



Comparative genetic study among *Origanum L.* plants grown in Egypt

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Abstract

In the present investigation we focus to study the phylogenetic and chemical variability (essential oil) among four *Origanum* species under Egyptian condition. Twenty-three components were identified across the four species of genus *Origanum*. Carvacrol, thymol, p-cymene, and γ -terpinene were found as major components in oils of *Origanum vulgare*, *Origanum vulgare* subsp. *Hirtum* and *Origanum syriacum* var. *sinaicum*, whereas, the volatile oils of marjoram 'cymyl'-compounds are almost completely absent and high percentages of 'sabinyll'-compounds are present. The grouping patterns between the four species provided by ISSR and SRAP were similar, while the combined data of diversity analysis was great accurate to distinguished between the individual species and sub-species. A set of 16 ISSR and 12 SRAP primers combination were compared in terms of their informativeness and efficiency for analysis of genetic relationships among the four *Origanum* species. The ISSR and SRAP exhibited a remarkable variation among the tested species. The SRAP exhibited effective and relatively higher level of assay efficiency index (72.50), effective multiples ratio (16.58) and marker index (16.08), than the ISSR. Considering the results of SRAP marker system seems to be more effective than ISSR for studies on intraspecific diversity and relationships among *Origanum* germplasm. SRAP had more sensitive, distinctive nature and higher discrimination capacity and could simultaneously detect several polymorphic markers per reaction. These findings can be very applicable to resolve the evolution genetic relationships, breeding strategies and management of its genetic resources within the genus *Origanum*.

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Introduction

The genus *Origanum* L. belongs to the family Lamiaceae and comprises 42 species and 18 hybrids widely distributed in Eurasia and North Africa (Din and Dogu, 2013).

This genus includes numerous species, subspecies, varieties, and hybrids that can be distinguished individually, but extensive variation still exists (Azizi *et al.*, 2009). *Origanum* species are usually subshrubs or woody perennial herbs that reach heights of up to 80 cm and have ovate leaves and white or purple flowers. Many of the studies confirmed the medicinal effects of oregano for human health (Azizi, 2010).

In Egypt, *Origanum syriacum* commonly known as 'Syrian marjoram' is an aromatic, herbaceous and perennial plant growing wild in the Sinai desert of Egypt (Tackholm, 1974; Chishti *et al.*, 2013). It is a very popular culinary herb that has been used through ages in traditional medicine mainly in Lebanon and Arab world (Chishti *et al.*, 2013). The genus *Origanum* contains two important aromatic plants with different sensorial qualities, marjoram (*Origanum majorana* L.) and oregano (*Origanum* sp.,) but also species from other genera with similar sensorial qualities are called 'oregano' (Novak *et al.* 2010). The *Origanum* genus, which are rich in essential oils, have been used for thousands of years as spices for food production and as local medicines in traditional medicine (Fleisher and Fleisher, 1988).

The essential oil of *Origanum* genus is composed of carvacrol and/or thymol as dominant components, followed by *y*-terpinene, *p*-cymene, linalool, terpinen-4-ol, and sabinene hydrate (D'Antuono *et al.*, 2000; Skoula and Harborne, 2002). Indeed, medicinal plants are becoming an important research area for novel and bioactive molecules for drug discovery. *Origanum* genus is a plant which has shown to possess several types of biological activity such as, antiradical, antifungal, anti hyperglycemic, antibacterial, antithrombin and the antioxidant

activity (Chishti *et al.*, 2013). Results of various studies indicated that the antioxidant effects of *Origanum* genus might be related to the dominant components, carvacrol and thymol (Elezi *et al.*, 2013). Recently, Tuncer *et al.*, (2013) indicate that *Origanum vulgare* has antitumor activity against breast cancer cell lines *in vitro* and *in vivo*.

The taxonomy of *Origanum* was found to be rather complex and nearly all of the sections are afflicted with some kind of taxonomic uncertainties (Lukas, 2010). Systematic and phylogenetic studies on medicinal and aromatic plants were usually based on morphological characters (Gurcharan, 2004). However, in the last decades, continuous advances in molecular biology have offered a set of new tools useful for investigating the relationships among these taxa and to characterize the peculiar chemical composition of related cultivars (De Mattia *et al.*, 2011). Presently, the advent of molecular markers overcame most of the problems associated with using morphological markers in which major phenotype-altering genes were used as genetic markers (Kumar *et al.*, 2013). Among PCR-based methods, inter-simple sequence repeats (ISSRs) has been found to be an efficient, low cost, simple operation, high stability and abundance of genomic information (Wang, 2010). ISSR technique is a PCR-based method which uses microsatellites as primers in a single reaction targeting multiple genomic loci mainly to amplify ISSR sequences of different sizes (Kumar *et al.*, 2013). ISSR is reliable technique for the identification of species or varieties, population authentication and population genetic structure, etc. (Liu *et al.*, 2009). In the context (Novak *et al.*, 2008a), reported that microsatellite loci may also be useful in other closely related Lamiaceae like mint, thyme, sage and savory which are valuable medicinal and aromatic plants.

Sequence-related amplified polymorphism (SRAP), is a PCR-based marker system which aimed for the amplification of open reading frames (ORFs) (Li and Quiros, 2001; Li *et al.*, 2013). The SRAPs is a simple and efficient marker system that can be adapted for a

variety of purposes including germplasm identification, map construction, genetic diversity and gene tagging in various crops, such as potato, rice, lettuce, cotton, most crops, tree species, ornamental and medicinal plants (Amar *et al.*, 2011; Li *et al.*, 2013). Rather, the result of SRAP markers was a moderate number of co-dominant markers (Agarwal *et al.*, 2008). Unfortunately no molecular marker technique is ideal for every situation. Each marker has advantage and disadvantage, thus combining different marker system were greatly better for diversity study in medicinal and aromatic plants.

In the present investigation, we sought to determine a new basis for the ongoing discussion about the taxonomic uncertainties concerning *Origanum* and *Majorana*. In more specifically, (1) to assess species limits and taxonomic status of and to *Origanum syriacum var. sinaicum*, *Origanum vulgare*, *Origanum vulgare* subsp. *Hirtum*, and *Origanum majorana*, (2) discuss the molecular evolutionary relationships and the discriminating capacity of each marker system within the section *marjoram* and *oregano* by linking molecular with phytochemical evidence.

Materials and methods

Plant materials

A panel of four *Origanum* plants species were grown in a greenhouse at the beginning of October (Desert Research Center, North Sinai Research Station, Egyptian Deserts Gene Bank, Egypt) (Table 1). After about a month, the successful seedlings were transferred to the plot in a separate line.

During the field work the above ground parts of individual plants of *Origanum syriacum var. sinaicum*, *Origanum vulgare*, *Origanum vulgare* subsp. *Hirtum*, and *Origanum majorana*, were collected at the beginning of flowering stage. The plants were dried at room temperature and leaves/flowers separated from stem and stored for further analysis of essential oil.

Seeds of *Origanum vulgare* and *Origanum vulgare* subsp. *Hirtum*, were kindly provided by AERI Institutional Linkage Project the holder of the U.S. Agency for International Development (USAID)-funded AERI/ILA Cooperative Agreement (ENZA ZADEN co). While *Origanum majorana* and *Origanum syriacum var. sinaicum* were provided by the Egyptian Deserts Gene Bank, Egypt.

Extraction of essential oil

The essential oil percentage was determined using dried plant material according to British Pharmacopoeia (1963). Satisfactory results were obtained by distilling 50gm of dried plant material for three hours in a flask of 1000 cc capacity.

GC/ Mass analysis of volatile oil.

The essential oil was analyzed on a VG analytical 70-250S sector field mass spectrometer, 70 eV, using a SPsil5, 25 m x 30 m, 0.25 µm coating thickness, fused silica capillary column, injector 222°C, detector 240°C, linear temperature 80°–270°C at 10°C/min. Diluted samples (1/100, v/v, in n-pentane) of 1 µl were injected, at 250 °C, manually and in the splitless mode flame ionization detection (FID) using the HP Chemstation software on a HP 5980 GC with the same type column as used for GC/MS and same temperature program. Identifications were made by library searches (Adams, 2007) combining MS and retention data of authentic compounds by comparison of their GC retention indices (RI) with those of the literature or with those of standards available in our laboratories (Adams, 2007).

Isolation of Genomic DNA (gDNA).

Total genomic DNA of *Origanum* sp. was extracted from young leaves (100 mg per plant) of five week-old plants following the CTAB procedure according to Doyle and Doyle, (1990). After RNase treatment, the DNA content was quantified by use of a Quawell Q5000 UV-Vis spectrophotometer (V2.1.4, USA). Genomic DNA of ten plants per species was bulked and diluted to 50 ng/µl working solution. Both the stock and diluted portions were stored at -20°C.

ISSR analysis

The ISSR-PCR amplification was carried out following method, previously described by Sankar and Moore, (2001). Among twenty ISSR primers, sixteen reselected primers which were synthesized by (Metabion, Germany), gave highly levels of polymorphism. ISSR amplifications were performed in a final volume of 25µl, containing 50 ng DNA, 0.3µM of ISSR primer, 200 µM of dNTPs, 3.5µl of Green PCR buffer, 1 unit of DreamTaq Green DNA Polymerase (Thermo Scientific Inc, Germany), and sterile doubled-distilled water.

The PCR reaction program was carried out in Agilent's (SureCycler 8800 thermal cycler, USA), consisted of: 1 cycle at 94 °C, 4 min; 35 cycles of 94 °C, 45 s; 45°C, 45 s; 72°C, 1 min; 1 cycle at, 72°C, 10 min; °C and 4°C for infinitive. Agarose gel electrophoresis (1.2%) used for resolving the PCR amplification products. Bands were detected using Bio Rad Gel Doc™ XR+ imaging system with Image Lab™ (USA), (Fig. 2a).

SRAP analysis

The SRAP analysis was performed as described by Li and Quiros (2001). SRAP primer combinations were screened using 25 different combinations which employed using five forward and reverse primers. All reagents and their buffers were supplied by Thermo Scientific Inc, (Germany). Each PCR contained a reaction mixture of 25µl made up of 30 ng of genomic DNA, 200 µM of dNTPs, 0.3 µM of each primer, 3.5µl of Green PCR buffer, 1 unit of Taq DNA polymerase, and sterile doubled-distilled water.

PCR cycling parameters included 4 min of denaturing at 94°C, five cycles of three steps: 1 min of denaturing at 94°C, 1 min of annealing at 35°C and 1min of elongation at 72 °C. In the following 35 cycles the annealing temperature was increased to 50 °C, and for extension, one cycle of 7min at 72 °C. A 100 bp DNA ladder was used as molecular standard in order to confirm the appropriate SRAP markers. The amplification products were separated by

electrophoresis in a 6% polyacrylamide gel visualized by a simplified silver staining method (Xu *et al.*, 2002) (Fig. 2b).

Band scoring and data analysis

Profiles for each species and marker technique (ISSR, SRAP) were constructed by scoring 0 and 1 for absence and presence of bands and assembled onto a data matrix. Comparisons of the discriminating capacity, level of polymorphism and informativeness of each marker system of ISSR and SRAP were calculated according to Belaj *et al.* (2003). However, polymorphism information content (PIC) values were calculated according to Smith *et al.* (1997), using the following formula as follows:

$$PIC = 1 - \sum f_i^2,$$

f_i is the frequency of the allele. PIC provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles but also the relative frequencies of those alleles. PIC values vary from 0 (monomorphic) to 1 (very highly discriminative, with many alleles in equal frequencies). To compare the efficiency of the two markers in *Origanum* species, we estimated the following parameters for each assay unit.

- Number of monomorphic bands (n_{mp})
- Number of polymorphic bands (n_p)
- Average number of polymorphic bands/assay unit (n_p/U)
- Number of allele (L)
- Number of allele /assay unit (n_u)
- Total number of effective alleles (N_e)
- Polymorphism information content (PIC)
- Expected heterozygosity of the polymorphic loci (H_e)
- Fraction of polymorphic loci (β)
- Assay efficiency index (A_i)
- Effective multiplex ratio (EMR)
- Marker index (MI)

The genetic similarity between individual pairs of species was analyzed by using the NTSYS pc 2.1 software (Rohlf, 2000). The dendrogram were

obtained through clustering analysis by the unweighted pair-group method (UPGMA). To verify the adjustment between similarity matrices and respective dendrogram derived matrices, the cophenetic correlation coefficient (*r*) was estimated using the software NTSYS pc 2.1.

Results

Essential oil content

In the present investigation the essential oil content (Table 1 and Fig. 1) presents the wide range of variability among *Origanum syriacum var. sinaicum*, *Origanum vulgare*, *Origanum vulgare* subsp. Hirtum, and *Origanum majorana* under Egyptian conditions. Twenty-three components were identified across the four species of genus *Origanum*. These components constituted 93.69. % (*Origanum syriacum var. sinaicum*) to 97.32% (*Origanum vulgare*) of the total oil. The main components of the oil in *Origanum vulgare* and *Origanum vulgare* subsp. Hirtum, were carvacrol (41.1-65.3%) followed by thymol (12-11%), γ -terpinene (9.50-4.20%) and p-cymene (7.1-3.7%) which was the most abundant components during the vegetative cycle (Table1 and

Fig. 1).While the major components of the oil in *Origanum syriacum var. sinaicum* was thymol (24%), α - and γ -terpinene (11-15%), carvacrol (9.26%) and p-cymene (6.9%). With respect to *Origanum majorana*, the main components of the oil was Terpinene-4-ol (26.1) followed by γ and α -terpinene (14-10%), Sabinene (8.14%) and p-cymene (6.86%).

Genetic similarity and phylogenetic relationships

Genetic similarity coefficients among four *Origanum* species examined were calculated separately using ISSR, SRAP and the combined data as shown in Table 4. The average similarity coefficients for ISSR, SRAP and the combined data were ranged from (0.58-0.65), (0.45-0.56) and (0.40-0.60) respectively. All genotypes, including bud sports, could be differentiated by each of the molecular markers.

Table 1. Essential oil Compounds among of four different *Origanum* species in Egypt.

Scientific name	<i>Origanum syriacum var. sinaicum</i>	<i>Origanum vulgare</i>	<i>Origanum vulgare</i> subsp. <i>Hirtum</i>	<i>Origanum Majorana</i>
Compound				
α- Pinene	2.55	1.6	0.56	1.11
Sabinene	—	—	0.79	8.14
Camphene	0.19	—	0.12	—
β- Pinene	0.27	0.88	—	4.88
Myrcene	2.4	1.42	1.68	1.14
Limonene	—	2.99	0.45	3.2
Thymol	24.05	12.21	11.33	—
γ- Terpinene	14.82	9.45	4.2	14.21
α- terpinene	11.22	3.17	0.75	10.22
Terpinene-4-ol	4.5	3.46	0.53	26.12
Borneol	—	0.53	0.18	—
Linalool	0.55	3.04	0.55	1.32
p-Cymene	6.85	7.05	3.67	6.86
Cis-sabinene hydrate	1.3	3.05	—	3.24

trans-Sabinene hydrate	0.91	—	0.45	1.54
1.8 cineole	0.4	2.22	0.34	0.55
Terpinolene	10.27	—	0.21	2.5
carvacrol	9.26	41.14	65.25	—
Carvone	—	0.33	0.35	0.2
Isoborneol	2.51	1.88	2.55	1.35
β-caryophyllene ne	1.64	2.26	0.7	1.2
α- Terpineol	—	—	0.26	6.56
α- thujene	—	0.64	0.81	0.67
Total	93.69	97.32	95.73	95.01
Max	24.05	41.14	65.25	26.12
Min	0.19	0.33	0.12	.0.2

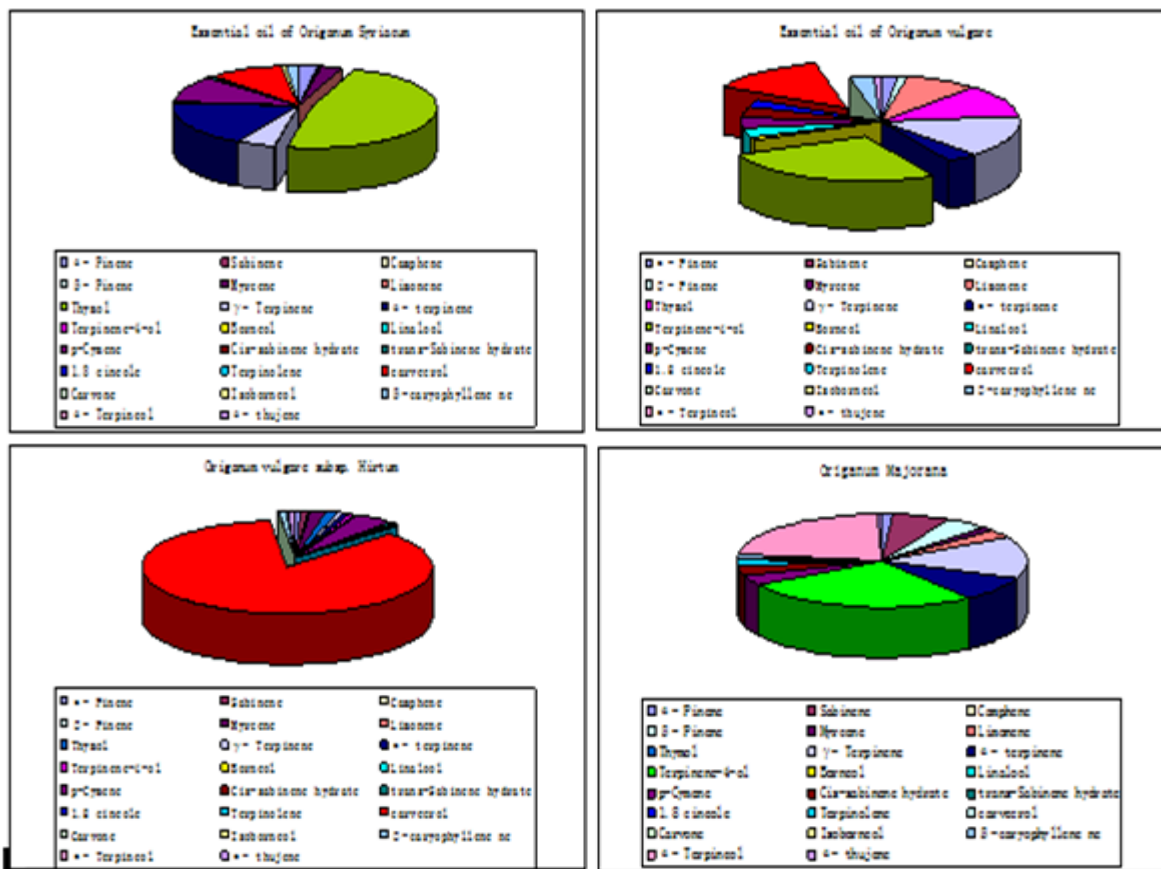
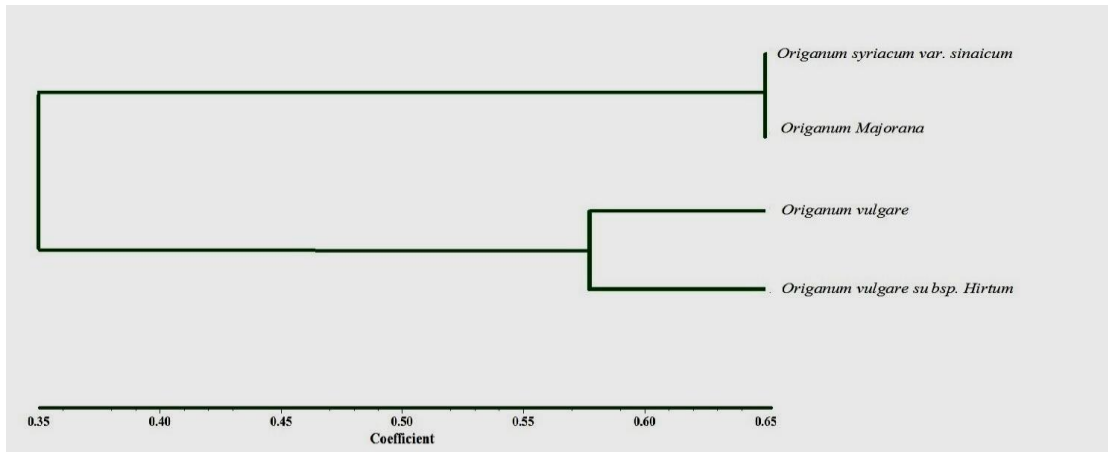
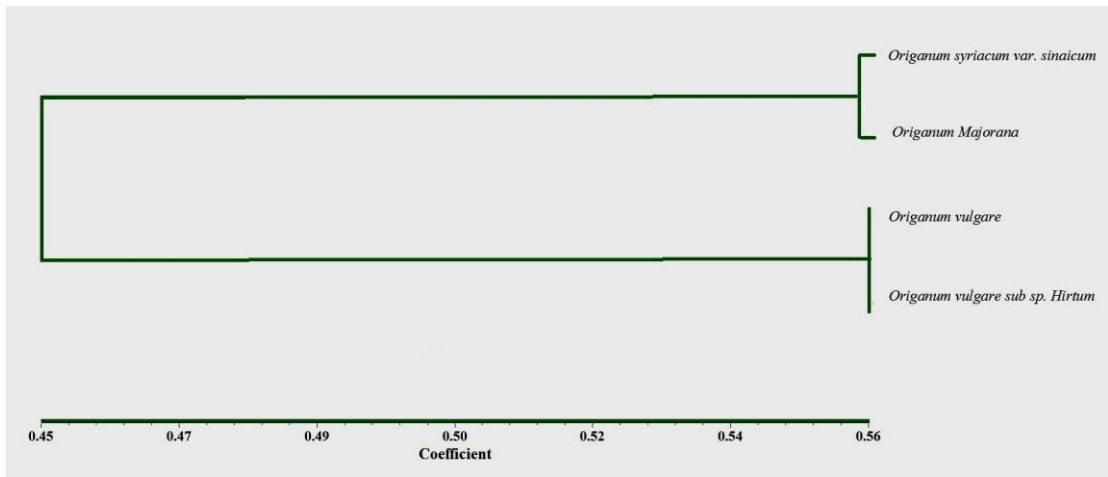


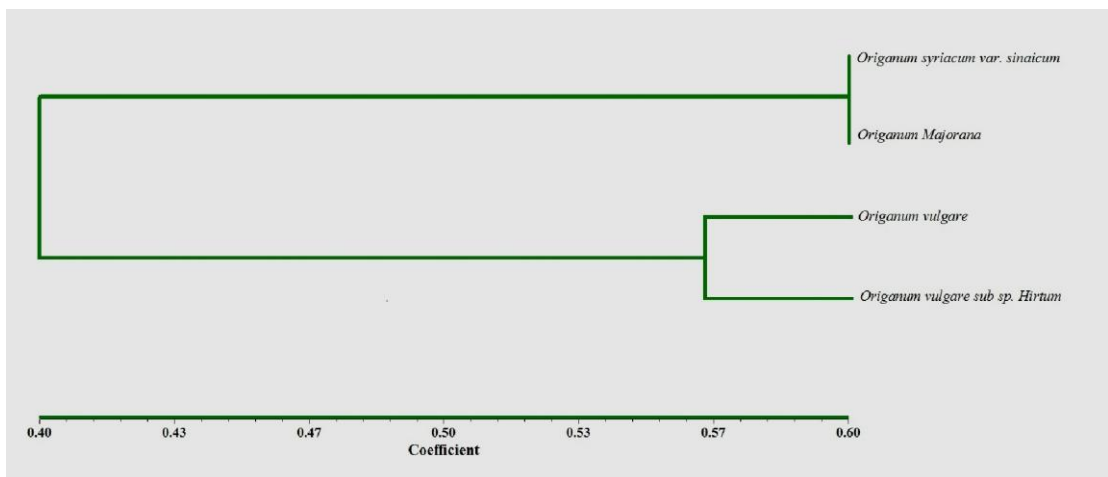
Fig. 1. Schematic representation of the essential oil content among four species of *Origanum*.



(a) ISSR.



(b) SRAP.



(c) Combined tree based on ISSR and SRAP.

Fig. 3. An unweighted pair-group method with arithmetic averages (UPGMA) dendrogram of genetic relationships among four species of *Origanum* based on the Dice similarity coefficients obtained using ISSR, SRAP and combined data.

Table 4. Average of minimum, maximum values of Dice similarity coefficients and correlations among matrices (ISSR and SRAP) four *Origanum* species.

Marker system			
Parameters	ISSR	SRAP	ISSR and SRAP
Minimum	0.58	0.45	0.40
Maximum	0.65	0.56	0.60
correlation (<i>r</i>)	0.89	0.76	0.67

The cophenetic correlation coefficient between the dendrogram and the original similarity matrix was significant for ISSR ($r = 0.89$), SRAP ($r = 0.76$) and the combined data of ISSR and SRAP ($r = 0.67$), giving a good degree of confidence in the association obtained for the *Origanum* germplasm.

Three dendrogram were constructed (Fig. 3 a, b and c) to express the results of cluster analysis based on data obtained from the ISSR, SRAP and the combined data. The result revealed that the ISSR, SRAP and the combined markers were able to cluster the two species from Egypt, *Origanum syriacum* var. *sinaicum* and *Origanum majorana* all together with narrow relationships in the first main cluster. Meanwhile, the two species from Germany, *Origanum vulgare* and *Origanum vulgare* subsp. *Hirtum* were assembled together in the second main cluster.

Comparison of polymorphic levels and informativeness obtained with ISSR and SRAP markers

The levels of polymorphism detected with each marker technique (ISSR and SRAP) and the index comparing their informativeness are reported in Tables 2 and 3. The two marker systems examined turned out to be useful tools for detection of polymorphism and assessing genetic diversity in *Origanum* germplasm, but the degree of resolution depended on the technique applied. We initially tested 20 ISSR and 36 SRAP primers combination

between the four *Origanum* species. Among all, 16 ISSR and 12 SRAP primers gave high levels of polymorphism as exposed in Fig. 2. The total numbers of polymorphic amplicons varied from 177 for ISSR to 199 for SRAP markers. The total number of amplicons scored for ISSR and SRAP was relatively high, 191 and 222, respectively. The average number of polymorphic amplicons per assay unit (n_p/U) correlate positively with the total number of amplicons; the average number of polymorphic amplicons per assay unit was 11.06 for ISSR and 16.58 for SRAP. In the context, the value of number of allele per assay unit was relatively higher in SRAP compared to ISSR markers system (18.50 and 11.93) respectively. With the view of the total number of effective alleles (N_e) was consistent with the total number of allele. In contrast, the average of PIC for ISSR and SRAP markers system was nearly similar and relatively high, 0.96 and 0.97, respectively. Herein, the above result revealed that SRAP markers were the most suitable marker in total polymorphic amplicons, the average number of polymorphic amplicons per assay unit, the number of allele per assay unit and PIC values.

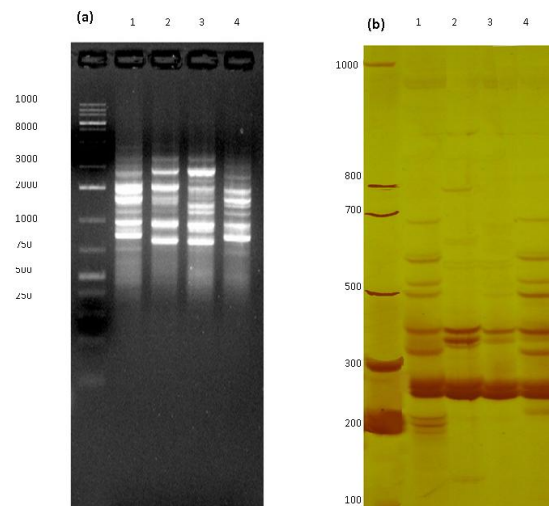


Fig.2. (a) ISSR, and (b) SRAP profiles of four species of *Origanum*.

Fig. 2. (a) ISSR, and (b) SRAP profiles of four species of *Origanum*.

Table 2. Levels of polymorphism and PIC value generated by ISSR and SRAP assays among four *Origanum* species.

Index with their abbreviations		Marker systems	
		ISSR	SRAP
Number of markers	U	16	12
Number of monomorphic amplicons	n_{np}	14	23
Number of polymorphic amplicons	n_p	177	199
Average number of polymorphic amplicons /assay unit	n_p/U	11.06	16.58
Number of allele	L	191	222
Number of allele /assay unit	n_u	11.93	18.50
Total number of effective alleles	N_e	783	870
Min of PIC	PIC	0.99	0.99
Max of PIC	PIC	0.88	0.87
Average of PIC value	PIC	0.96	0.97

Comparison of the discriminating capacity of ISSR and SRAP markers

A comparative scenario of the discriminating capacity of ISSR and SRAP markers are presented in Table 3. Effective number of alleles per locus and the expected heterozygosity of the polymorphic loci for ISSR and SRAP were nearly similar value (2.17, 2.13) and (0.54, 0.53) respectively. This was reflected in higher values of the expected heterozygosity (H_{ep}) for ISSR and SRAP markers. Meanwhile, the very high values of assay efficiency index (72.50), effective multiples ratio (16.58) and marker index (16.08) for SRAP marker highlights the distinctive nature of these markers. This is due to the simultaneous detection of several polymorphic markers per single reaction.

Discussion

Conventional and biotechnological plant-breeding techniques can be applied at the genetic level to improve yield and uniformity of medicinal herbs to bring them into cultivation, and also to modify pharmaceutical potency or toxicity (Canter *et al.*, 2005). Exploitation of the genetic potential of these plants is still in its initial stage, and classical breeding

approaches prevail due to the availability of high natural diversity (Pank, 2007). Subsequently, the useful DNA markers that can correlate DNA fingerprinting data with selected phytochemical compounds would have extensive applications in breeding of medicinal plants based on marker assisted selection (MAS) (Azizi, 2010). *Origanum* L. genus contains two important spices commonly used as spices for cooking with different secondary metabolite content: marjoram and oregano. The aromatic quality of marjoram is generally found in one species in the section Majorana only (*Origanum majorana* L.) (De Mattia *et al.*, 2011). In contrast to marjoram, the quality of oregano arises from many different species, subspecies, varieties, and hybrids that can be distinguished individually, although extensive variation still exists. However, the best qualities of *Origanum* come from different subspecies of *O. vulgare*, *O. onites* and *O. syriacum* (Baser *et al.*, 1993; Azizi *et al.*, 2009; De Mattia *et al.*, 2011). Phytochemical characters are often helpful in phylogenetic considerations (Larsson, 2007). In the present investigation we focus to study the phylogenetic and chemical variability (essential oil)

among four *Origanum* species under Egyptian condition. For chemical characterization in *Origanum vulgare* and *Origanum vulgare* subsp. Hirtum the essential oil profiles was a 'cymyl' type, with carvacrol and/or thymol as main compounds. This result is in agreement with the previous results of (Lucks, 2010; Azizi, 2010) finding carvacrol and/or thymol types were in good correlation to high essential oil yielding. Recently, Crocoll (2011) reported that the pathway for thymol and carvacrol is proposed to start with the formation of γ -terpinene by a monoterpene synthase. These two monoterpenes, their precursors p-cymene, γ -terpinene and β -caryophyllene represented the bulk of the essential oil compounds. Many of the studies reported that carvacrol, thymol, p-cymene, and γ -terpinene were found as major components in oils of *Origanum vulgare* (Baser *et al.*, 1993; Ceylan *et al.*, 2003; Demirci *et al.*, 2004; Toncer *et al.*, 2009). On behalf to *Origanum syriacum* var. *sinaicum* (Baser *et al.*, 2003) reported that γ -terpinene and thymol was to be intermediate in its essential oil composition. Our results were in line with the results of Lukas *et al.*, (2009) who confirmed that, the average values for carvacrol are in a range of 2.7-69.8%, whereas thymol values were between 0.3 and 57.6%. In subsequent studies, Lukas *et al.*, (2010) confirmed that *Origanum syriacum* var. *sinaicum* from Egypt is probably of hybridogenous origin as it appeared intermediate between *O. syriacum* and *O. majorana*. Within the *Origanum majorana* possesses a very different essential oil rich in 'sabinyl'-compounds (cis-/transsabinene hydrate and cis-sabinene hydrate acetate) (e. g. Fischer *et al.*, 1987; Novak *et al.*, 2008b) that are responsible for the specific flavors of *majorana*. Recently, Badee *et al.*, (2013) confirmed that *majorana* volatile oil is rich in terpinen-4-ol, sabinene hydrate, γ -terpinene, p-cymene, α -terpinene, and α -terpineol. Our results demonstrated

that the volatile oils of marjoram 'cymyl'-compounds are almost completely absent and high percentages of 'sabinyl'-compounds (bicyclic monoterpenoids, mainly sabinene, cis- and transsabinene hydrate and cis-sabinene hydrate acetate deriving from the 'sabinyl'-pathway) are present (Azizi, 2010).

The taxonomic within the genus *Oregano* and *Marjoram* seem to remain a considerable problem for breeding programmes and exploring its potential for utilization (Azizi, 2010). Therefore, DNA based molecular markers, which are not affected by environmental conditions; could be employed for the resolving the problem (Azizi *et al.*, 2009). These markers are useful for phylogenetic studies to distinguish plant species and subspecies. Especially the reports on molecular markers in *Origanum* species are rare (Azizi *et al.*, 2009). In the present investigation, one of the main aims in the study was to investigate the efficiency of ISSR and SRAP marker systems in surveying DNA polymorphisms and in detecting genetic relationships among the four species of the genus *Origanum*. The grouping patterns between the four species provided by ISSR and SRAP were similar, while the combined data of diversity analysis was great accurate. Several previous results confirmed that combining different marker system were greatly better for diversity study (Agarwal *et al.*, 2008; Amar *et al.*, 2011).

In view of the performance of the combined tree, *Origanum vulgare*, *Origanum vulgare* subsp. Hirtum, were the stable and independent character of this species, concurrence with the reports of (Novak *et al.*, 2008a; Azizi *et al.*, 2009; Azizi, 2010) confirmed with our results of *Origanum vulgare*, and *Origanum vulgare* subsp. Hirtum, were highly correlated to each other.

Table 3. Comparison of information obtained with and discriminating capacity of ISSR and SRAP markers among four *Origanum* species.

Index with their abbreviations		ISSR	SRAP
Average of the allele frequency	pi_2	0.459	0.468
Effective number of alleles per locus	ne	2.17	2.13
Expected heterozygosity of the polymorphic loci	He	0.54	0.53
Fraction of polymorphic loci	β	0.92	0.89
Expected heterozygosity	Hep	0.003	0.002
Total number of effective alleles	N_e	783	870
Assay efficiency index	A_i	48.93	72.50
Effective multiples ratio	EMR	11.06	16.58
Marker Index	MI	10.62	16.08

For *Origanum majorana* a close relationship to *O. syriacum* var. *sinaicum* was observed. This was especially evident from the microsatellite results where *Origanum majorana* were quite close genetic distance to *Origanum syriacum* var. *sinaicum* (Lucks, 2010). Recently the results of ITS sequence data of Lucks *et al.*, (2010) explain that *Origanum majorana* directly derived from *O. syriacum*. Our findings agreed with the previously reported phylogenetic relationship in the genus *Origanum*.

Comparisons of molecular markers for measuring genetic diversity have been carried out in several plant species (Powell *et al.*, 1996; Milbourne *et al.*, 1997; Belaj *et al.*, 2003). Thus, the marker index (MI) is a convenient estimate for marker efficiency (Milbourne *et al.*, 1997). To obtain a measure of the usefulness of the marker systems, a comparison of the overall efficiency of ISSR and SRAP marker systems was provided by the marker index (MI). As is well known, the marker index value is correlated with the expected heterozygosity, assay efficiency index and effective multiplex ratio (Belaj *et al.*, 2003; Biswas *et al.*, 2011; Amar *et al.*, 2011). In this study the SRAP technique showed comparatively higher value of N_e , A_i , EMR and MI than ISSR. These results suggested that the SRAP had a higher discrimination capacity and could simultaneously detect several polymorphic markers per reaction, which is in concurrence with earlier reports in many plant species (Powell *et al.*, 1996; Li and Quiros, 2001; Belaj *et al.*, 2003; Azizi *et*

al., 2009; Du *et al.*, 2009; Amar *et al.*, 2011; Wang *et al.*, 2012).

In recently studies Li *et al.*, (2013) who confirmed that SRAP could preferentially amplify gene-rich regions in a genome. Consequently, SRAP markers could be more advantageous over ISSR markers due to a big difference in the numbers of polymorphic loci detected by individual SRAP primers.

In conclusion, the present study was basically designed to assess the genetic diversity of the oregano germplasm based on molecular markers and chemical compounds of essential oils. A relatively high correlation between chemotypic patterns and genetic markers was identified. Considering the results of SRAP marker system seems to be more effective than ISSR for studies on intraspecific diversity and relationships among *Origanum* germplasm. SRAP had more sensitive and higher discrimination capacity and could simultaneously detect several polymorphic markers per reaction. To our knowledge, no such studies have been reported yet about comparison of discriminating capacity, efficiency and ability of SRAP and ISSR markers system in *Origanum* germplasm. Consequently, a better understanding of the effectiveness of the different molecular markers is considered a priority step toward *Origanum* germplasm characterisation and classification, and a prerequisite for more effective breeding programs.

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