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a b s t r a c t

Oregano herbs and essential oils are considered of great interest for their medicinal virtues as well as for their culinary properties. The present investigation aims to study the chemical diversity of the essential oils (EO) of a rare *Origanum* species (*O. grosii*) endemic to the North-West of Morocco based on a large sample size (68 individual plants from 8 natural populations). Besides, 8 individual plants (genotypes) with defined EO yield and composition have been transplanted in the experimental field to show the impact of changing environmental conditions on their EO characteristics. All EOs samples were analyzed by Gas Chromatography with Flame Ionization Detector (GC-FID), Gas Chromatography/Mass Spectrom- etry (GC/MS) and Nuclear Magnetic Resonance (^{13}C NMR). The EO yields of *O. grosii* extracted by hydrodistillation ranged from 1.71% to 2.41% with an average of 2%. The analysis showed that chemical profile of *O. grosii* from natural accessions is dominated by carvacrol (0.48–85.66%), *p*-cymene (3.01–77.49%), thymol (0–75.56%) and γ-terpinene (0–28.9%), and to a lesser extent with thymohydroquinone, thymoquinone and carvacryl methyl oxide in some individual plants with remark- able intraspecific and intrapopulation variability. The EO production of the transplanted genotypes was positively affected by the experimental field conditions. In addition, the transplantation induced changes in the chemical composition of their major components (γ-terpinene, *p*-cymene, carvacrol, thymol and thymoquinone) where we stated mainly an increase of γ-terpinene and carvacrol against a decrease of *p*-cymene. The present findings exhibit the chemical profile of *O. grosii* in relation with their geograph- ical origins, besides they provide useful data concerning the impact of the culture on their EO characteristics.

1. Introduction

Due to their recognized positive effects on human life and health, plant natural products have numerous and distinct applications in various fields such as cooking, food products, agriculture, medicine and perfumery. Traditional medicinal and aromatic plants with an important crop production and/or bio-chemical compounds yield are targeted by modern world agriculture. In this context, oregano is one of the most important and most used medicinal and aromatic plants in the world due to their important and efficient biological and culinary proprieties such as antibacterial, antioxidant, antiparasitic (Bouhdid et al., 2008; Bouyahya et al., 2017), antifungal (Fadel et al., 2013), anti-inflammatory, immunomodulatory and anticancer (Han and Parker, 2017).

The genus *Origanum* is known by its restricted geographical distribution around the Mediterranean. Except *O. vulgare*, which has the largest distribution in comparison to all *Origanum* species, the great majority of taxa are endemic or having a specific local distribution with an exclusively abundance in the East Mediterranean sub-region. However, in North Africa, three taxa occur in Libya (*O. akhdarense*, *O. cyrenaicum* and *O. pampaninii*), two grow in Algeria and Tunisia (*O. vulgare* subsp. *glandulosum* and *O. floribundum*) and five are present in Morocco: three are endemic (*O. elongatum*, *O. grosii* and *O. × font-queri*) and two other occur also in Iberian peninsula (*O. compactum* and *O. vulgare* subsp. *virens*) (Ietswaart, 1980; Kokkini, 1997).

Ietswaart (1980) has regrouped *Origanum* taxa within 3 groups and 10 sections according to their morphological characters. *Elongatispica* section includes *O. floribundum*, *O. elongatum* and *O. grosii*. *Origanum*

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grosii is one of the rarest *Origanum* taxa which grows in very restricted areas in North–West (NW) Morocco, exactly in Western Rif Mountain range, mostly in high elevations and under humid climate (Bakha et al., 2017). Morphologically, this species is characterized by its short and slightly pubescent stems (up to 55 cm long), more or less long branches reaching 12 cm (4–30), and also by their few large bracts of about 3 mm (2–5) and small leaves length 11 mm (5–19) (Ietswaart, 1980). As almost Moroccan oregano species, *O. grosii* is also overharvested from wild populations because of its mentioned virtues.

Interestingly, the cultivation of oregano under controlled conditions is considered to be the best way to produce herbs or EOs with relatively stable yield and composition. Such crop requires the selection of chemically defined plants (genotype/chemotype) that are greatly used in both industrial and pharmacological areas (Benlarbi and Elmtili, 2012; Benlarbi et al., 2014). Furthermore, the advantages of the cultivation are the elevation of pressure exerted on wild populations in order to ensure their conservation and a sustainable utilization of this valuable medicinal plant, besides the socio-economical benefits. This approach was successfully applied in numerous aromatic plants such as lavender, mint, basil and hyssop (Moro et al., 2011).

Chemically, the essential oils (EOs) of *Origanum* are characterized by noticeable quantitative and qualitative variability at the inter- and intra-specific scales. The chemical profiles of almost all *Origanum* taxa contain compounds such as carvacrol, thymol and their precursors; *p*-cymene and γ-terpinene, as dominant (Skoula et al., 1999). Moreover, numerous other compounds were detected in the EOs of many *Origanum* taxa reaching in some cases higher rates such as α-terpineol (up to 73%) in *O. majorana* (Novak et al., 2008) and linalool (up to 76%) in *O.vulgare* subsp. *virens* (Figuérédo et al., 2006a). It has been proved that the chemical polymorphism of EOs is influenced by genetic and environmental factors (Aboukhalid et al., 2017; Crocoll et al., 2010; Davidenco et al., 2017; Kofidis et al., 2003). In addition, agronomic and technical practices, mainly fertilization, irrigation, harvest period, drying techniques, extraction and conservation methods could also influence the EOs composition of several medicinal plants (Azizi et al., 2009; Bahreininejad et al., 2014).

For the *Origanum* taxa, realized studies on the EOs variability in term of chemical composition and productivity have mainly concerned species with extent geographical distribution and diversified pedoclimatic and environmental conditions such as; *O. vulgare* (Lukas et al., 2013), *O. onites* (Vokou et al., 1988) and *O. compactum* (Aboukhalid et al., 2016). However, species with more restricted distribution and relatively homogeneous environmental conditions could provide useful information for a better comprehension on EO production on quite limited scale. This present investigation is the first one which aims to (1) reveal the intraspecific chemical diversity of EOs of the Moroccan endemic *O. grosii* using individual plants sampling from different populations; and (2) study the impact of transplantation on the quantitative and qualitative characteristic ofits EOs.

Table 1
Geographic and climatic data, sample numbers and mean EO yield of investigated wild populations of *O. grosii*.

Accession no	Accession location	Number of samples	Samples order	Elevation (m)	Climate	EOs %
G1	Talassemtane Park	5	G1.1–G1.5	1302	Humid	1.82
G2	Jbel Lakraa	7	G2.6–G.212	1898	Humid	1.71
G3	Jbel Tissouka	11	G3.13–G3.23	1257	Humid	1.87
G4	Loubar	13	G4.24–G4.36	834	Humid	2.41
G5	Jbel Kalaa 1	10	G5.37–G5.46	1021	Humid	2.26
G6	Jbel Kalaa 2	9	G6.47–G6.55	1070	Humid	2.1
G7	Tissemlale	7	G7.56–G7.62	960	Humid	1.8
G8	Talamboute	6	G8.63–G8.68	402	Humid	2.04

2. Materials and methods

2.1. Plant material

Individual plant samples of *O. grosii* have been collected from eight natural populations in the NW of Morocco during July 2015 (Table 1). Taxonomic identification was performed following the key of *Origanum* taxonomy of Ietswaart (1980). A total of 68 individual plants were collected, among which several ones have been transplanted at the INRA experimental field in Annoceur (Sefrou-Morocco; 1450 m, 33°41′02.2″ N 4°51′19.2″W). The transplanted genotypes were irrigated to minimize the impact of precipitation. The aerial parts of all collected individual plants were harvested during the flowering period when oregano produces normally a maximal biomass. Besides, eight transplanted genotypes (G3.20, G4.30, G4.31, G4.32, G4.33, G5.42, G5.43 and G6.54) were selected to study the impact of changing of natural conditions on EOs yield and composition where each plant among the transplanted genotypes was harvested also during the flowering stage (10/07/2016).

2.2. Essential oils extraction and analysis

Essential oils were extracted from aerial part of the individual plants by hydrodistillation and then were analyzed via Gas Chromatography with Flame Ionization Detector (GC-FID), Gas Chromatography/Mass Spectrometry (GC/MS) and Nuclear Magnetic Resonance (^{13}C NMR).

Gas chromatography (GC) analysis – GC analyses were performed on a PerkinElmer Clarus 500 gas chromatograph (FID) equipped two fused silica gel capillary columns (50 m × 0.22 mm, film thickness 0.25 μm), BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60 to 220 °C at 2 °C/min and then held isothermal at 220 °C for 20 min, injector temperature: 250 °C; detector temperature: 250 °C; carrier gas: hydrogen (1.0 mL/min); split: 1/60. The relative proportions of the oil constituentswere expressed as percentages obtained by peak area normalization, without using correcting factors. Retention indices (RIs) were determined relative to the retention times of a series of *n*-alkanes with linear interpolation ('Target Compounds' software of PerkinElmer).

Mass Spectrometry – The EOs were analyzed with a PerkinElmer TurboMass detector (quadrupole), directly coupled to a PerkinElmer Autosystem XL, equipped with a fused silica gel capillary column (50 m × 0.22 mm i.d., film thickness 0.25 μm), BP-1 (dimethylpolysiloxane). Carrier gas, helium at 0.8 mL/min; split: 1/75; injection volume: 0.5 μL; injector temperature: 250 °C; oven temperature programmed from 60 to 220 °C at 2 °C/min and then held isothermal (20 min); ion source temperature: 250 °C; energy ionization: 70 eV; electron ionization mass spectra were acquired over the mass range 40–400 Da.

NMR analysis – ^{13}C-NMR analyses were performed on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.623 MHz for ^{13}C, equipped with a 5 mm probe, in $CDCl_3$, with all shifts referred to internal tetramethylsilane (TMS). ^{13}C-NMR spectra were recorded with the following parameters: pulse width (PW): 4 μs(flip angle 45°); acquisition time: 2.73 s for 128 K data table with a spec-tral width (SW) of 220.000 Hz (220 ppm); CPD mode decoupling; digi-tal resolution 0.183 Hz/pt. The number of accumulated scans ranged 2000–3000 for each sample (around 40 mg of oil in 0.5 mL of $CDCl_3$). Exponential line broadening multiplication (1.0 Hz) of the free induction decay was applied before Fourier transformation.

2.3. Soil analysis

The soil samples were dried and sieved (2 mm). The part of soil finer than 2 mm was used for chemical and granulometry analysis. The pH was determined in a 1:1 soil/water volume ratio. Texture, Organic Matter (OM), K_2O, P_2O_5 and cationic exchange capacity were determined by standard methods (Black, 1965).

2.4. Statistical analysis

The principal component analysis (PCA), the hierarchical cluster analysis (HCA; *Ward*'s method) and the Analysis of Variance (ANOVA) were performed with PAST (Paleontological Statistics Software Package) 3.14 version (Hammer et al., 2001).

3. Results

3.1. Intraspecific chemical variability

All the studied populations grow under humid climate and elevation level between 400 and 1900 m (Table 1). The dry aerial parts of the spontaneous *O. grosii* (20–60 g) produce a mean population EOs yield (*w*/*w*) ranging from 1.71% to 2.41% with a species average of 2%. The statistical analysis showed that there are no correlation between the EO yield in *O. grosii* and elevation (p N .05). The highest mean population EO yield was detected in G4 (Table 1). Three groups of populations were statistically different in EO yield ($F_{2,26} = 7.47$, $p = .0027$). The lowest yields (average: 1.7%) were obtained in populations G1 (1302m) and G2 (1898m), intermediate yields (average of 2%) in populations G6 (1070m), G7 (960m) and G8 (402m), and the highest yields (average: 2.5%) in populations G3 (1257m), G4 (834m) and G5 (1021m) (Fig. 1).

The 68 EOs samples of *O. grosii* have been analyzed by GC-FID (on polar and apolar columns), GC/MS and ^{13}C-NMR. In total, 28 compounds were identified that account for 96.94% (mean) of the total composition of the EOs samples. The chemical profile of *O. grosii* was found mainly represented by oxygenated monoterpenes (M 64.03%, 9.08–90.82%) and monoterpene hydrocarbons (M 31.60%, 6.33–86.07%). However, the sesquiterpene have been detected in much lower concentrations (M 1.31%, 0–5.58%). In fact, carvacrol (M 42.96%, 0.48–85.66%),*p*-cymene (M 22.66%, 3.01–77.49%), thymol (M 15.45%, 0–75.56%) and γ-terpinene (M 5.03%, 0–28.9%) constituted the major compounds, while thymoquinone, thymohydroquinone and carvacryl methyl oxide were detected in appreciable amounts in some EO samples. The results are summarized in Table 2.

In order to study the intraspecific chemical variability within and between the studied populations, two statistical analyses were carried out, namely the Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). The PCA showed that the total variance is highly represented by the two first principal components (PC1 = 69.8%) and (PC2 = 27.4%), where PC1 is positively correlated with carvacrol and negatively with thymol contents, while PC2 is positively related to carvacrol and thymol, and negatively to *p*-cymene (Fig. 2). The HCA using *Ward*'s method showed that the studied samples were divided into three groups according to similarity of their chemical components (Fig. 3). For each group, the Mean, Minimum and Maximum values of the different compounds are presented in Table 2.

The first group is the largest one and consists of 30 individual plants which represent 44.12% of all analyzed samples. The profile of their EOs is characterized by the dominance of carvacrol (M 72.96%, 61.74–85.66%) with amount higher than 80% for five individual plants(G2.10, G4.34, G4.35, G4.36 and G5.41). Besides, this group contains also *p*-cymene (M 10.74%, 3.01–22.78%) and γ-terpinene (M 3.86%, 0–13.69%) at noticeable amounts. Thymohydroquinone was detectedat appreciable percentages in three individual plants: G2.12 (4.05%), G2.6 (4.71%) and G2.8 (5.02%).

The 22 EO samples of Group II were found rich in *p*-cymene (M 47.19%, 26.07–77.49%). Percentages of *p*-cymene higher than 60% have been detected in G8.64 (62.03%), G8.63 (65.59%) and G8.65 (77.49%). The EOs samples of this group were also characterized by the presence of carvacrol (M 25.54%, 0.48–58%) and thymol (M 6.54%, 0–25.82%) in relatively high amounts. Particularly, six individual plants contain an amount of thymoquinone superior than 4% (G3.13, G5.38, G5.39, G6.49, G6.54 and G8.67) among which G6.54 had the highest value (12.18%). Besides, a considerable amount of carvacryl methyl oxide has been detected in G6.47 (14.57%).

The third group includes 16 individual plants containing EOs dominated by thymol (M 54.81%, 23.68–75.56%) where the highest amounts were detected in G1.2 (72.14%) and G1.1 (75.56%). In addition, *p*- cymene (M 11.30%, 3.12–21.27%), carvacrol (M 10.66%, 2.55–44.66%) and γ-terpinene (M 10.53%, 2.77–28.90%) have been detected in noticeable amounts in this group. The maximal amounts of γ-terpinene (N 20%) were revealed in two samples; G7.57 (20.62%) and G7.56 (28.9%). On the other hand, only one individual plant (G2.9) from thisgroup reached an appreciable percentage of thymohydroquinone (5.32%).

The carvacrol and *p*-cymene chemotypes (group I and II) were detected in seven populations out of eight studied populations. Individual plants rich in carvacrol were predominant in four populations (G2, G3, G4 and G7), while *p*-cymene is prevalent in two popula- tions (G6 and G8). On the other hand, the thymol chemotype (Group III) was represented in six populations with great abundance in G1 and G5 (Fig. 4). As previously mentioned, all EOs with an ap- preciable amount of thymohydroquinone belong to the population G2.

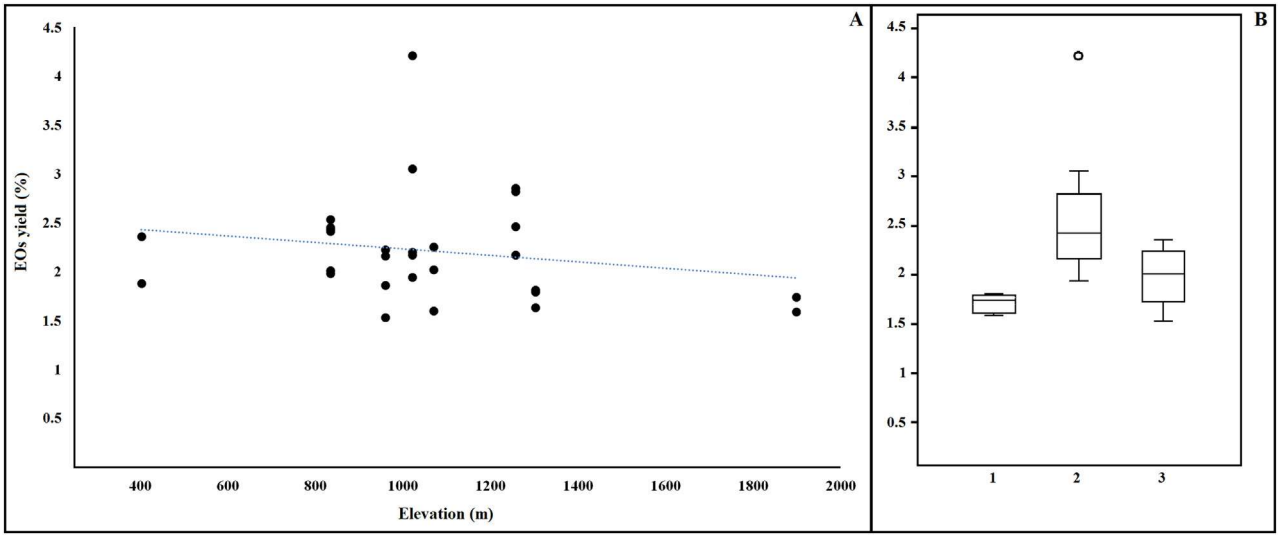

Fig. 1. (A) EO yields of 29 samples of *O. grosii* from the different studied populations related to their altitudinal gradient. (B) The three obtained groups of population in terms of EO production (see text for detailed of the group compositions).

Table 2
Chemical composition and chemical groups of *O. grosii* EO (Content [%]: Mean (Min–Max)).

Compounds[a]	RIa	RIp	Total (n = 68)	Group I (n = 30)	Group II (n = 22)	Group III (n = 16)
α-Thujene	925	1017	0.78 (0.1–1.62)	0.6 (0.1–1.37)	1.11 (0.34–1.62)	0.67 (0.16–1.39)
α-Pinene	932	1014	0.44 (0–0.93)	0.38 (0–0.93)	0.58 (0.22–0.83)	0.36 (0–0.58)
Camphene	945	1064	0.08 (0–0.23)	0.06 (0–0.23)	0.12 (0–0.23)	0.06 (0–0.13)
1-Octen-3-ol	962	1446	0.45 (0–1.99)	0.31 (0–0.66)	0.53 (0–1.22)	0.62 (0.19–1.99)
3-Octanone	965	1254	0.51 (0–1.4)	0.38 (0–0.93)	0.64 (0–1.34)	0.57 (0.14–1.4)
β-Pinene	972	1111	0.12 (0–0.21)	0.09 (0–0.19)	0.16 (0.01–0.21)	0.1 (0–0.16)
3-Octanol	979	1389	0.06 (0–1.32)	0.06 (0–1.32)	0.05 (0–0.32)	0.08 (0–0.4)
Myrcene	983	1159	0.63 (0–2.18)	0.59 (0–1.60)	0.46 (0–1.86)	0.97 (0–2.18)
α–Phellandrene	999	1164	0.07 (0–0.28)	0.06 (0–0.19)	0.07 (0–0.28)	0.11 (0–0.28)
δ-3-Carene	1007	1147	0.09 (0–0.57)	0.06 (0–0.46)	0.16 (0–0.57)	0.06 (0–0.19)
α-terpinene	1011	1179	1.28 (0–3.81)	0.83 (0–1.90)	1.76 (0–3.81)	1.45 (0.39–3.33)
p-cymene	1014	1270	22.66 (3.01–77.49)	10.74 (3.01–22.78)	47.19 (26.07–77.49)	11.30 (3.12–21.27)
Limonene	1023	1200	0.42 (0–0.84)	0.28 (0–0.56)	0.65 (0.47–0.84)	0.35 (0–0.77)
γ-Terpinene	1050	1243	5.03 (0–28.9)	3.86 (0–13.69)	2.63 (0–18.18)	10.53 (2.77–28.9)
Trans sabinene hydrate	1054	1461	0.6 (0–1.49)	0.71 (0.24–1.49)	0.32 (0–0.85)	0.78 (0–1.39)
Linalool	1085	1544	1.03 (0–2.7)	1.06 (0.31–2.7)	0.78 (0–1.77)	1.32 (0.35–2.45)
Bornéol	1149	1696	0.19 (0–0.86)	0.24 (0–0.86)	0.11 (0–0.34)	0.19 (0.05–0.32)
Terpinen-4-ol	1163	1598	0.61 (0–1.21)	0.57 (0–0.89)	0.57 (0–1.21)	0.75 (0.34–1.17)
α–Terpineol	1173	1691	0.48 (0–3.6)	0.59 (0–3.60)	0.35 (0.09–2.15)	0.44 (0.07–1.45)
Thymoquinone	1216	1571	1.02 (0–12.18)	0.37 (0–2.20)	2.61 (0–12.18)	0.06 (0–0.42)
Carvacryl methyl oxide	1226	1601	0.24 (0–14.57)	0.00 (0–0.02)	0.73 (0–14.57)	0 (0–0)
Thymol	1271	2179	15.45 (0–75.56)	0.99 (0–11.24)	6.54 (0–25.82)	54.81 (23.68–75.56)
Carvacrol	1279	2206	42.96 (0.48–85.66)	72.96 (61.74–85.66)	25.54 (0.48–58)	10.66 (2.55–44.66)
(E)-b-Caryophyllene	1417	1590	0.89 (0–2.76)	0.62 (0–1.24)	1.29 (0.58–2.76)	0.82 (0.21–1.73)
α-Humulene	1450	1662	0.02 (0–0.15)	0.01 (0–0.08)	0.03 (0–0.15)	0.02 (0–0.1)
β-Bisabolene	1501	1720	0.05 (0–0.99)	0.04 (0–0.99)	0.08 (0–0.36)	0.05 (0–0.33)
Thymohydroquinone	1516	2176	0.42 (0–5.32)	0.65 (0–5.02)	0.17 (0–1.11)	0.34 (0–5.32)
Caryophyllene oxide	1569	1974	0.35 (0–2.35)	0.20 (0–0.94)	0.58 (0.1–2.35)	0.34 (0.01–1.49)
Total identified [%]			96.94 (72.21–99.15)	97.32 (85.54–99.15)	95.79 (72.21–98.7)	97.82 (92.42–98.93)
Monoterpenes hydrocarbons			31.6 (6.33–86.07)	17.55 (6.33–30.56)	54.87 (29.39–86.07)	25.96 (6.62–56.77)
Oxygenated monoterpenes			64.03 (9.08–90.82)	78.9 (67.43–90.82)	38.95 (9.08–65.81)	70.63 (40.58–87.94)
Sesquiterpene hydrocarbons			0.96 (0–3.23)	0.67 (0–2.04)	1.4 (0.59–3.23)	0.9 (0.29–2.08)
Oxygenated sesquiterpene			0.35 (0–2.35)	0.2 (0–0.94)	0.58 (0.1–2.35)	0.34 (0.01–1.49)

[a] Order of elution and percentages have been given on apolar column, all compounds are identified by GC-FID, GC/MS and ^{13}C-NMR (for compounds over 1%), RIa, RIp retention indices on apolar and polar column.

The PCA realized on soil characteristics of six studied populations showed that G4 and G5 have more or less similar soil. The EOs of samples from these two populations have only *p*-cymene and carvacrol as major chemotypes. In contrast, the other populations regroup individual plants which contain EOs rich in thymol, carvacrol and *p*-cymene (Fig. 5). The correlation analysis between EO composition and elevation showed that elevation gradient was highly correlated (p b .001, r = − 0.516) with the production of *p*-cymene in *O. grosii*, where *p*-cymene increased with the decrease of elevation. Nevertheless, no significant correlation was detected between elevation and any other compound (p N .05).

3.2. Influence of transplantation on EO characteristic

In order to study the impact of plant transplantation on the EO yields and compositions in *O. grosii*, eight individual plants were transplanted

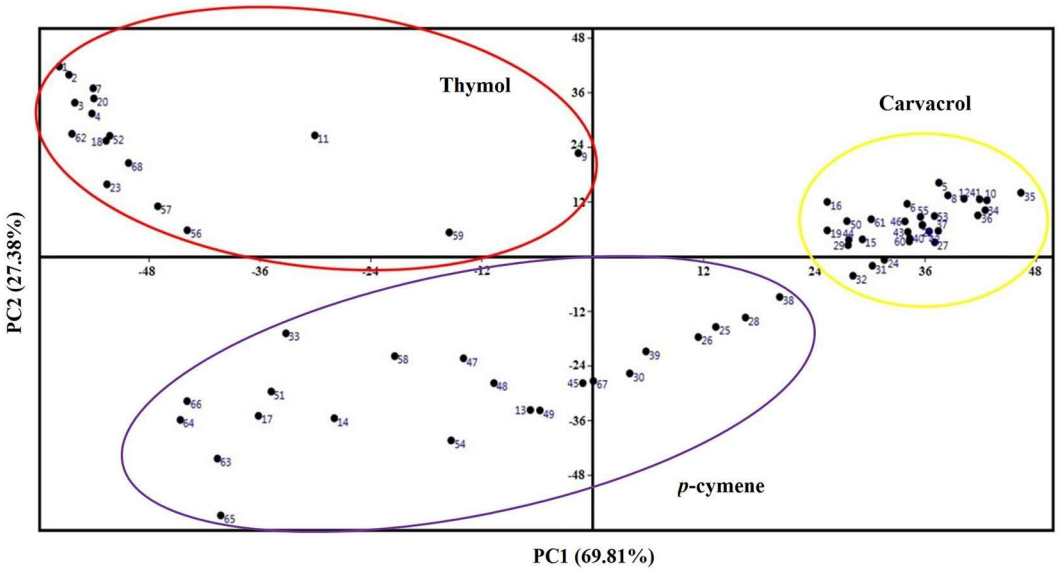

Fig. 2. PCA of the chemical composition of 68 EO samples from eight studied populations of *O. grosii*.

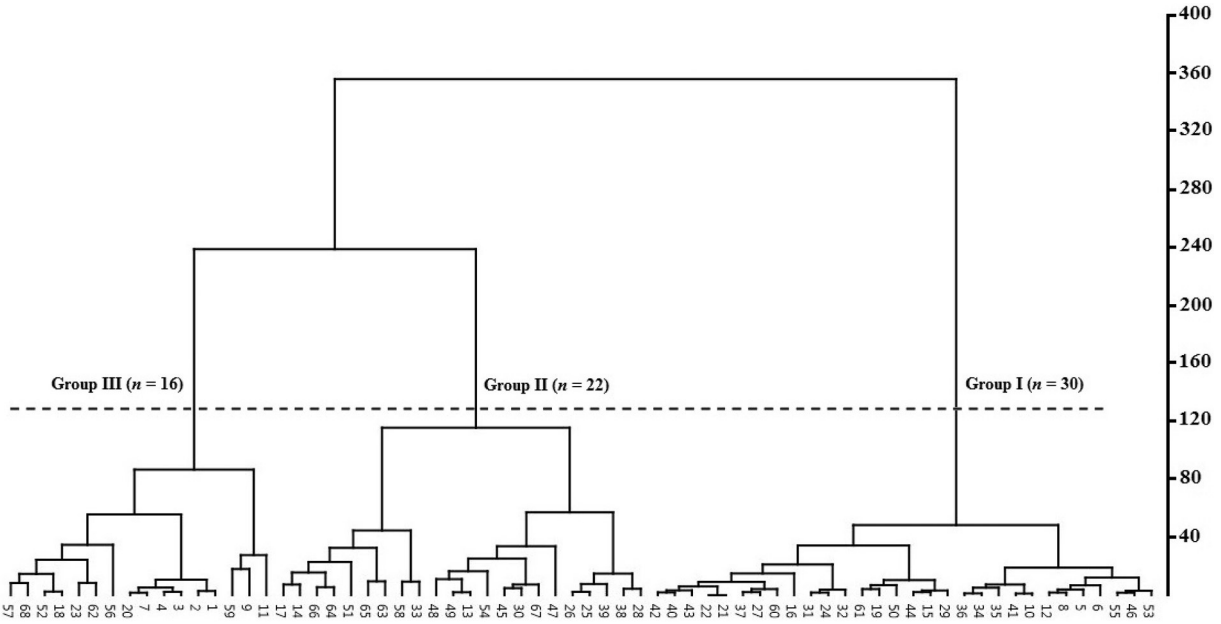

Fig. 3. Dendrogram obtained by cluster analysis, representing chemical composition similarity relationships between the 68 EO samples of *O. grosii* (Group I: carvacrol, Group II: *p*-cymene and Group III: thymol).

at the INRA experimental field in Sefrou (Morocco). The chemical composition of these transplanted genotypes was dominated either by carvacrol (G4.31, G4.32, G5.42 and G5.43), *p*-cymene (G4.30, G4.33 and G6.54) or by thymol (G3.20) from original plants in the wild (before transplantation). Two principal pedoclimatic factors were considered significantly different between spontaneous populations and the experimental field. In natural conditions, the studied populations were characterized by clay-silt and poor soils with periodic water stress (Table 3, Fig. 5). In contrast, in the experimental field the transplanted plants have been regularly irrigated, and the soil was silt-clay and rich in K_2O and P_2O_5 (Table 3, Fig. 5).

The results exhibited that the biomass production had greatly increased under experimental field conditions (dry biomass; 60–180 g). Moreover, the EO production was positively affected by transplantation (Table 4). Furthermore, the composition of the EOs obtained after the transplantation showed considerable variability in the major compound contents: *p*-cymene, γ-terpinene and carvacrol, and to a lesser extent in thymol and thymoquinone (Table 4). Nevertheless, no noticeable variability has been detected in the percentages of minor compounds. In fact, significant decrease in *p*-cymene production was observed after transplantation in the majority of the studied genotypes among which those having *p*-cymene as the main compound in the spontaneous state (G4.30, G4.33 and G6.54). In contrast, a remarkable increase of γ-terpinene and carvacrol contents was observed after transplantationin almost all studied genotypes. Thymol production increased after transplantation in genotype G4.33, while it has decreased in genotype G3.20. Moreover, the amounts of thymoquinone decreased in individual plants which contain low concentrations (G4.30, G4.32 and G4.33), whereas it remained relatively stable after transplantation in genotype (G6.54) which was characterized by a noticeable amount of this compound.

4. Discussion

The present study is considered to be the first investigating the intraspecific chemical variability of the EOs of *O. grosii* isolated from individual plants harvested in their natural habitat. Furthermore, this investigation reported an initiative data for domestication of this species by studying the impact of transplantation on the EO yield and composition of defined chemotypes.

4.1. The characteristics of EOs from natural populations

The EO production in *O. grosii* is considered not negligible in comparison to other *Origanum* taxa. Bellakhdar and Il Idrissi (1990) reported a lower EO yield (1.6%) in one population (Jbel Magou–NW of Morocco, 1600 m) of *O. grosii*. Our previous studies of two other Moroccan *Origanum* taxa showed that, the Moroccan endemic *O. elongatum* produced an EO yield ranging between 0.81% and 3.12% (Bakha et al., 2018), and from 0.67% to 2.88% for *O. compactum* (Aboukhalid et al., 2016). In fact, previous works noticed that the highest EO yields have been obtained at the full-flowering stage in many *Origanum* taxa such as *O. majorana* (Sellami et al., 2009; Soliman et al., 2009) and *O. onites* (Kizil et al., 2008), as well as in many other taxa from the Lamiaceae family such as *Satureja rechingeri* (Sefidkon et al., 2007) and *Thymus vulgaris* (Ozguven and Tansi, 1998).

The EOs chemotypes of *O. grosii* found in this study (carvacrol, thymol and *p*-cymene) have been previously reported in many other *Origanum* taxa (Hazzit and Baaliouamer, 2009; Skoula et al., 1999) with some differences in the amounts of the major com- pounds. The specificity of *O. grosii* could be however much more related to the chemotypes rich in *p*-cymene. We report in *O. grosii*the second highest percentage of *p*-cymene (75.56%) in the genus *Origanum* taxa after one composition reported by Figuérédo et al. (2006b) in *O. saccatum* (83.7%). In addition, carvacryl methyl oxide and thymoquinone were present in appreciable amounts in *O. grosii*. The maximum amount of carvacryl methyl oxide in *O. grosii* (14.57%) is higher than in *O. elongatum* (12.66%) (Bakhaet al., 2018). However, the values of carvacryl methyl oxide de- tected in *O. grosii* are lower than those found in *O. vulgare* subsp. *glandulosum* (22.9%) (Houmani et al., 2002) and in *O. compactum* (36.2%) (Aboukhalid et al., 2016). Moreover, the maximum per- centage of thymoquinone (12.18%) is the third highest one foundin the genus *Origanum* after those detected in *O. dictamnus* (22.9%) (Skoula et al., 1999) and in *O. syriacum* (27.7%) (Zgheib et al., 2016).

Bellakhdar and Il Idrissi (1990) found that the EO in one population of *O. grosii* (Jbel Magou-NW of Morocco, 1600 m) was characterized by thymol (35.5%), *p*-cymene (28.5%) and γ-terpinene (13.7%), while Figuérédo (2007) reported one EO from Talassemtane park (Morocco) rich in carvacrol (47.7%), *p*-cymene (13.7%), thymol (12.5%) and

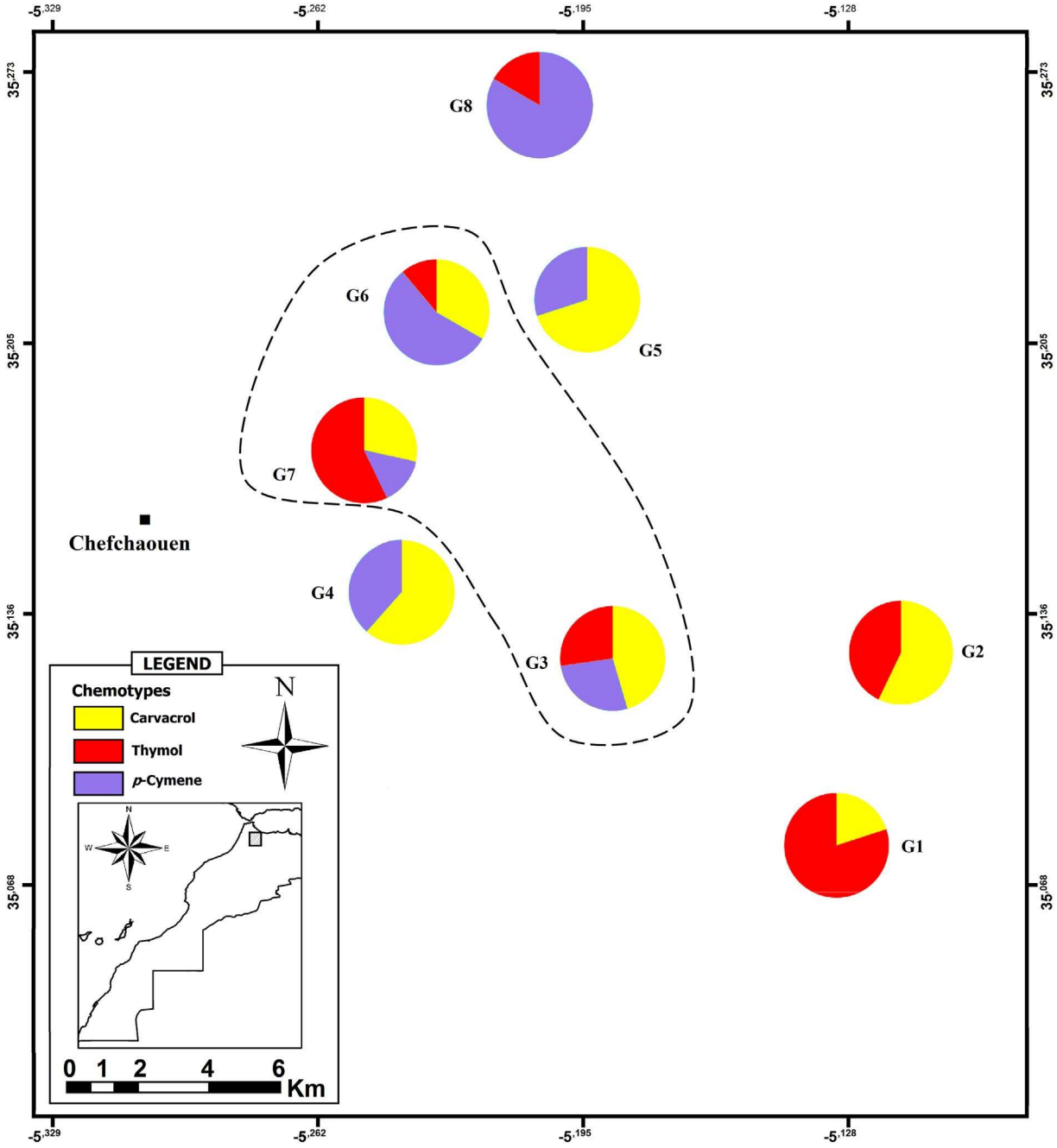

Fig. 4. Geographical distribution of the identified chemotypes of *O. grosii* according to their populations.

γ-terpinene (10.4%). This variability of EOs compositions seems to be mainly due to population differences. Now further studies are needed to assess whether such chemical differences are due to environmental and/or genetic factors. In comparison to the Moroccan *Origanum* taxa, our results indicated that *O. grosii* is chemically closer to *O. elongatum* than to *O. compactum*. In fact, both *O. elongatum* and *O. grosii*, belonging to section *Elongatispica*, contain thymoquinone and thymohydroquinone while *O. compactum* (section *Prolaticorolla*) differs by (E)-β-caryophyllene (up to 11.5%) and germacrene D (up to 12%), and also by high amounts of carvacryl methyl oxide (up to 36.2%) and α-terpineol (up to 25.8%) (Aboukhalid et al., 2016; Bakha et al., 2018). This reinforces the finding of Skoula et al. (1999) who showed that *Origanum* species belonging to the same section share close chemical profiles. Regarding the very important studies about *O. vulgare*, the most widely distributed species and also the most variable, results indicated that the chemical profiles of this species change depending on the harvesting period, geographical origin and from one subspecies to another which makes comparison withthe Moroccan *Origanum* taxa complex (De Martino et al., 2009; Baranauskienė et al., 2013; Lukas et al., 2013). For instance, the wild Iranian *O. vulgare* subsp. *viridulum* (named as *viride)* is characterized by linalyl acetate, sabinene, γ-terpinene, trans-β-ocimene, and cis-β-ocimene (Afsharypour et al., 1997). *Origanum vulgare* subsp. *hirtum* from Italy was found rich in carvacrol/thymol, thymol/ α-terpineol and linalyl acetate/ linalool (De Martino et al., 2009), while *O. vulgare* subsp. *vulgare* from different countries have been reported with various chemotypes such as sabinene, β-ocimenes, β-caryophyllene, germacrene D, cis-sabinene hydrate, α-terpineol, *p*-cymene, carvacrol and thymol (Baranauskienė et al., 2013; Lukaset al., 2013).

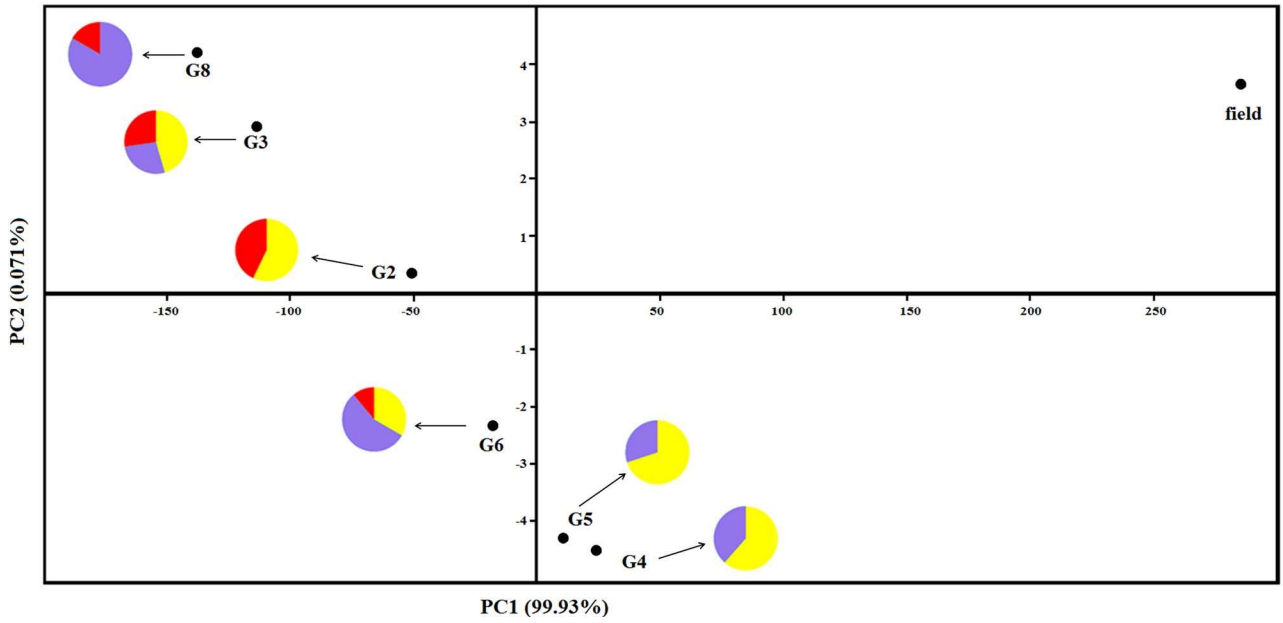

Fig. 5. PCA of the soil characteristics of six natural populations of *O. grosii* and the experimental field conditions (see Table S1) related to the chemical profile of each natural population.

Despite the fact that environmental conditions were relatively homogenous in each population, all studied populations were found chemically heterogeneous and regrouped individual plants from two or three chemical profiles. Moreover, the no correlation between thymol and carvacrol with elevation in *O. grosii* contrasting the finding of Giuliani et al. (2013) who reported that the EO contents of these two compounds were affected by the elevation gradient in *O. vulgare* in Italy, as in the Moroccan *O. compactum* by Aboukhalid et al. (2017). Indeed, it is well recognized that elevation is related to many environmental factors: temperature, precipitations, humidity, wind and sun exposure (Aboukhalid et al., 2017). Similar observations regarding the factors affecting *O. vulgare* essential oil were previously described in the literature (De Martino et al., 2009; Tibaldi et al., 2011). In addition, Aboukhalid et al. (2017) reported that the physical and chemical properties of soil affected notably the EO yield and composition of *O. compactum*. Such result lack of correlation in *O. grosii* may be due to the very restricted geographical distribution area studied (~460 km²) and not affected by the wide elevation gradient (402–1898 m asl) suggesting maybe some fixed genetic factors controlling EO compositions. Further studies on the variations of the environmental conditions along the elevation gradient are requested for a better comprehension of this observed pattern.

4.2. The stability of EOs under field conditions

The increase of EO production in *O. grosii* after transplantation could be mainly attributed to the regular irrigation. Azizi et al. (2009) reported that an optimal irrigation during cultivation could increase the yield of EOs in *O. vulgare*. The same results were reported in geranium (*Pelargonium capitatum* × *P. radens*) by Eiasu et al. (2008). In addition, Bahreininejad et al. (2014) found also that the water stress decreases the essential oil production in *Thymus carmanicus*. In addition to irrigation, the silt-clay soil texture and the important amounts of K_2O and P_2O_5 may also influence the EOs production in *O. grosii* under field conditions.

In the case of the present transplanted genotypes, the transplantation affected the production of the precursors γ-terpinene and *p*-cymene and their conversion to carvacrol and thymol, where the percentages of *p*-cymene decreased drastically after cultivation against a significant increase in carvacrol and γ-terpinene content. The increase of γ-terpinene and carvacrol after transplantation could be explained by the impact of irrigation. Bahreininejad et al. (2014) stated that high increased irrigation also increase carvacrol and γ-terpinene production in *Thymus carmanicus*. In contrast, Azizi et al. (2009) found that carvacrol and thymol were not significantly affected by water stress in *O. vulgare*. On the other hand, the decrease of *p*-cymene production after transplantation in *O. grosii* could be due to the age of transplanted plants. In this regard, Mechergui et al. (2016) found the same results in *O. vulgare* subsp. *glandulosum* from Tunisia where the amounts of *p*-cymene decreased from one harvest year to another.

Table 3
Chemical and physical characteristics of soil from natural populations and experimental field.

Soil parameters	Natural populations	Experimental field
Depth (cm)	0–30	0–30
pH	7.81–8.18	7.7
K_2O (ppm)	108–270	530
P_2O_5 (ppm)	6.93–9.24	35.1
Organic Matter (%)	2.37–3.36	4.94
Electrical Conductivity (dS/m)	0.33–0.59	1.38
Texture	Clay-silt	Silt-clay

Table 4
The amounts of major compounds of the transplanted chemotypes in the spontaneous state (S) and in the experimental field (T).

	G3.20		G4.30		G4.31		G4.32	
	S	T	S	T	S	T	S	T
EO yield (%)	2.46	2.75	2.01	2.17	2.45	2.57	1.98	1.84
p-Cymene	8.39	16.49	43.35	6.81	20.61	9	22.78	9.32
γ-Terpinene	4.2	13.4	0	4.38	0.09	7.16	0	4.34
Thymoquinone	0.04	0	1.81	0	0	0	1.25	0.08
Thymol	68.37	52.49	0.12	0.36	0.18	0.28	0.16	0.34
Carvacrol	8.62	3.53	42.09	81.12	69.04	73.14	66.93	77.31
	G4.33		G5.42		G5.43		G6.54	
	S	T	S	T	S	T	S	T
EO yield (%)	2.53	2.89	1.94	2.05	2.17	2.02	2.25	2.54
p-Cymene	43.51	7.79	12.7	8.92	12.31	8.97	55.87	4.05
γ-Terpinene	0	5.51	2.47	6.68	5.25	9.73	0	0.4
Thymoquinone	2.5	0.04	0	0	0	0	12.18	12.61
Thymol	25.82	44.78	0.19	0.07	0.19	0.37	0	1.8
Carvacrol	13.62	33.43	74.96	74.03	72.25	68.88	21.19	69.34

Regarding the effect of the soil constitution on EO composition, Aboukhalid et al. (2017) noticed that silt and sand contents are negatively related to thymol and carvacrol production in *O. compactum*, while in our case the important rates of the silt, K_2O and P_2O_5 in the experimental field soil were related to a decrease of *p*-cymene and an increase of carvacrol and γ-terpinene contents. The nitrogen fertilization and growth conditions are important factors that could also affect EO production under field conditions. Results reported by Omer (1999) exhibited that nitrogen fertilization affected the composition of the EO of *O. syriacum* by increasing thymol and carvacrol with a simultaneous decrease of *p*-cymene and γ-terpinene. However, in *O. vulgare* subsp. *hirtum* nitrogen fertilization significantly increased carvacrol and decreased thymol and *p*-cymene amounts (Karamanos and Sotiropoulou, 2013). In contrast, Azizi et al. (2009) showed that EO composition of *O. vulgare* was not affected by nitrogen fertilization. On the otherhand, some differences in the EO composition of *O. vulgare* have been detected according to the growth conditions; plants cultivated on single rows produced sabinene-rich oil, while those grown in double rows were richer in ocimenes (De Falco et al., 2013).

Furthermore, plant growth stage is another factor to take into account that could influence the chemical composition of the EOs. Within the genus *Origanum*, different responses have been shown in EO composition according to the harvest time. Carvacrol was identified as major compound in *O. onites* at the flowering period (Yaldiz et al., 2005). In *O. syriacum*, thymol and γ-terpinene reached their highest level in the flowering stage, whereas *p*-cymene was the main component in the early spring (Toncer et al., 2010). On the other hand,Casiglia et al. (2015) reported that *p*-cymene and γ-terpinene decrease and carvacrol increase from May to July in *Thymus capitatus*. Althoughthe biosynthesis of the EOs compounds is genetically controlled, the variation of environmental conditions could affect their gene expression and consequently influences their biosynthesis pathway. Therefore, the choice of chemically defined plants with relatively stable composition and yield could only be guaranteed under high controlled conditions.

5. Conclusions

This investigation provides the first database concerning the chemical variability of EOs of the endemic *O. grosii* which has a restricted geographical distribution in NW of Morocco. The present results showed that wild growing *O. grosii* is characterized by an important production of EO during the flowering stage. Furthermore, domestication of this species favored biomass and EOs production. The chemical analysis of EOs pointed out an appreciable intraspecific and intrapopulation diversity. As a result, carvacrol, thymol, *p*-cymene and γ-terpinene represented the most prevalent compounds, whereas thymoquinone, thymohydroquinone and carvacryl methyl oxide were detected at considerable concentrations in some individual plants. The revealed major compounds are known for their strong biological activities, especially carvacrol and thymol, consequently they could be used in pharmaceutical and food industries as natural products. On the other hand, the transplantation of chemically defined genotypes induced some changes in their EO composition. Generally, an increase of carvacrol and γ-terpinene contents was noticed whereas *p*-cymene decreased. This variation could be attributed to irrigation and soil composition in the experimental field.

Variations in biomass production and, EO yield and composition were observed to occur both in natural conditions and transplantation experiment despite the fixation of the genotype effect. It appears that the chemical conditions of at least some chemotype (e.g. *p*-cymene) can be affected by abiotic factors such as soil nutrient and water stress.More studies are needed to quantify the effects of the different abiotic parameters on the chemical composition of EO. It is also not excluded that biotic interactions with for example phytophagous insects could also affect the chemical composition as a defense response. Finally, the effects of culture conditions (density, shading, irrigation …) on the production and quality of EO are primordial to understand to develop the optimal conditions for domestication of this species.

Conflicts of interest

The authors declare that they have no conflict of interest.

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