volatile extract of marjoram is used in pharmacology, medicine, clinical microbiology, pathology and food preservation (Dafarere et al., 2000).

Currently, besides drought, there is an expansion of salt-affected soils which cover about 10% of the total area in Tunisia (Hachicha, 2007). These two environmental constraints affect the growth, nutrition, antioxidant activities and anatomy structure of medicinal, aromatic and the majority of cultivated plants. Thus, the study of plants responses to these constraints is required. In a previous study, referring to the set of the physiological and biochemical behaviour of the marjoram observed in

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Abbreviations: CV, Canadian variety; **TV,** Tunisian variety.

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responses to the variation of NaCl concentration in the culture medium, the concentration of 75 mM was kept for the continuation of the experiment (Bâatour et al., 2010). In another study, based on fatty acid and essential oil composition, Bâatour et al. (2011) observed that at this sodium chloride concentration (75 mM), Canadian variety (CV) seemed to be more tolerant than the Tunisian variety (TV). The purpose of the present work was to confirm the tolerance of CV by studying the: (i) growth, (ii) water content (iii) mineral status (iv) enzyme assays and (v) anatomical structure.

MATERIALS AND METHODS

Canadian and Tunisian Marjoram (***O. majorana*** L.) were cultured individually in a hydroponic system containing a complete Hoagland's medium (Hoagland and Arnon, 1950) diluted eightfold in a culture chamber (16 h light/8 h dark at 22/18°C). After 20 days of acclimation, 75 mM NaCl was added to nutritive solution. The aerial parts of ***O. majorana*** were harvested after 17 days of treatment. Subsequently, the dry weight (DW) of different organs (leaves, stems and roots) was measured. Ions were extracted with 0.5% HNO_3; K^+, Na^+ and Ca^{2+} concentrations were assayed by flame photometry (Eppendorf apparatus).

Tissue ion content and ion selectivity

Major cations and chloride in dried leaf and roots materials were extracted with 0.5% HNO_3 and were assayed with flame photometry as previously described by M'rah et al. (2006). The ability of the plants to maintain tissue K^+ concentration in saline conditions is indicated as the K^+ selectivity. It is defined as the ratio of K^+ / (K^+ + Na^+) in the tissue divided by the ratio of K^+ / (K^+ + Na^+) in the external medium (Ashraf and McNeilly, 1990).

Determination of enzyme activity

Fresh leaves were homogenised with 5 ml of extraction buffer containing 50 mM K phosphate buffer, pH 7.5, 100 mM ethylenediaminetetraacetic acid (EDTA), 5% polyvinylpyrrolidone (PVP), 5% glycerol and 1 mM dithiothreitol (DTT). The homogenate was centrifuged at 15000 ***g*** for 15 min, and the supernatant fraction was used to assay various enzymes. All steps in the preparation of enzyme extracts were performed at 4°C. Protein concentrations in the enzyme extract were determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Catalase activity (CAT) was determined by monitoring the disappearance of H_2O_2 according to the method of Cakmak and Marschner (1992). The final reaction mixture contained 50 mM sodium phosphate buffer (PH 7.0) and 2% H_2O_2. The activity was expressed as units (µmol H_2O_2 consumed per minute) per mg of protein. Guaiacol peroxidase activity (GPX) according to Srinivas et al. (1999) was assayed using guaiacol as an electron donor, with a reaction mixture containing 20 mM phosphate buffer (pH 7.0) and 30 mM H_2O_2. The increase of absorbance due to tetraguaiacol formation was recorded at 470 nm. One unit of peroxidase activity catalyzes the oxidation of 1 µmol of guaiacol.

Measurement of malondialdehyde (MDA)

MDA was determined for aerial parts following a published procedure (Locquin and Langeron, 1978). Briefly, fresh tissue (0.2 g) was homogenized in 2 ml of a mixture containing 20% 2-thiobarbituric acid and 0.5% trichloroacetic acid. Extracts were incubated at 95°C for 30 min, the reaction was stopped on ice and then centrifuged at 4000 g for 30 min at 4°C, and the absorbance of the supernatant was measured at 532 and 600 nm. The MDA concentration (µmol g^{-1} FW) was calculated using a molar extinction coefficient at 532 nm (155 mM cm^{-1}). The absorption at 600 nm resulting from non specific absorption was subtracted from the optimal absorption at 532 nm.

Light microscopy

Fresh control and treated leaves (level 4) were subjected to various treatments: (1) some leaves were fixed in formaldehyde-acetic-acid (FAA), cut with a freezing microtome then stained with acetocarmine (Locquin and Langeron 1978); (2) other leaves, were fixed with 4% glutaraldehyde in sodium cacodylate buffer, and then post fixed with 1% osmium tetroxide (OsO_4) buffered with the same product, dehydrated in acetone and subsequently embedded in Spurr resin. Sections were made with a glass knife and stained with Boracic toluidine blue. These sections were observed with a Leitz Ortholux (LM) equipped with a camera.

Statistical analysis

All extractions and determinations were conducted in triplicate and data was expressed as mean ± standard deviation (SD). The means were analysed using the one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. Means were statistically compared using the STATISTICA (v 5.1) (Statsoft, 1998) program with Student's ***t***-test at the $p < 0.05$ significance level.

RESULTS

Growth and water content

Both varieties of ***O. majorana*** produced the same amount of dry weight (about 1200 mg. $plant^{-1}$) under controlled condition (Figure 1). A very important part of this biomass is allocated to the stems (about 50%) and leaves (40%). Plant biomass was significantly decreased following salt treatment for two weeks, but it was more pronounced in Tunisian variety as compared to the Canadian. Besides, the degree of sensibility decreased gradually from stems to roots. Moreover, there was a significant decrease in water content in leaves and stems of TV under salt constraint. As compared to the other organs, its roots appeared less sensitive to NaCl, whereas CV seemed insensitive to salt in all organs (Figure 2). This salt hydration insensitivity reflected a greater capacity for osmotic adjustment in plants tissues grown in the presence of salt. In addition, the effect of salt on the water content seemed more pronounced than dry biomass and can better discriminate between the two varieties in terms of their response to salt stress.

Ionic concentrations

O. majorana plants cultivated with NaCl 75 mM, absorbed

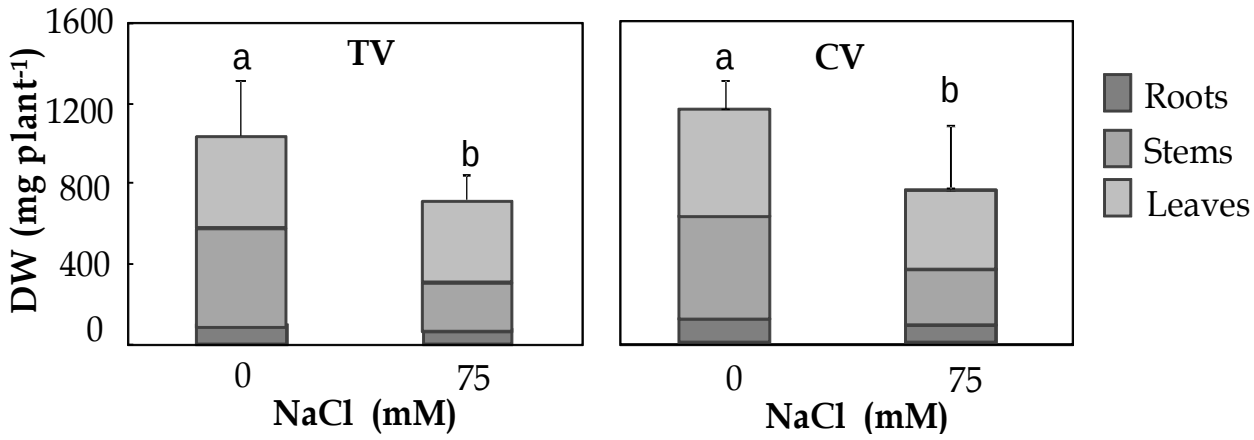

Figure 1. Dry weights (DW) in roots, stems and leaves of ***Origanum majorana*** L. plants grown over 17 days in the presence of 75 mM NaCl concentrations. Data represent the means of 8 replicates ± standard error at P ≤ 5%.

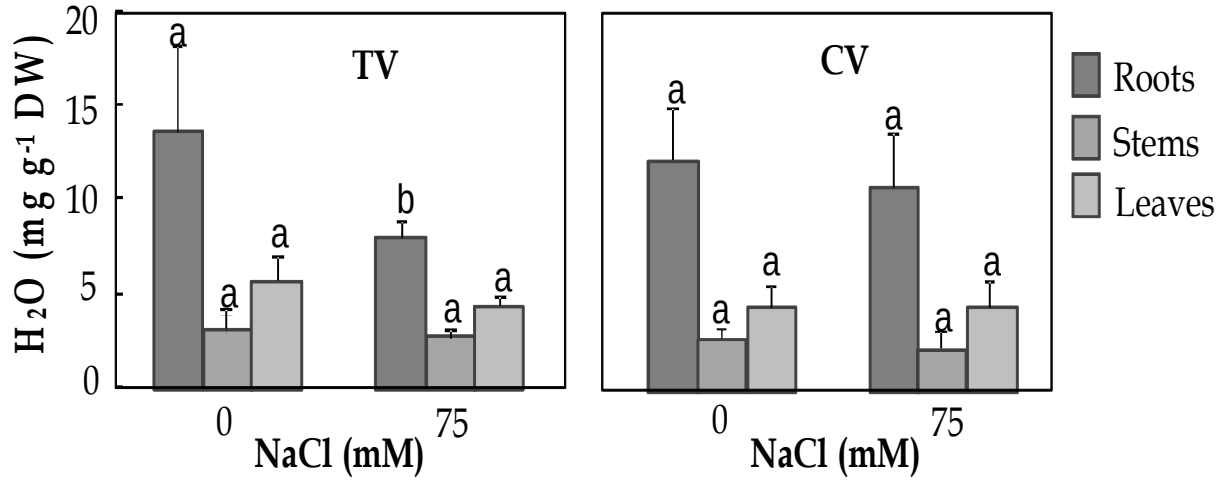

Figure 2. Tissue hydration in roots, stems and leaves of ***Origanum majorana*** L. plants grown over 17 days in the presence of 75 mM NaCl concentrations. Means of 8 replicates ± standard error at P ≤ 5%.

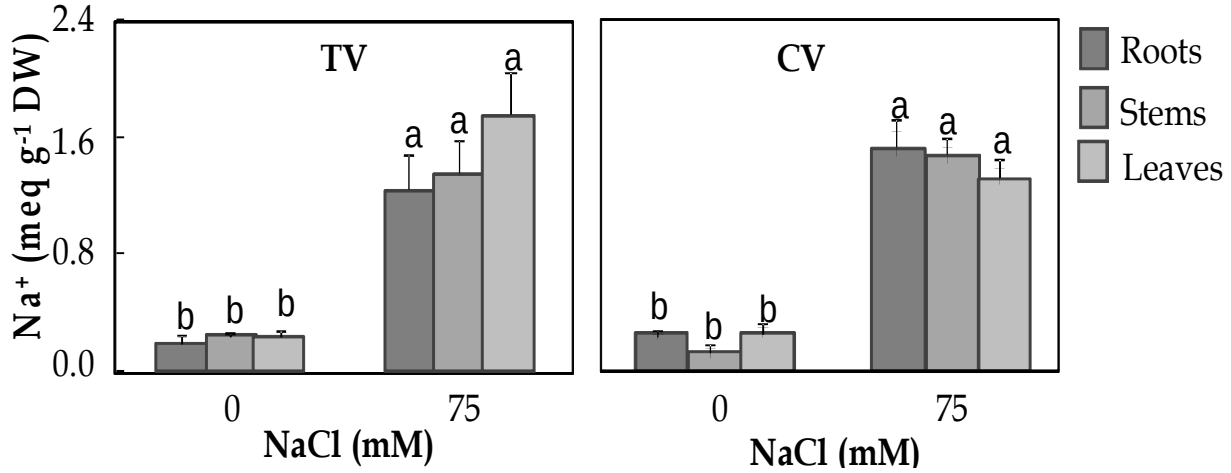

Figure 3. Na^+ concentrations in roots, stems and leaves of ***Origanum majorana*** L. plants grown over 17 days in the presence of 75 mM NaCl concentrations. Data represent the means of 8 replicates ± standard error at P ≤ 5%.

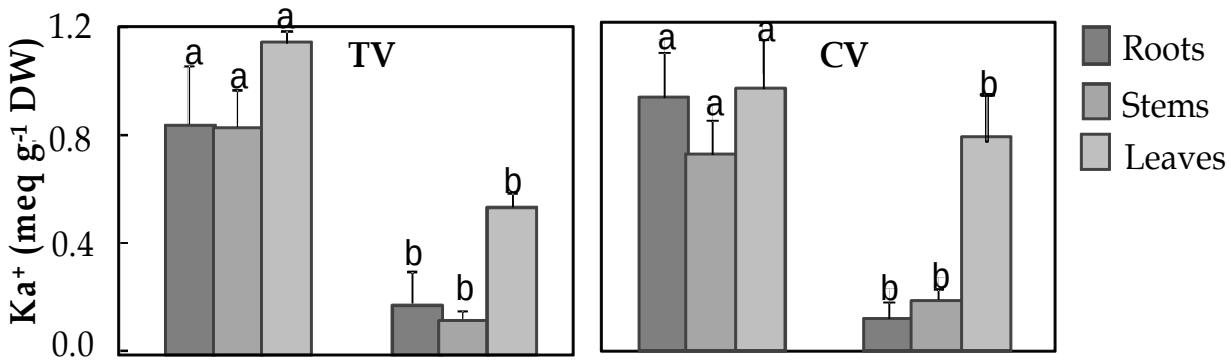

Figure 4. K^+ concentrations in roots, stems and leaves of ***Origanum majorana*** L. plants grown over 17 days in the presence of 75 mM NaCl concentrations. Data represent the means of 8 replicates ± standard error at P ≤ 5%.

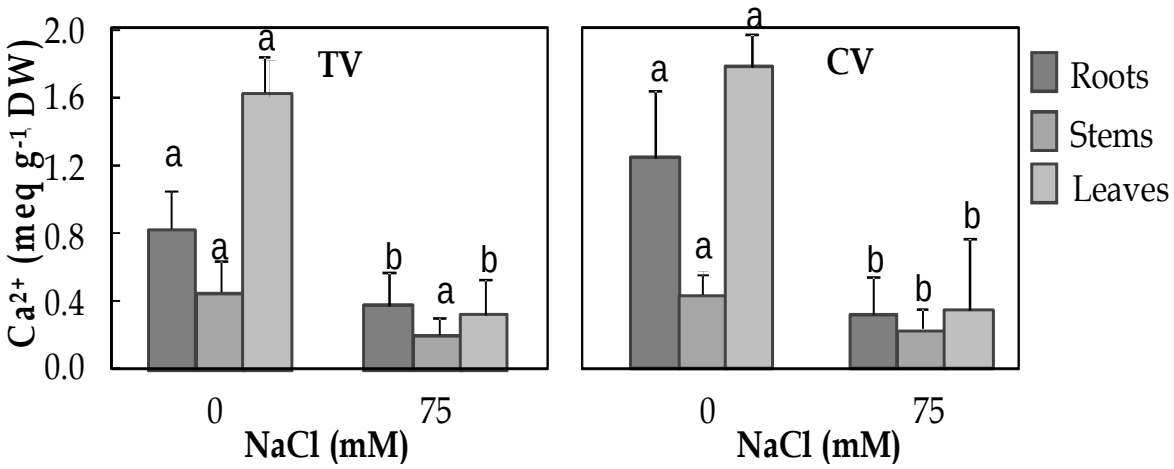

Figure 5. Ca^{2+} concentrations in roots, stems and leaves of ***Origanum majorana*** L. plants grown over 17 days in the presence of 75 mM NaCl concentrations. Data represent the means of 8 replicates ± standard error at P ≤ 5%.

and accumulated sodium in their different organs (Figure 3). However, the leaves with sodium accumulation were higher in Tunisian variety (about 1.8 meq.g^{-1}DW) against only (1.2 meq.g^{-1} DW) in their roots. Conversely, in CV, the Na^+ accumulation reached comparable levels in all the three organs (about 1.5 meq^{-1}. g^{-1} DW). This result suggests that CV has the ability to control Na^+ absorption and transport it from roots to shoots parts. Under control conditions, both marjoram varieties have the same K^+ content in their different organs (leaves, stems and roots) (Figure 3). Salt treatment significantly decreased K^+ accumulation in the three organs of TV, but only in the roots and stems in CV. A decrease of K^+ accumulation about 50% in TV leaves was correlated with a greater accumulation of Na^+.

Calcium plays an important role in plant tissues regulating the function of Na^+ and K^+ (Cachorro et al., 1994; Grattan and Grieve, 1993). In saline soils with abundant Ca^{2+}, deficiency arises due to the competitive effects of K^+ / Na^+ selectivity that were associated with plant salt tolerance (Ashraf and Naqvi, 1991). In this experiment, the effect of salt stress on Ca^{2+} contents of the different organs (leaves, stems and roots) were illustrated in Figure 5. Ca^{2+} accumulation in shoots (leaves and stems) were similar in the two varieties. In contrast, roots Ca^{2+} accumulation were higher in Canadian variety compared with the Tunisian variety, although NaCl decreased significantly the content of Ca^{2+} in different organs of both varieties. In fact, it showed a strong inhibition of absorption and transport of calcium which would be due to Na^+ competition. Sodium reduced Ca^{2+} influx by binding it to the plasma membrane, inhibiting influx and increasing efflux of Ca^{2+} by depleting the internal Ca^{2+} concentration (Cramer et al., 1989).

Our results also show that the Canadian variety had the best performance (better growth) in salt constraint due to a better selectivity for K^+ in their leaves compared with Tunisian one (Figure 6). However, disturbances of potassium nutrition induced by salt treatment were not very remarkable. In fact, it is probable to suggest that the

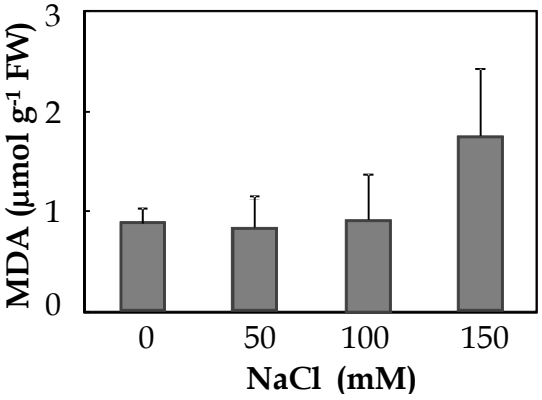

Figure 6. MDA content in leaves of ***Origanum majorana*** L. plants grown over 17 days in the presence of 75 mM NaCl concentrations. Data represent the means of 8 replicates ± standard error at P ≤ 5%.

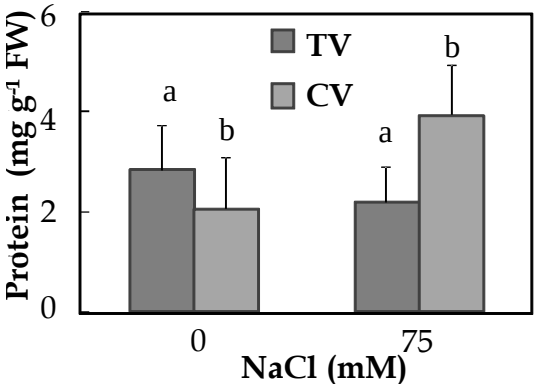

Figure 7. The total protein content in leaves of ***Origanum majorana*** L. plants grown over 17 days in the presence of 75 mM NaCl concentrations. Data represent the means of 8 replicates ± standard error at P ≤ 5%.

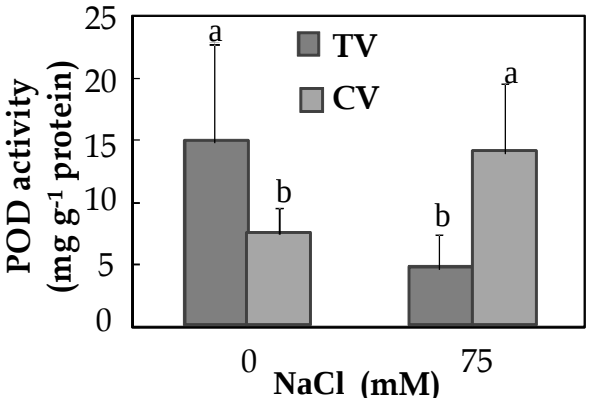

Figure 8. Guaiacol peroxidase (POD) activity in leaves of ***Origanum majorana*** L. plants grown over 17 days in the presence of 75 mM NaCl concentrations. Data represent the means of 8 replicates ± standard error at P ≤ 5%.

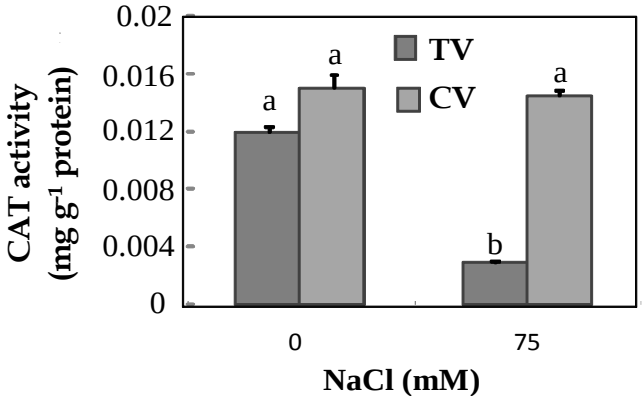

Figure 9. Catalase activity (CAT) in leaves of ***Origanum majorana*** L. plants grown over 17 days in the presence of 75 mM NaCl concentrations. Data represent the means of 8 replicates ± standard error at P ≤ 5%.

inhibition of ***O. majorana*** L growth was due to Ca^{2+}limitation of provision.

Membrane integrity

Membrane lipid peroxidation resulted in elevated levels of MDA, a generic biomarker for membrane damage (Elkahoui et al., 2005). The leaves MDA content was much higher in CV (about 0.92 µmol g^{-1} FW) than in TV (Figure 6). NaCl treatment reduced and stimulated the MDA content by 42 and 29%, respectively, in Canadian and Tunisian varieties.

Total protein and antioxidant enzyme activities

Leaves protein content of both ***O. majorana*** varieties are represented in Figure 7. The results show that NaCl increased the protein content in the CV and decreased those in the Tunisian variety. GPX and CAT are the major antioxidant enzymes associated with scavenging active oxygen species (ROS) (Marschner, 1995). Therefore, to determine the response of ***O. majorana*** to salt-induced oxidative stress, GPX and CAT activities were measured in leaves treated with or without 75 mM NaCl. Our results (Figure 8) show that GPX activity was increased in the presence of salt in CV, whereas it decreased significantly in TV. This response could explain the protective leaves response in CV against the accumulation of reactive oxygen species. In the absence of salt, the highest catalase activity was observed in CV leaves (0.016 mg g^{-1} FW) than Tunisian variety (Figure 9). NaCl treatment, tended to decrease catalase activity by three-fold in TV, but it remained constant in Canadian variety.

Anatomical structure

Cross sections of leaves and stems were investigated in

Table 1. Extent of different leaf tissues in Canadian variety in the absence and presence of 75 mM NaCl in µM.

NaCl (mM)	Cu d	Cu v	Epd	Epv	PL	PP	L (PP)	l (PP)	Co	Ep F
CV (0)	0.22[a]	0.12[a]	0.25[a]	0.6[a]	0.24[b]	0.6[a]	0.6[b]	0.1[b]	0.11[b]	2.84[a]
CV (75)	0.13[b]	0.10[a]	0.15[b]	0.20[b]	0.27[a]	0.7[a]	0.83[a]	0.15[a]	0.2[a]	2.73[b]

CV, Canadian variety; Cu d, dorsal cuticule; Cu v, ventral cuticule; Epd, dorsal epidermis; Epv, ventral epidermis; PL, spongy parenchyma; PP, palisade parenchyma; L, length ; l, widh; Co, collenchyma; Ep F, thickness of the vessel.

Table 2. Extent of different leaf tissues in Tunisian variety in the absence and presence of 75 mM NaCl in µM.

NaCl (mM)	Cu d	Cu v	Epd	Epv	PL	PP	L (PP)	l (PP)	Co	Ep F
TV (0)	0.056[b]	0.06[a]	0.17[b]	0.36[a]	1.2[a]	1.4[b]	0.4[b]	0.2[a]	0.14[b]	3.99[a]
TV (75)	0.1[a]	0.054[a]	0.18[a]	0.35[b]	1.3[a]	1.5[a]	0.6[a]	0.15[b]	0.53[a]	3.76[a]

TV, Tunisian variety; Cu d, dorsal cuticule; Cu v, ventral cuticule; Epd, dorsal epidermis; Epv, ventral epidermis; PL, spongy parenchyma; PP, palisade parenchyma; L, length; l, width; Co, collenchyma; EpF, thickness of the vessel.

Table 3. Extent of different stem tissues in Canadian variety in the absence and presence of 75 mM NaCl in mM.

Variety	Cu	Ep	Co	PC	MO	T
CV (0)	0.11[a]	0.99[b]	1.00[a]	0.53[a]	0.92[a]	0.60[b]
CV (75)	0.04[b]	1.00[a]	0.08[b]	0.17	0.30[b]	0.83[a]

CV, Canadian variety; Cu, cuticule; Ep, epidermis; PC, cortical parenchyma; Co, collenchyma; T, entire stem.

detail. Our findings show that both leaves varieties were bifacial covered by a thick cuticle on the upper and lower surfaces, and followed by a single layered epidermis and three or four layered palisade. Spongy parenchyma cells are lined-up and occupy a wide area in the mesophyll. Vascular bundles were surrounded by a sheath of parenchyma cells. Upper and lower parts of central vessel were surrounded by collenchymatous cells (Figures 1 and 2). The ventral epidermal cells were larger than dorsal ones in CV (Table 1) and TV (Table 2), at the control medium. The treated leaves were characterized by a spongy parenchyma with small lacunae. In CV, salt induced an increase of lengthening of palisade cell and lacunae in spongy parenchyma as shown in Figure 1A and Table 1. However, in the Tunisian variety, leaf thickness remained substantially unchanged due to an increase in the size of the dorsal epidermal cells associated with elongation of palisade parenchyma cells. In addition, salt induced thickening of the cuticle covering epidermis (Figures 2A and B; Table 2).

Stems

For the control, cross sections showed that Tunisian variety (Figure 3A) had a less large diameter than the Canadian one (Figure 3E). It consisted of a unilayered epidermis (Ep) with more or less rounded cells, covered by a thick cuticle in both varieties. Under the epidermis lied a unilayered collenchyma (Co) all around the stem, but thicker at the corners where it was formed by two to three layers. Cortical parenchyma (CP) was of meatus type, composed of three to four layers of cells with a thin pectocellulosique wall. In the vascular cylinder, vascular system showed primary structure. The phloem (Ph) was surrounded by the side, by an arc composed of fibres (F) more numerous in the Tunisia variety (Figure 3B). Between the phloem and xylem, there was a cambial zone (CZ) which extended laterally in the interfascicular regions. The development of the cambial zone was more advanced in the Tunisian variety (*). The centre of the stem was occupied by a pith (P) with large cells; larger at the Canadian variety (Table. 3). Ripe stem diameter increased in the two varieties (Figures 3D and H), by the increase of the amount of vascular tissues. It became subquadrangular in the Tunisian variety (Figure 3C) and quadrangular in the Canadian variety (Figure 3G).

In both varieties (Figures 3D and H), collenchyma were layered (seven to eight cells) at the corners, and sclerenchymatic in the Canadian variety (Figures 3G and H). Conducting tissues form a continuous vascular cylinder, where the wood (W) is the dominant element. They are surrounded externally by a layer of cells (+ +, Figure 3D and H), distinguished by their large size (they are larger in the Tunisian variety and with sclerenchymatic wall). The pith in the Canadian variety, is reduced to a diamond-shaped area (P, Figures 3G and H), but in the Tunisian variety, its cells are absorbed in

Table 4. Extent of different leaf tissues in Tunisian variety in the absence and presence of 75 mM NaCl in mM.

NaCl (mM)	Cu	Ep	Co	PC	MO	T
TV (0)	0.039	0.08	0.098	0.158	0.29	0.4
T V (75)	0.041	0.97	0.12	0.140	0.24	**0.6**

TV, Tunisian variety; Cu cuticule; Ep, epidermis; PC, cortical parenchyma; Co, collenchyma; T, entire stem.

their place to form a central gap (P, Figures 3C and D).

Salt induced a decrease in stem diameter (Figures 3A and C) due to a reduction in cortical parenchyma cells (Pc) in both varieties. Besides, we observed an increase and a decrease respectively, in the thickness of the Ep and the light of the Co (Figures 4B and D), In the Canadian variety, we also observed a decrease of the pit (P) (Figures 4A and B), vessels size associated and a thickening of F above the Ph (Tables 3 and 4).

DISCUSSION

The ability of the two varieties to maintain their hydration status at 75 mM NaCl could be due to their osmotic adjustment capacity as is the case of wheat (Bouslama et al., 2004). However, the decrease in biomass depending on the severity of stress could be an adaptive strategy. Indeed, plant reduces its exchange surface with the external environment in order to conserve a better water content. In aromatic and medicinal plants, recent studies emphasized sensitivity to salt stress; illustrated by a decrease in growth, such as in ***Mentha pulegium*** (Oueslati et al., 2010), an halophyte behavior in ***Sesuvium portulacastrum*** (Messeddi et al., 2004) or by a reduction in the biomass production by 25 and 38% as compared to control plants at 50 and 75 mM NaCl, respectively (Ben Taarit et al., 2010). Similarly, Hendawy and Khalid (2005) found a significant decrease in sage dry weight at 50 mM NaCl ranging between 34 and 48%. A growth reduction by salt constraint is considered by many authors as critical for discrimination between species or cultivars, tolerant and sensitive (Paradossi et al., 1999; Royo and Aragüés, 1999). Our findings suggest that CV was less sensitive to salt than TV at a moderate NaCl concentration.

Besides, as in a ***Thellungiella halophila*** (M'rah et al., 2006), a competition in absorption and transport of (K^+/Na^+) from the roots to aerial parts was found in variety CV. According to Kaddour et al. (2009), a good selectivity for K^+ is necessary to maintain better plant growth on saline medium. Indeed, a law biomass production due to Na^+ competition with K^+ for uptake and transport causes a deficiency of potassium (Shiyab et al., 2003). Since potassium as suggested by Ashraf and Orooj, (2006) and Oueslati et al. (2010) is one of the most growth-limiting factors under saline conditions, salinity at some extend can induce ROS, which may be reduced by antioxidant enzymes. GPX and CAT are the major antioxidant enzymes associated with scavenging ROS (Marschner 1995). In CV, NaCl constraint increased GPX and CAT activities, whereas it decreased it significantly in TV. This increase in GPX was also observed in ***M. pulegium*** (Oueslati et al., 2010) and has been previously described in cotton cultivars (Meloni et al., 2002); in this latter, peroxidase activity was stimulated by 76 and 94% of the control, at 100 and 200 mM NaCl respectively.

MDA is regarded as a marker for evaluation of lipid peroxidation or damage to plasmalemma and organelle membranes that increases with environmental stresses. Our findings indicate a lower degree of membrane damage observed in CV, which is indicated by low MDA content and high peroxidase activity. According to their results, Demiral and Turkan (2005), showed that the lowest MDA content is correlated with salt tolerance in resistant cultivar of rice, compared with the susceptible cultivar IR28. Similarly, lettuce Verte variety was more tolerant than Romaine on by limiting the accumulation of MDA and enhancing the accumulation of antioxidant enzymes (Mahmoudi et al., 2010). Sreenivasulu (1999) showed that a high peroxidase activity associated to a low MDA content contribute to better salt tolerance.

In order to confirm and better explain the tolerance of CV as compared to TV, we carried out a study on the anatomical structures of leaves and stems of these two varieties. The marjoram leaves structures were similar to ***Origanum onites*** (Gonuz et al., 1999), but it differed from ***Origanum vulgare*** (Romanes et al., 2008). Stem has a subquadrangular shape in TV and quadrangular in the Canadian variety. Our findings in CV was similar to ***O. onites*** L., characterized by quadrangular stem (Gonuz et al., 1999). The stem of ***O. vulgare*** L. plants presented a quadratic contour to in transverse section, with four (more or less prominent in its upper third and attenuated in the rest of its length) (Romanes et al., 2008). According to Kofidis et al. (2003), the combined effects of altitude and season on growth of oregano showed that the increasing elevation resulted in a progressive decrease of plant height, while during the growing period, plants in autumn are some how shorter than plants in summer. A comparatively histo-anatomical observations at the spontaneous ***O. vulgare*** L. testify the fact that the thickness of the aerial stem (resulting especially from the development of the pith) progressively decrease at once with the increase of the altitude where the plants grow (Romanes et al., 2008). In the same time, our comparatively study reveals

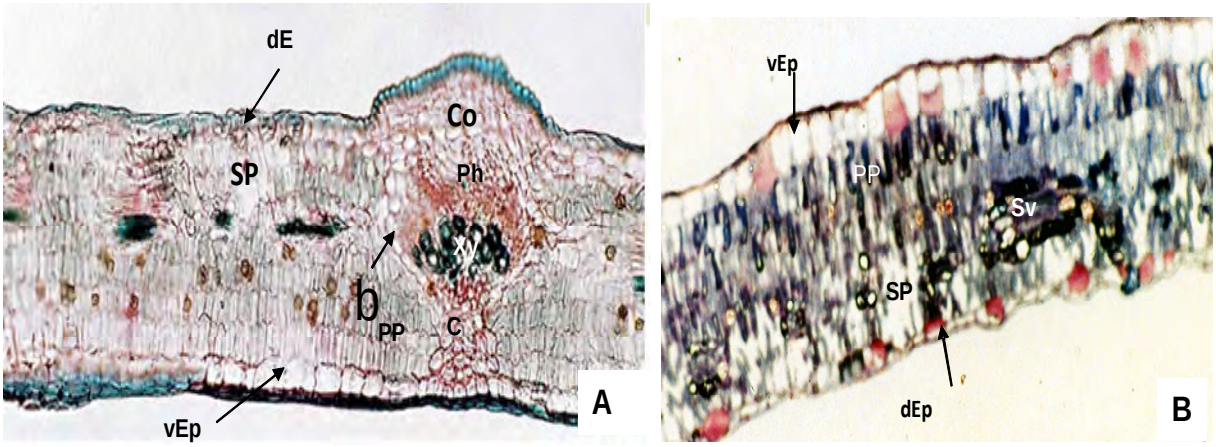

Figure 10. Anatomical structure of the control (A) and treated leaves (B) of the Canadian variety (G* 500), A, section fixed in FAA and stained with aceto-carmine. B, section fixed in 4% glutaraldehyde buffered with Na-cacodylate, post fixed with et post 1% OsO_4 buffered with the same buffer and then stained with boric toluidine blue. Epd, Dorsal epidermis; Epv, ventral epidermis; Co, collenchyma; PP, palisade parenchyma; PL, spongy parenchyma; NS, small vein; G, bundle-sheath; FAA, formaldehyde-acetic-acid.

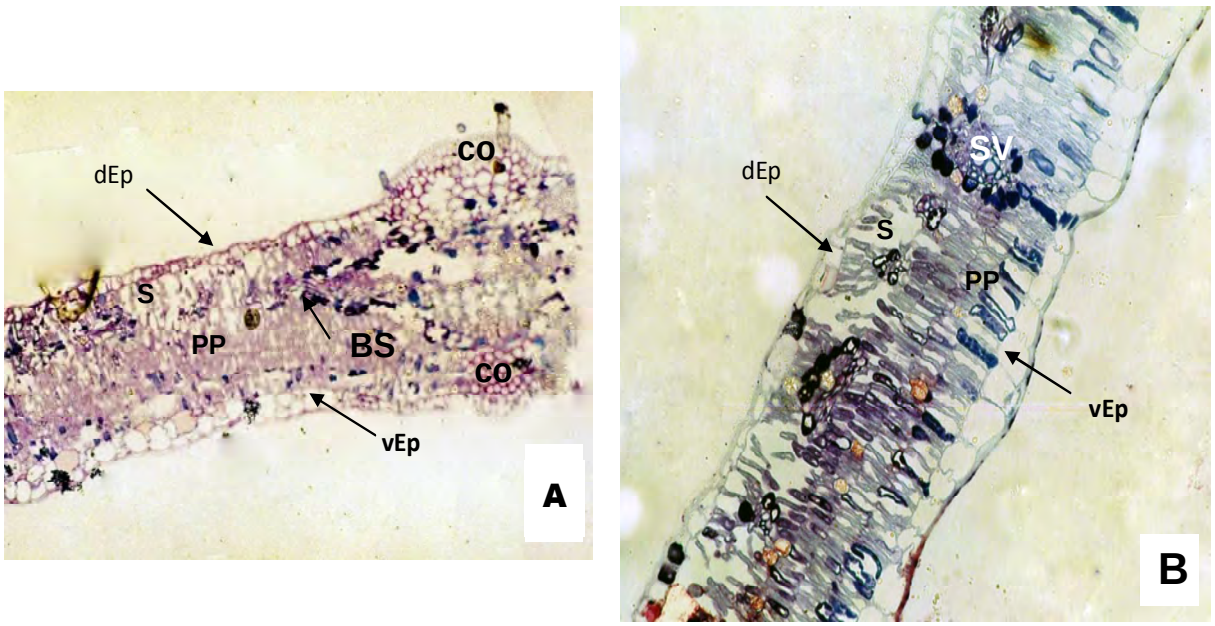

Figure 11. Anatomical structure of the control (A) and the treated leaves (B) of Tunisian variety (G* 500). Sections were fixed in 4% glutaraldehyde buffered with Na-cacodylate, then post fixed with et post 1% OsO_4 buffered with the same buffer and stained with toluidine blue boric. dEp, Dorsal epidermis; vEp, ventral epidermis; Co, collenchyma; PP, palisade parenchyma; SP, spongy parenchyma; SV, small vein; G, bundle-sheath.

some anatomical differences of the stem and leaves which discriminate between the both varieties of ***Origanum majorana*** L. (Figures 10 to 13).

Conclusion

Our results demonstrate that the two varieties, CV and TV, responded differentially to NaCl treatment. The CV, however, accomplished the followings: i) maintained organs hydration under saline condition; ii) maintained its K^+ status in leaves; iii) restricted the accumulation of Na^+ in its aerial parts; IV) maintained a high selectivity in favour of K^+, which was demonstrated by limiting the MDA accumulation and enhancing the peroxidase activity; V) had thicker ventral and dorsal cuticle in the absence or presence of salt and increase in the lengthening of palisade cell accompanied by an increase

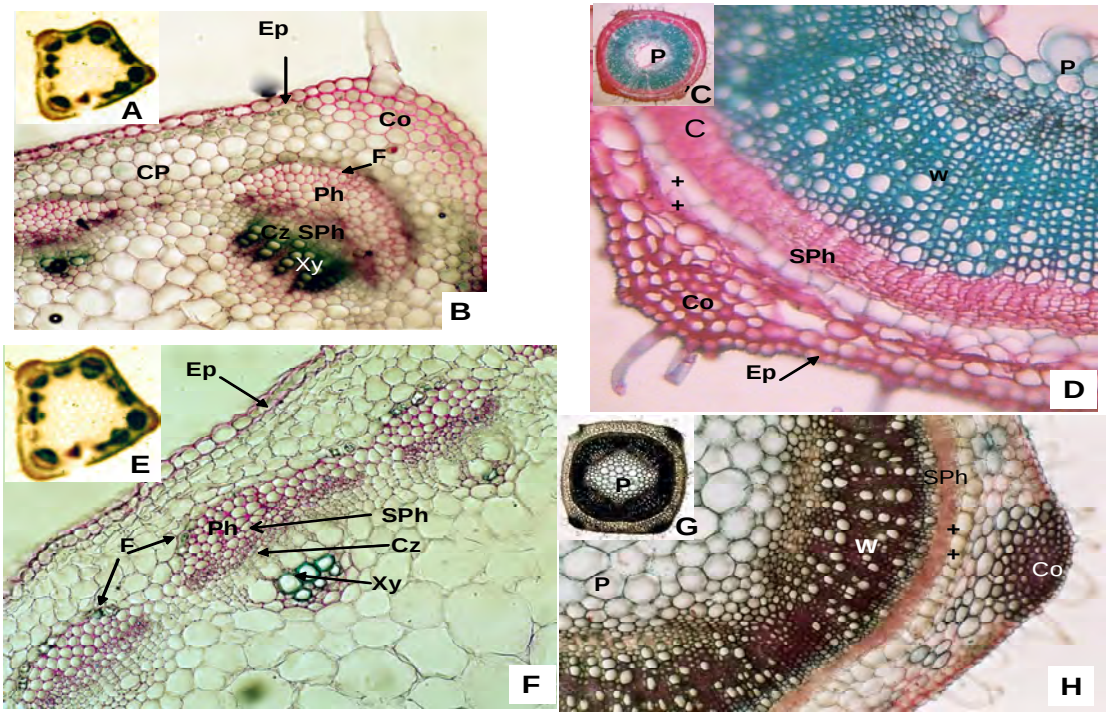

Figure 12. Control stem of the two varieties (A - D Tunisian variety, E - H, Canadian variety). A and E, overview of young control stems cross-section, G×38; B and F, detail of portions from Figures A - E respectively, showing their anatomical structures, G*400; C to G, overviews of ripe stems cross-sectional, G×38; D - H, detail of portions from Figures C and D respectively, showing their anatomical structures, G*270. Sections fixed in FAA and stained with aceto-carmine. Ep, Epidermis; CZ, cambial zone; Xy, xylem; Ph, phloem; SPh, secondary phloem; P, pith, w, wood; Co, collenchymas; FAA, formaldehyde-acetic-acid.

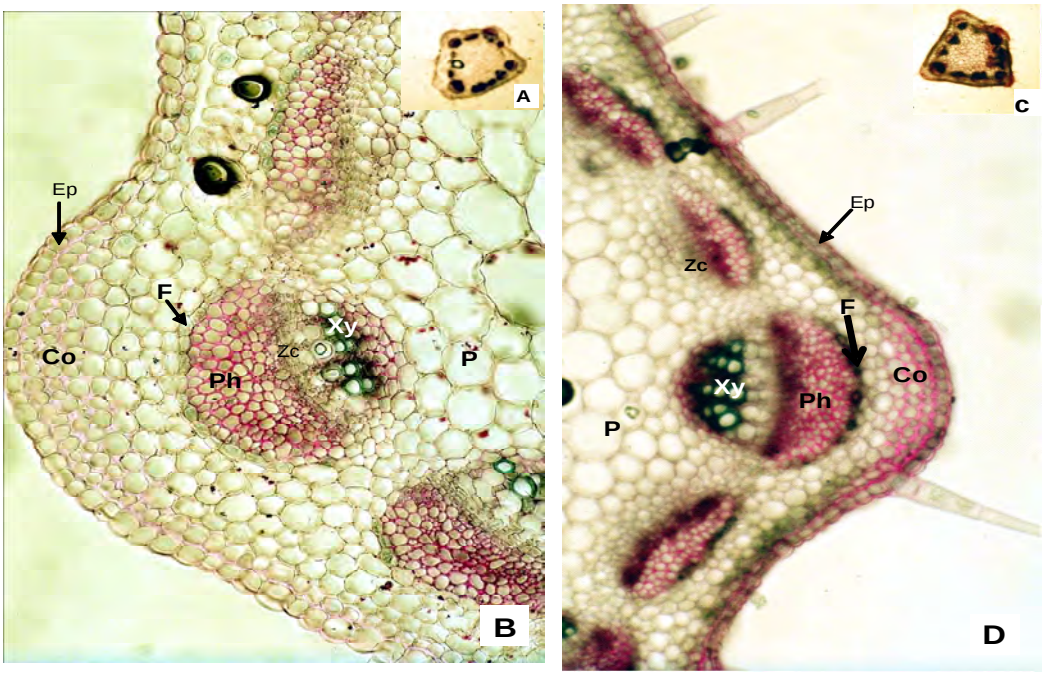

Figure 13. Transverse sections of treated stems. A and B, Canadian variety; C and D, Tunisian variety; A and C, overview of cross-sectional stems, G*20; B and D, details of a portion of the stem showing its anatomical structure, G*750. In A to D, sections were fixed in FAA and stained with aceto-carmine. FAA, Formaldehyde-acetic-acid.

of lacunae in spongy parenchyma.

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