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Full Length Research Paper

Essential oil yield and trichomes structure in two sweet marjoram (*origanum majorana* L.) varieties under salt stress

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***Origanum majorana* L. shoots were investigated for their essential oil (EO) yield, composition and the structures responsible for its biosynthesis. Shoots EO was extracted by hydrodistillation and composition was determined by gas chromatography–mass spectrometry (GC-MS) method. Fresh Leaves were observed by light microscopy (LM). Plants were cultivated for 17 days in a basal medium supplemented with 75 mM NaCl. Salt decreased the essential oil yield in Tunisian variety (TV), but an increase was shown in Canadian variety (CV). Salt constraint induced a change in EO chemotype of two varieties. Their anatomical study showed three types of trichomes: (i) non-glandular, simple hairs; (ii) small, capitate glandular trichomes; (iii) and peltate glandular trichomes. Non-glandular trichomes provided of three types at the TV against two at the Canadian one at control. Indeed, with TV we observed; multicellular (M), bicellular (B) and unicellular (U) trichomes. Bicellular trichomes (BT) were observed in CV, and multicellular (MT) and bicellular (UT) in TV at salt constraint. The increase and the decrease of EO yield in CV and TV was respectively due to the presence of three type of EO secretion which seemed to make defect in Tunisian one.**

Key words: Trichomes, non-glandular, capitate, peltate, glandular, salt, chemotype.

INTRODUCTION

Salinity is one of the most important environmental stresses which affected nearly half of the irrigated surface (Flagella et al., 2002). Moreover, in irrigated areas, the bad quality of irrigation water charged with dissolved salts has resulted unfortun6ately in soil secondary salinization responsible for decreases in productivity (Mtimet, 2001). It limits crop productivity by decreasing the water potential of the root medium, the ion toxicity due to excessive sodium and chloride uptake, and the nutrient ion imbalance by the disturbance of essential intracellular ion concentrations (Greenway and Munns, 1980). Salinity on medicinal and aromatic plants can affect plant growth (Ashraf and Orooj, 2006), morphological, physiological, biochemical processes, anatomical structures (Tester and Davenport, 2003) and essential oil biosynthesis and secretions (Heuer et al., 2002). One of the most common medicinal and aromatic species is sweet marjoram (*Origanum majorana* L. Syn. *Majorana hortensis*), it is an herbaceous, perennial plant, native of Cyprus and the Eastern Mediterranean (Novak et al., 2000). It's a most appreciate herb for its essential oil used in perfumery and for its spicy herbaceous notes. It is used as fungicides or insecticides in pharmaceutical and industrial products (Vera and Chane-Ming, 1999). As all Lamiaceae species, it has glandular trichomes responsible for biosynthesis, secretion and accumulation of essential oil (McCaskill et al., 1992; Serrato-Valenti et al., 1997).

In our present study, we prompt to determinate the

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correlation between trichomes anatomy structures and essential oil yield of two varieties of *O. majorana* areal parts and to evaluate their exudates under saline and nom saline conditions.

MATERIALS AND METHODS

Plant material

At 6 leaves stage, plants from two varieties of *O. majorana* originating from Canada and Tunisia were transferred into eight-strength Hoagland's (1950) nutrient solution (1.25 mM KNO_3, 1.25 mM $Ca(NO_3)_2$ $4H_2O$, 0.50 mM $MgSO_4$ $7H_2O$, 0.25 mM KH_2PO_4, 0.01 mM H_3BO_3, 0.001 mM $MnSO_4$ $4H_2O$, 0.0005 mM $CuSO_4$ $5H_2O$, 0.0005 mM $ZnSO_4$ $6H_2O$, and 0.00005 mM $(NH_4)_6Mo_7O_{24}$ $4H_2O$), in a culture chambers with a 16 h photoperiod (150 $\mu mol.m^{-2}.s^{-1}$). Temperature and average relative moisture are respectively 22°C and 40% during the day and 18°C and 86% at the night. After 20 days of acclimation, 75 mM NaCl was added to nutritive solution. The areal parts of *O. majorana* were harvested 17 days later.

Essential oil isolation identification and quantification

Essential oil (EO) was extracted by hydrodistillation of fresh shoots (50 g) during 90 min according to Msaada et al. (2007). The distillate was submitted to a liquid–liquid extraction with diethyl ether and the organic phase was concentrated at 35°C using a Vigreux column. In order to quantify EO and its constituents, 6-methyl-5-hepten-2-one was used as an internal standard. Essential oils obtained were stored at -20°C prior to analysis. Each extraction was made in triplicate.

Gas chromatography-flame ionization detector (GC-FID)

Gas chromatography analysis was carried out on a Hewlett–Packard (HP) 6890 gas chromatograph equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. A polar HP Innowax (PEG) column and an apolar HP-5 column (30 m × 0.25 mm, 0.25 µm film thickness) were used. The nitrogen carrier gas flow rate was 1.6 ml min^{-1} and the split ratio was 60:1. EO analysis was performed using the following temperature program: oven at 35°C for 10 min, from 35 to 205°C at the rate of 3°C min^{-1} and isotherm at 225°C for 10 min. Injector and detector temperatures were held, respectively, at 250 and 300°C.

GC–MS

GC–MS analysis was performed on a gas chromatograph HP 5890 (II) interfaced with a HP 5972 mass spectrometer with electron impact ionization (70 eV). A HP-5MS capillary column (30 m ×0.25 mm, 0.25 µm film thickness) was used. The column temperature was programmed to rise from 50 to 240°C at a rate of 5°C min^{-1}. The carrier gas was helium with a flow rate of 1.2 ml min^{-1}; split ratio was 60:1. Scan time and mass range were 1 s and 40 to 300 m/z, respectively.

Light microscopy

Fresh control and treated leaves (level 4) were gathered from the growing plants. They were divided in to three plots. Leaves of the first plot were viewed on their ventral surface to show trichomes distribution. From the second plot, upper epidermis layer was removed from a scalpel-cut leaf fragment and immediately mounted in glycerined water. The third plot was cut by a freezing microtome and stained with aceto-carmin (Locquin and Langeron, 1978). Freezing and fresh leaves were observed under a Leitz Ortholux (LM) equipped with a camera.

Statistical analysis

All extractions and determinations were conducted in triplicate. Data were expressed as means ± S.D. Means were statistically compared using the STATISTICA (v 5.1) 1998 program with Student's t-test at the $p < 0.05$ significance level.

RESULTS

Essential oil yield and compound amounts

The essential oil (EO) yield in the two marjoram varieties CV and TV shoots were 0.19% (w/w) and 0.11% in the control, based on dry weight respectively. At 75 mM NaCl, this yield increased by 15% in CV, whereas a decrease by 40% was observed in TV (Table 1). At the control medium, twenty-five and thirty eight compounds were identified in *O. majorana* shoots and listed in Table 1. Cis-sabinene hydrate (11.44 µg/g DW) was the major compound, followed by terpinene 4-ol (7.01 µg/g DW) in TV. The main compound in CV was sabinene (13.10 µg/g DW) followed by trans-sabinene hydrate (6.64 µg/g DW) and terpinene-4-ol (3.84 µg/g DW).

Salinity had a significant effect on the content of these constituents. In TV, the cis-sabinene hydrate and terpinene-4 ol contents decreased respectively by 38. 2 time folds in the presence of NaCl 75 mM. But, we showed an increase by 5 times folds in sabinene. Salinity increased evidently the content of sabinene, trans-sabinene hydrate and terpinene-4-ol by 2, 16 and 6 times folds, respectively in CV. Thus, the chemotype was sabinene/trans-sabinene hydrate and cis-sabinene hydrate/terpinene-4-ol respectively in CV and TV. Under saline conditions, the chemotype changed to trans-sabinene hydrate/terpinene-4-ol and sabinene/trans-sabinene hydrate, in CV and TV, respectively (Table 1). TV shoots EO, was primarily made up of monoterpene hydrocarbons (41.36 µg/g DW) represented by sabinene (Table 2). The oxygenated monoterpenes (11.30 µg/g DW) were the second aim class and contained trans-sabinene hydrate and cis sabinene hydrate. But, the main classes in CV were monoterpenes hydrocarbons (26.79 µg/g DW) and oxygenated monoterpenes (151.13 µg/g DW). At NaCl 75 mM, these chemical classes decreased significantly in TV and increased in CV (Table 2).

Non glandular trichomes

Non glandular trichomes were distributed over all the ventral faces of leaves of the both varieties (Figure 1A and B, double arrows); but they were denser and more concentrated around leaf veins (*, Figure 1A and B).

Table 1. ANOVA analysis and essential oil amount (µg/g DW) of Canadian and Tunisian variety shoots cultured under NaCl 75 mM (means of 3 replicates).

				NaCl (mM)				
				TV		CV		
No.	Compounds	RI[a]	RI[b]	0	75	0	75	Identification
	Essential oil yield (% DW)			**0.11**[a]	**0.07**[b]	**0.19**[b]	**0.22**[a]	
1	Ni	Nd	Nd	Nd	Nd	0.71[a]	nd	GC-MS
2	tricyclene	927	1014	0.83[a]	0.25[b]	3.57[a]	0.83[b]	GC-MS
3	α pinene	931	1035	0.19[a]	nd	0.39[b]	0.83[a]	GC-MS
4	Ni	Nd	Nd	Nd	Nd	0.15[a]	nd	GC-MS
5	α thujene	939	1032	Nd	Nd	0.14[b]	0.82[a]	GC-MS
6	sabinene	976	1132	1.54[b]	7.72[a]	13.10[b]	27.25[a]	GC-MS
7	Δ-3-carene	1011	1159	0.138[a]	0.234[a]	0.12[b]	2.24[a]	GC-MS
8	myrcene	991	1174	Nd	037[a]	0.06[b]	0.47[a]	GC-MS
9	α –phellandrene	1006	1176	0.11[a]	0.19[a]	0.26[b]	1.76[a]	GC-MS
10	α terpinene	1016	1188	Nd	Nd	0.34[a]	0.49[a]	GC-MS
11	Limonene	1030	1203	0.094[a]	0.059[a]	Nd	0.66[a]	GC-MS
12	1.8 cineole	1033	1213	Nd	0.05[a]	Nd	0.35[a]	GC-MS
13	γ-terpinene	1062	1266	0.55[a]	0.42[a]	0.11	3.02[a]	GC-MS
14	heptane-2-one	892	1.264	Nd	Nd	0.55[a]	0.15[b]	GC-MS
15	p-cymene	1026	1280	Nd	Nd	Nd	0.32[a]	GC-MS
16	cyclohexanol	899	1296	Nd	Nd	0.12[b]	0.80[a]	GC-MS
17	terpinolene	1088	1290	0.123[a]	0.091[b]	0.71[a]	Nd	GC-MS
18	cis-p-menth-2 -1-ol	1129	1562	0.083[a]	nd	Nd	Nd	GC-MS
19	trans-p-menth-2 -1-ol	1146	1586	2.36[a]	0.96[b]	Nd	Nd	GC-MS
20	tridecane	1300	1300	Nd	Nd	2.14[a]	0.28[b]	GC-MS
21	linalool	1098	1553	0.07[a]	0.05[a]	1.11[a]	0.29[b]	GC-MS
22	Ni	Ni	Ni	Nd	Nd	0.20[a]	nd	GC-MS
23	Ni	Ni	Ni	Nd	Nd	0.06[b]	nd	GC-MS
24	cis sabinene hydrate	1082	1556	11.44[a]	0.30[b]	1.30[b]	17.96[a]	GC-MS
25	Ni	Ni	Ni	Nd	Nd	0.35[b]	nd	GC-MS
26	trans sabinene hydrate	1053	1474	1.65[b]	4.85[a]	6.64[b]	95.48[a]	GC-MS
27	linalyl d'acetate	1257	1556	0.74[a]	0.07[b]	0.77[b]	5.82[a]	GC-MS
28	bornyl d'acetate	1295	1597	0.07[a]	Nd	0.26[b]	2.81[a]	GC-MS
29	hexadecane	1600	1600	Nd	Nd	3.61[a]	1.37	GC-MS
30	carvone	1245	1598	0.01[b]	0.18[a]	0.29[b]	0.54[a]	GC-MS
40	β-elemene	1391	1601	0.36[b]	0.17[b]	Nd	Nd	GC-MS
41	terpinene 4-ol	1176	1611	7.01[b]	2.86	3.84[b]	36.31[a]	GC-MS
42	β caryophyllene	1419	1612	0.12[a]	0.05[b]	Nd	0.27[a]	GC-MS
43	α-humulene	1454	1687	Nd	Nd	0.11[b]	0.70[a]	GC-MS
44	phenylacetaldehyde	1490	1690	Nd	Nd	Nd	1.68[a]	GC-MS
45	α-terpineol	1189	1709	0.05[a]	0.02[a]	0.06[b]	0.47[a]	GC-MS
46	bicyclogermacreme	1494	1755	1.35[a]	0.57[b]	0.90[b]	9.85[a]	GC-MS
47	neryl acetate	1385	1733	0.44[a]	0.16[b]	1.00[a]	0.38[b]	GC-MS
48	geranyl acetate	1383	1765	0.06[a]	0.02[a]	0.07[b]	0.30[a]	GC-MS
49	Ni	Nd	Nd	Nd	0.077[a]	Nd	Nd	GC-MS
50	*cis* piperitone	1228	1797	0.092[a]	0.043[a]	0.06[b]	0.30[a]	GC-MS
51	nerol	1228	1797	Nd	Nd	0.57[a]	0.46[a]	GC-MS
52	geraniol	1255	1857	Nd	Nd	0.09[b]	0.63[a]	GC-MS
53	Ni	Nd	Nd	Nd	Nd	Nd	0.77[a]	GC-MS
54	nonadecane	1581	1900	Nd	Nd	0.16[b]	0.63[a]	GC-MS

Table 1. Contd.

55	Ni	Nd	Nd	Nd	Nd	0.64 [a]	0.40 [b]	GC-MS
56	phenol	-	-	0.067 [a]	0.037 [a]	0.82 [a]	0.78 [b]	GC-MS

Volatile compound proportions were calculated from the chromatograms obtained on the HP Innowax column. ND not detected. NI not identified. Values (means of three replicates) with different superscripts (a–b) are significantly different at P < 0.05. Note: Retention indices relative to n-alkanes on [a]apolar column HP-5MS and [b]polar column HP-Innowax.

Table 2. ANOVA analysis and chemical classes (µg/g DW) of *O. majorana* areal part cultured under NaCl 75 mM.

		NaCl (mM)			
		TV		CV	
		0	75	0	75
Classes	Monoterpene hydrocarbones	41.36[a]	14.32[b]	26.79[b]	151.13[a]
	Oxygenated monoterpenes	11.30[a]	2.94[b]	5.12[b]	37.71[a]
	Sesquiterpenes hydrocarbons	2.00[a]	0.62[b]	0.90[b]	9.85[a]
	Aliphatiques hydrocarbons	5.53[a]	0.225[b]	2.50[a]	1.45[b]
	Sesquiterpenes oxygenated	Nd	Nd	0.57[a]	0.46[a]
	Cetones	8.37[a]	2.13[b]	3.68[a]	4.66[a]
	Esters	4.66[a]	4.28[a]	2.11[b]	9.33[a]

Volatile compound proportions were calculated from the chromatograms obtained on the HP Innowax column. ND not detected. NI not identified. Values (means of 3 replicates) with different superscripts (a–b) are significantly different at P < 0.05. Note: Retention indices relative to n-alkanes on [a]apolar column HP-5MS and [b]polar column HP-Innowax.

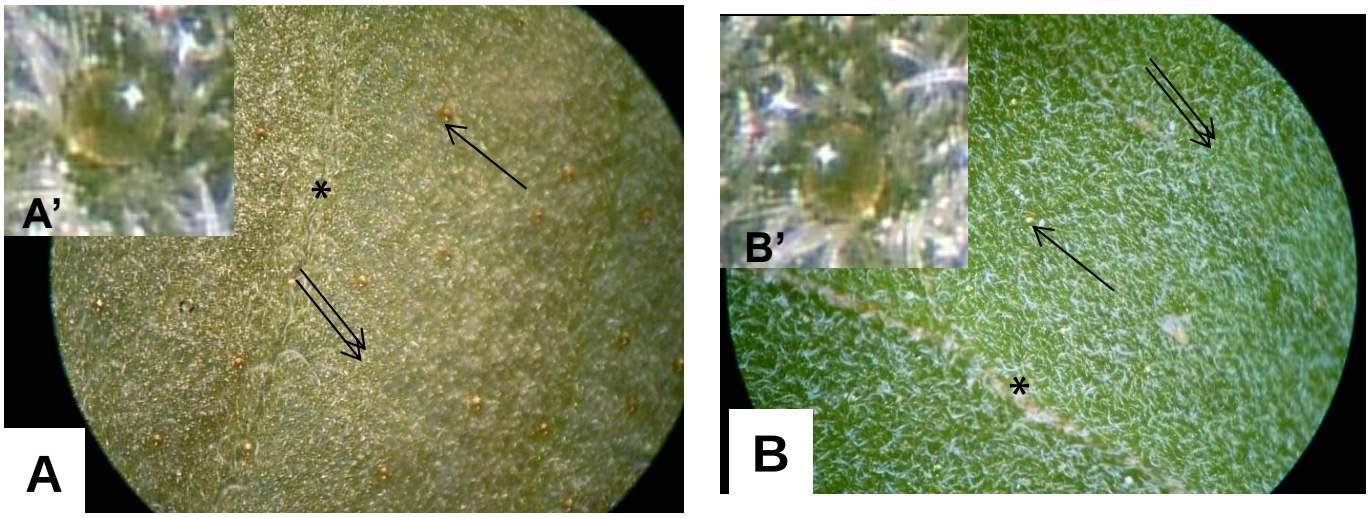

Figure 1. Binocular magnifying glass micrographs (350×) showing non glandular (double arrows) and glandular trichomes (arrow) on ventral sides of Canadian (A) and Tunisian (B) varieties of *O. majorana* mature leaves under non-saline conditions. A' and B' secretary gland at higher magnication of VC and VT, respectively (1300*).

Thus they seemed to have a protective role. Control transverse sections of leaves, strained with red soudan, showed three types of non-glandular trichomes at TV variety against two in the Canadian one (Figure 2A and C). But at saline conditions, in the Canadian variety only BT were shown (Figure 2B); whereas in the Tunisian one we found besides, long MT (Figure 2D).

Glandular trichomes

In our two varieties, beside non glandular trichomes we

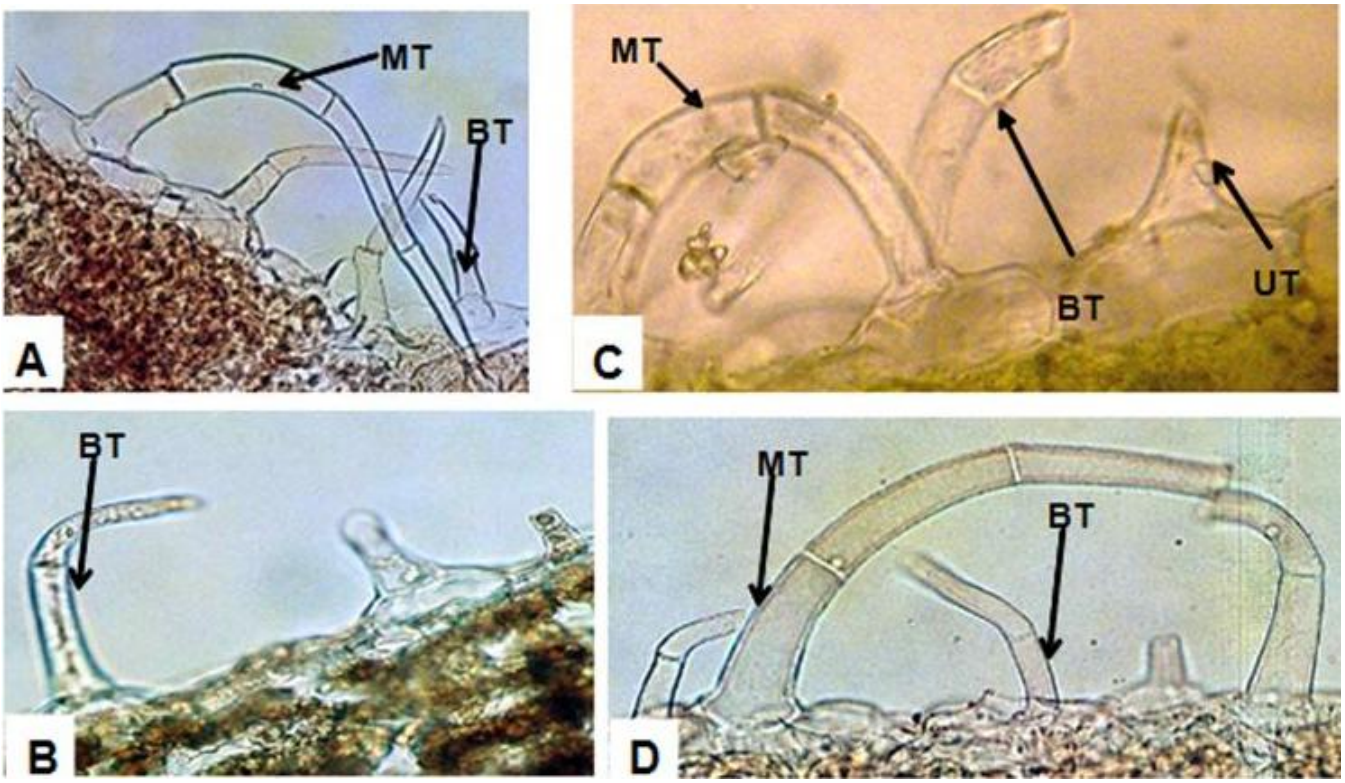

Figure 2. Non glandular trichomes in VC (A and B) and VT (C and D) at non saline (A and C) and saline (B and D) conditions. (A): Two types of non glandular trichomes are obvious on upper epidermis of the leave: multicellular (MT), bicellular (BT) (1200*) (B): only bicellular trichome (BT) are seen (C): multicellular (MT), bicellular (BT) and unicellular (UT) obvious on upper epidermis of VT leave (1700*). (D): multicellular (MT) and bicellular (BT) trichomes (1000*).

found two types of glandular (peltate and capitates), sites of biosynthesis and accumulation of the essential oil. Theses trichomes appeared at low magnification as lipid droplets, on the ventral faces of leaves (arrow, Figure 1A, B, A', B'). But, at high magnification, we can distinguish the peltate from the capitates (Figures 3, 4 and 5).

Under control conditions, capitates glandular trichomes of the leaves were very small (Figure 3A, C, A', C'). Each of them, was formed, in both varieties, by a basal cell (BC), a short unicellular stalk cell (P) and a relatively bigger avoid head (H). Even the same types of capitates trichomes were observed at salt constraint (Figure 3B and D).

Peltate glandular trichomes (Figure 4) were bigger than the capitates ones (Figure 3). Under control conditions, at the mature stage and at upper view, each trichome was composed in VC (Figure 4B) or in VT (Figure 5B) of two concentric circles of cells (12 cells) with cuticle. This latter seemed not obvious at the young stage neither in VC (Figure 4A) nor in VT (Figure 5A). These peltate glandular trichomes were protected by non glandular (TNG) (Figure 5C and D) in TV and surrounded with stomata (ST). Their presences on leaves surface suggest their involvement in the production of EO. Even, their cells heads were the sites of active secretions of EO inside a pocket (*) covered with a cuticle (Cu) (Figure 4B). Referred to their contrast, in VC, three types of secretions (Figure 4C and D) appeared at the control medium:

(i) Spherical shape lipids droplets with clear and homogeneous content (*, Figure 4C).
(ii) Ovoid shape of dark red droplets (+, Figure 4D).
(iii) Secretions of lucid appearance (++, Figure 4D).

Essential oil secretions were obvious in CV at control (Figure 4C and D) as well as salt condition (Figure 4C and G); but it seemed to be lacking in TV when it was observed with light microscopy (Figure 5E and F).

DISCUSSION

Changes in essential oil yield and compositions have been reported to be influenced by environmental conditions (Gil et al., 2002). In our finding, EO yield increased and decrease in CV and TV, respectively under salt constraint. In the paper by Baatour et al. (2010), Table 2 demonstrated that EO yield was affected by both 50 and 100 mM NaCl, it was reduced from 0.12%

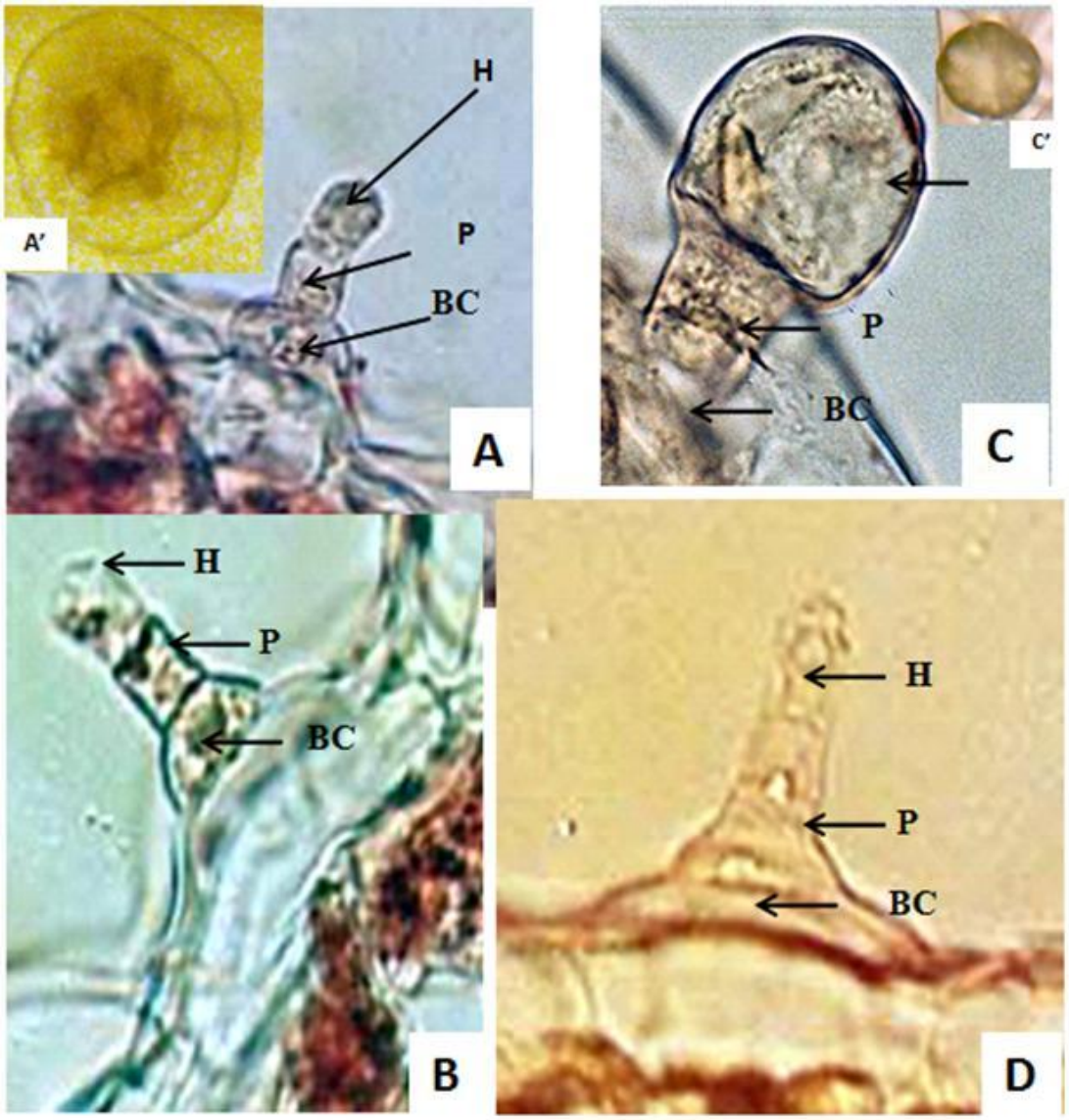

Figure 3. Glandular trichomes in VT (A and B) and VC (C and D) observed at side (A, B, C and D) and upper views (A' and C'). (A-D), capitates glandulars trichomes showing a basal cell (BC), a short unicellular stalk cell (P) and a relatively bigger avoid head (H). (A), (4200*), and (B) (5000*), (C), (3600*). (A') and (C'), détail of A and C respectively at higher magnification of capitate glandular trichomes (5600*) and (2200*) respectively under saline conditions: in VC and D : in VT (2000*).

(a) to 0.10% (b) and 0.05% (c) by 50 and 100 mM, respectively.

According to Karray et al. (2009), the essential oil (EO) yields of *Mentha. pulegium* increased significantly. An increase of essential oil yield by salinity has been reported earlier in other plant species, e.g. sage (*Salvia officinalis* L.) (Hendawy and Khalid, 2005), and peppermint (*Mentha piperita* L.) (Abou El-Fadl et al., 1990). In our investigation, we found that at control, *O. majorana* shoots exhibited a cis-sabinene hydrate-terpinene-4-ol and sabinene-trans sabinene hydrate chemotyped EO in TV and CV, respectively. Under salt treatment, the chemotype became sabinene/trans-sabinene hydrate and trans-sabinene hydrate-terpinene-4-ol in TV and CV, respectively.

Our results showed that some of the above main constituents were found in numerous EO of *O. majorana*. In fact, according to Trivino and Johnson (2000) terpinen-4-ol was the major component in marjoram EO. In Novak et al. (2002) studies the main compounds are the epimeric monoterpene alcohols trans sabinene hydrate, cis-sabinene hydrate and cis-sabinene hydrate acetate. According to Banchio et al. (2008), the main components were terpinen-4-ol, trans-sabinene hydrate in

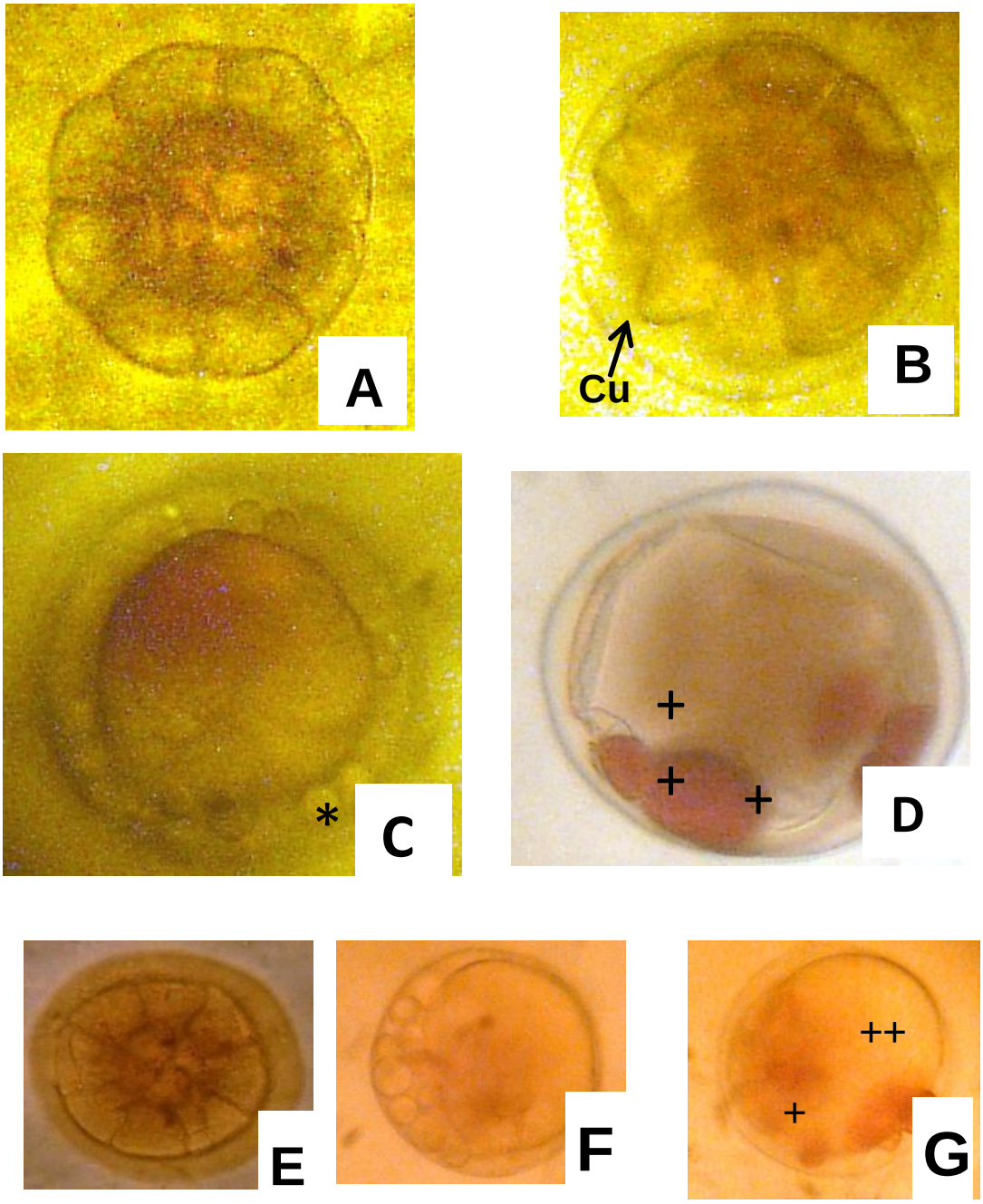

Figure 4. Upper views of peltate glandular trichomes in VC; A to D, under non-saline conditions and E-F, under saline conditions. (A): trichome with 12 head cells, before cuticle edification (6300*). (B): cuticle (*) formed all around head cells (7300*) (C): lipids droplets (*) with clear and homogeneous content (7300*). (D): Two others types of secretions (+, ++) (5600*). (E): peltate trichome with 12 head cells (4800*). (F): lipids droplets (*) with clear and homogeneous content (4800*). (G): others productions (+) and (++) (4000*).

O. majorana EO originated from Argentina. In Tunisian marjoram, Hamrouni et al. (2009) reported that the major component were terpinen-4-ol and cis sabinene hydrate. In our previous work, Bâatour et al. (2010) investigated that, trans-sabinene hydrate and terpinene-4-ol were the chemotype of marjoram EO originated from Canada. In order to find an explanation for this increase (improvement) and decrease in EO yield in shoots of CV and TV, respectively, we carried out a study on the structures responsible for the production of these secondary metabolites.

Trichomes appeared in three types; multicellular,

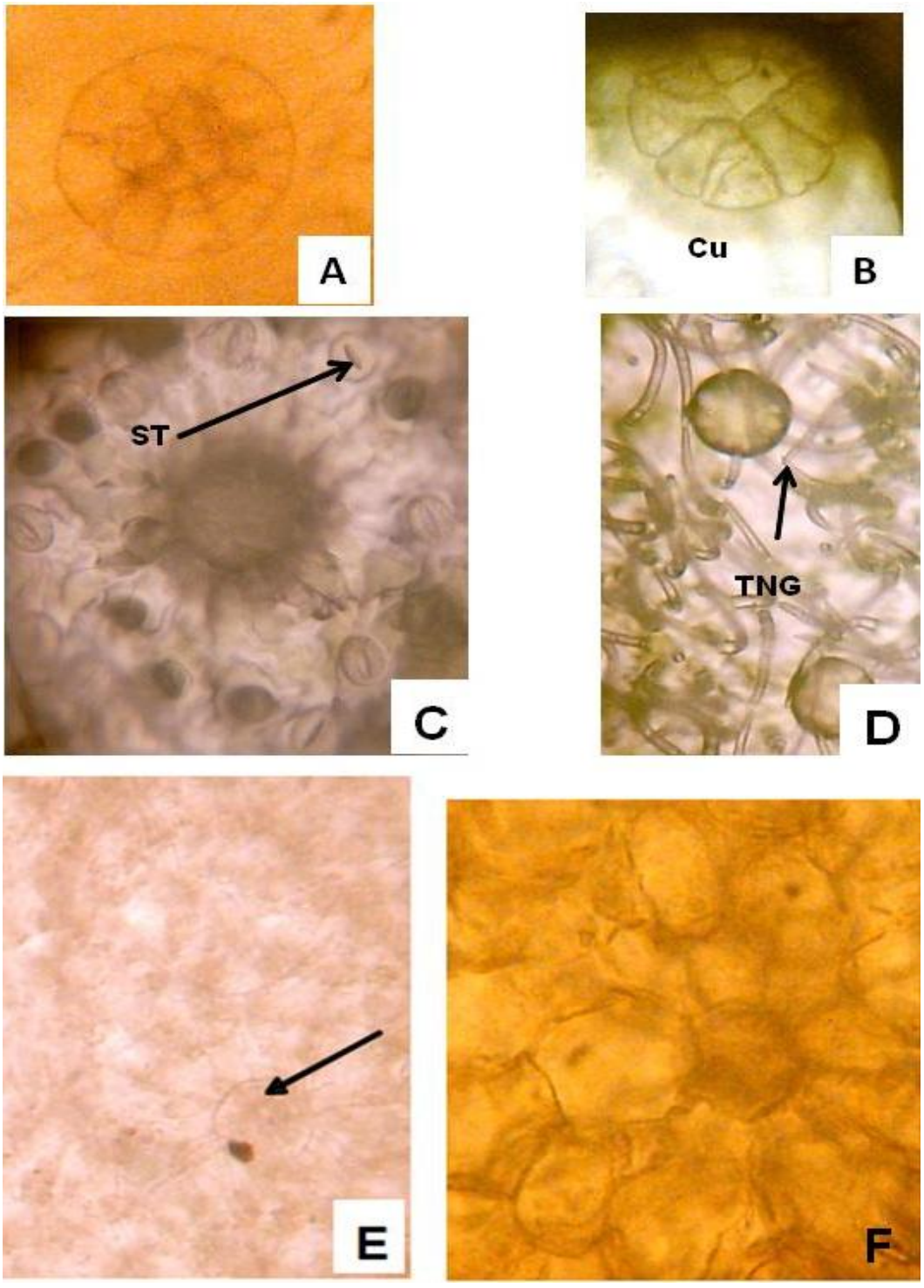

Figure 5. Upper view of peltate glandular trichomes in VT A-D: under non-saline conditions and (E to F) under saline conditions, (A): trichome before cuticle edification (5900*) (B): trichome after cuticle edification. (6200*). (C) Trichome surrounded by stomata (ST) (2300*), (D) and protective by non glandular trichomes (TNG) (2250*) (E to F) under saline conditions (4000*) and (1000*), respectively.

bicellulaire and unicellular. Multicellular, obvious in TV, were described in *Origanum vulgare* (Gales et al., 2008). A digitiform Type was also found in some Lamiaceae species such as *Plectranthus ornatus* (Ascensão et al., 1999). These latter, seemed to protect aerial parts of plants against foraging insects and airborne propagules

of fungi (Delamare et al., 2005).

Capitate glandular trichome with unicellular celled stalks and unicellular elongated heads, have been found in our two varieties of *O.majorana* under non saline and saline conditions. This type seemed the commonest one in *Calamintha menthifolia* according to Handilou et al. (1991). Werker et al. (1985), observed in *Lamiaceae* species examinated glandular hairs with 2-celled stalks and unicellular elongated heads. According to (Karray et al., 2009), capitate trichomes on *Mentha pulegium* leaves and stems belong to short stalk and small secretary head; structure described before in *M. spicata*, *M. spicata×suaveolens*, and *M. piperita* (Martins, 2002). Our findings showed that *O. majorana* peltate type consist of a basal cell, short stalk cell and a head of 12 secretory cells arranged on two concentric rings or discs (4 to 8). The storage space of peltate trichomes was filled with EO and was formed by separation of the cuticle from the apical walls of the disc cells. This finding was similar to Turner et al. (2000)'S one. Glandular scales appear in other species but with a variable number of head cells. Mature leaf peltate trichome's exhibited 8 in *M. piperita*, *M. spicata* and *M. spicata×suaveolens* (Martins, 2002), 10 cells; in *M. pulegium (*Turner et al., 2000), 12 cells and 16 in *Prostanthera ovalifolia* (Gersbach, 2002), ranging from 14 to 18 in *Micromeria fruticosa* L. (Werker et al., 1985). The type found in *C. menthifolia* and in *Satureja thymbra* L (12-celled head) seems to be the most common one and is also described for the leaves of other *Origanum* species (Bosabalidis and Tsekos, 1984).

In our investigation, salinity was shown to improve the types of secretions on leaves of CV. These secretions can be belongs to two types of chemical classes (hydrocarbons monoterpenes and oxygenated monoterpenes). The improvement of secretions in CV under salt stress could explain the enhancement of EO yield and these two chemical classes. The stimulation of essential oil production under moderate salinity could be due, as referred to Charles et al. (1990), to a higher oil gland density and an increase in the absolute number of glands produced. In our material, the absence of the three types of secretions in TV could be explained by a decrease of essential oil yield and the amounts of these two chemical classes. But their presence in CV could be due to improvement of EO yield and chemical classes. Heuer et al. (2002) found that salinity increased oil yield in *Ooenothera biennis*, decreased it in *Salvia hispanica*, and did not change it in *Matthiola tricuspidata.* In *Mentha. Pulegium,* the enhancement of essential oil yield could explain the improvement of density of glandular trichomes (karray et al., 2009). The correlation between EO yield and structure of trichomes differed from a medicinal plant to another. According to Najoua et al. (2009), in *Mentha pulegium*, the rise of glandular trichomes was correlated with an enhancement in essential oil yield in leaves of salt-treated plants. Thus, salinity was shown to improve the density of glandular trichomes. Escensão and Pais (1987), the positive correlation between glandular trichomes and EO yield under salinity conditions was genetically fixed. Farooqi et al. (1999) explained the increase of the glandular trichoms on leaves under environmental stress conditions by the fact that salt-treated plants exhibit less leaf area. Therefore, a positive correlation between EO yield and structure of trichomes was observed in Canadian Variety, but, the contrary was observed with the Tunisian ones.

Conclusion

Three types of trichomes were found in *O. majorana*: non glandular, glandular peltate and glandular capitate trichomes. The head cells of peltate trichomes were the sites of active secretions of EO inside a pocket covered with a cuticle. Three types of secretions were observed in VC, referred to their contrast (dark red droplets, clear lipids droplets, secretion of lucid appearance). These essential oil secretions were obvious in CV at control and salt condition but it was absent in Tunisian one. The enhancement in essential oil yield in CV could be explained by the presence of these secretions. It seemed that under salinity, leaves are more involved in essential oil production in CV than in TV.

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