Genetic study, chemical composition and volatile oil of two parsley cultivars (*Petroselinumcrispum* L.) Used in botanical gardens and cultivated under Egyptian conditions.

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Abstract: The present study was conducted aiming to find out the concordance between vegetative growth and yield production, phytochemical compounds, volatile oil and molecular genetic (RAPD- and ISSR-PCR) characteristics of two varieties of parsley the plain leaf type (sspneapolitanum, Danert) and the curly leaf type (sspcrispum) were cultivated at the Experimental Farm of Agriculture Faculty, Cairo University. The highest oil percentage of fresh parsley herb obtained from the plain leaf type (sspneapolitanum, Danert) in the first and second seasons inin the three cuts. Comparing the curly cultivar, the plain leaf cultivar of parsley was superior in producing higher yield of herb than curly cultivar. GC analysis of the volatile oil prepared by hydrodistillation from aerial parts of parsley, the major constituents of curly leaf type (sspcrispum) essential oil were Myristicin (15.05%), 2-Allyl-4-methyl phenol (10.15%), Isolongipholene (8.59%) and β-Caryophyllene (6.43%). In the volatile oil of (sspneapolitanum, Danert) the major compounds were Myristicin (12.65%), 2-Allyl-4-methyl phenol (10.77%), Apiol (9.5%) and Isolongipholene (9.45%). Generally, the high quality was observed in plain- leafed parsley if compared to curly leafed parsley, which had higher phytochemical contents (chlorophyll a, chlorophyll b, carotenoids, β-Carotene, flavonoids, phenols, total antioxidant capacity, free radical scavenging activity and vit C.). For molecular study Random Amplified Polymorphic DNA (RAPD) was performed which was efficient in detecting polymorphism and genetic variation within and between the two parsley varieties. In RAPD analysis, 5selected primers displayed a total of 76 amplified fragments, in which 49fragments were found to be useful as cultivar specific markers.. The number of total amplified fragments scored per primer ranged from 13 (primer OP-C12) to 18 (primer OP-B07). The tested ISSR primers generated variable banding patterns. 4selected primers displayed a total of 60 amplified fragments, in which 26 fragments were found to be useful as cultivar specific markers. The results indicated thatplain leafed parsley demonstrated higher quality than curly-leafed.

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1. Introduction

Parsley (*Petroselinum crispum* (Mill) Nym) is a biennial herb (A member of the Apiaceae (Umbelliferae) native to Europe and Western Asia, which is cultivated widely asa biennial plant. Botanical gardens all over the world cares about the three main types of parsley are the plain leaf type (ssp*neapolitanum*, Danert) and the curly leaf type (ssp*crispum*), which are cultivated for their foliage, and the turnip-rooted or 'Hamburg' type (ssp. *tuberosum* (Bernh.) Crov), primarily grown for its roots. In addition to their use as a fresh or dried herb, parsley leaves (also seeds) contain essential oils that can be used in perfumes, creams and soaps. Moreover, parsley possesses medicinal properties, first-mentioned by the ancient Greeks **(Simon, 1990).** It is used as a carminative, diuretic, hypertensive, hypotensive, stomachic, nervine, emmenagogic, abortifacient and nutritive agent **(Robbers & Tyler, 1999; Kreydiyyeh & Usta, 2002)** and in decorative design of the botanical gardens and uses as a ground cover provides protection of the topsoil from erosion and drought (Wayne and Sylvia, 2014). Parsley like many other herbs is highly seasonal in nature. It is a very rich source of vitamins C and E, b-carotene, thiamin, riboflavin and organic minerals **(Bakowski & Michalik, 1986; Wills *et a*l., 1986; Michalik & Dobrzan´ ski, 1987; Athar *et al*., 1999).** Fruits and vegetables are primary sources of carotenoids in human diet, and intake of lutein and β-carotene has been associated with decreased risks of cancer and chronic disease. **(Sommerburg, *et al*., Mortensen *et al*., 2001). Gazzani (1994)** found that parsley showed weak antioxidant activity in groundnut oil under various heating conditions. **Fejes *et al*. (1998)** and **(Wong and Kitts, 2006)** investigated the in vitro

antioxidant effect of various extracts prepared from different vegetative organs of parsley and observed that the essential oil plays a significant role in the scavenging effect. The distinctive flavor of parsley comes from p-1,3,8-menthatriene, the dominate volatile oil in the leaves **(López *et al*., 1999).** Monoterpene hydrocarbons, such as β-phellandrene, 1,3,8-p-menthatriene, p-α-dimethylstyrene and terpinolene, are considered to be primarily responsible for the characteristic aroma of fresh parsley **(Freeman *et al*., 1975 and Kasting *et a*l., 1972).** More recently, apiole, **(Macleod *et al*., 1985)** myristicin and myrcene have also been identified as major aroma constituents **(Simon and Quinn, 1988).** Genetic relations between different varieties of parsley are still unknown. The studies of general genetic variety of parsley using molecular markers are relatively few. Methods based on analysis of polymorphism of an amplified DNA fragment is widely applied to identify phylogenetic variability and relations between plants. Methods like application of RAPD and ISSR **(Williams *et al*., 1999).**

Here, for the first time, we describe the similarity and diversity in terms of RAPD and ISSR profiles of two ssp. of parsley, the plain leaf type (ssp*neapolitanum,* Danert) and the curly leaf type (ssp*crispum*), investigate genetic diversity among them and determine whether secondary metabolites such as essential compounds, phytochemical compounds and vegetative growth would be used as taxonomic markers in these varieties and elucidate relationships between genetic and chemical diversity by comparing their hierarchical structures.

2. Materials and Methods

1-Plant materials:

Two varieties of parsley the plain leaf type (ssp*neapolitanum*, Danert) and the curly leaf type (ssp*crispum*) were used in the present study. Table (1) shows the physical and chemical analysis of the experimental soil and Table (2) shows the chemical analysis of the irrigation water.

Table (1): Some physical and chemical properties of the experimental soils.

Characteristics	Giza area
Particle size distribution (%)	Normal soil
Coarse sand	1.18
Fine sand	25.82
Silt	34.22
Clay	38.30
Texture class	Loam clay
Organic matter (%)	0.58
Total $CaCO_3$ (%)	1.63
Bulk density (g cm^{-3})	1.32
Field capacity (%)	27.1
Wilting point (%)	13.0
Available water (%)	14.1
pH	7.88
Electrical conductivity (dS m^{-1})	2.96
Soluble cations (mmoleL^{-1})	
Ca^{2+}	13.8
Mg^{2+}	10.2
Na	4.30
K	1.30
Soluble anions (mmoleL^{-1})	
CO_3^{2-}	--
HCO_3^-	2.60
Cl^-	8.00
SO_4^{2-}	19.00
Available nutrients (mg kg^{-1})	
N	38.71
P	3.89
K	189.33
Fe	3.10
Mn	1.63
Zn	0.68
Cu	0.025

Table (2): The chemical analysis of the used irrigation water.

Characters	EC dS_m^{-1}	pH	Cations (meq / l)				Anions (meq / l)			
			Ca^{2+}	Mg^{2+}	Na^+	K^+	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}
Value	0.387	8.35	1.8	1.0	0.86	0.17	0.00	2.70	1.00	0.14

In October 2014, at the Experimental Farm of Agriculture Faculty, Cairo University the seedlings of the two varieties of parsley were obtained from the Medicinal and Aromatic Plants Section at El-Kanater El-Khairia, Kalubia Governorate, Horticulture Research Institute, A.R.C. The seedlings were 10 – 15 cm in length, with 5 – 8 leaves and were planted in a randomized complete block design (RCBD) with three replicates. The herb was cut at three times (the first half of December, January and March). In October 2015, the seedlings from each cultivar were planted by same previous method. Data of (2014-2015) and (2015-2016) seasons were recorded on the follow traits:

1-Growth parameters:

1-Plant height (m) (P.H)
2- Number of branches/plant
3- Fresh weight / plant (g) (F.W/P)
4-Dry weight /plant (g) (D.W/P)
5- Fresh yield of herb /fed (ton) (F. y/fed)

6- Dry yield of herb /fed (ton) (D. y/fed)

7- Volatile oil percentage in fresh herb (%) (V.O.P)

2-Chemical analysis:

Sample preparation and extraction for phytochemical study:

•Chlorophyll (a, b) and carotenoids determination

The protocol devised by **(Nagata and Yamashta, 1992)** was followed to determine chlorophyll a, b and carotenoids contents. 0.2 gram parsley leaf sample was ground in 10 mL of 80% acetone and filtered through Whatman No. 1 filter papers. The filtered extract was transferred in cuvette and absorbance was noted at 662, 644 and 440 nm by using UV-spectrophotometer. Following formulae were used to calculate chlorophyll a, chlorophyll b and carotene contents.

Chlorophyll a = 0.999 A662 – 0.0989 A644

Chlorophyll b = -0.328 A662 + 1.77 A644

Carotenoids = 4.695 X A 440 – 0.268 X A664 + A662

• β-Carotene and lycopene content

β-Carotene and lycopene were determined according to the method of **(Nagata and Yamashta, 1992).**

•For methanolic extraction: grounding (2 g) green leaves in a pestle with 20 ml of 80% methanol. The homogenate was filtered to obtain methanolic extraction colorless.

Spectrophotometric measurements

The spectrophotometric measurements were performed using an ultraviolet-visible spectrophotometer (model MA9523-SPEKOL 211, ISKRA, Horjul, Slovenia).

• Total phenols

The total phenolics content of methanolic extract was determined according to the method described by **(Singleton *et al*., 1999)** by folin-ciocalteu reagent. The absorbance was recorded at 725nm.

• Total flavonoids

Total flavonoids were estimated using method of **(Woisky and Salation, 1998)** using aluminum chloride; the absorbance was measured at 420 nm.

1. Ascorbic acid content

Ascorbic acid was determined according to the method of **(Klein and Perry, 1982)**

• Total antioxidant capacity

The total antioxidant capacity of moringa leaves extracts was evaluated by the phosphomolybdenum method by **(Prieto *et al*., 1999).** The absorbance of the solution was measured at 695 nm with a spectrophotometer against methanol as the blank. Ascorbic acid (AA) was used as the standard.

2. Diphenyl-1-picrylhydrazyl free radical scavenging activity

The capacity to scavenge the "stable" free radical DPPH was monitored according to the method of Takao et al. [23] adopted with suitable modifications from **(Kumarasamy *et al.*, 2007).**

Determination of essential oil content and composition:

Essential oil percentage was determined in fresh herbs according to the method described in the **British Pharmacopoeia (1963**). Oil yield per plant was calculated by multiplying oil percentage by herb yield/plant and expressed as ml/plant. Samples taken for the essential oil obtained in the second season were analyzed using DsChrom 6200 Gas Liquid Chromatography equipped with a flame ionization detector for separation of volatile oil constituents. The analysis conditions were as follows:-

The chromatograph apparatus was fitted with capillary column BPX-5, 5% phenyle (equiv.) polysillphenylene-siloxane 30m X 0.25 mm ID X 0.25µm film. Temperature program ramp increase with a rate of 10°C/ min from 70º to 200º C. Flow rates of gases were nitrogen at 1 ml / min, hydrogen at 30 ml/ min and 330 ml / min for air. Detector and injector temperatures were 300ºC and 250ºC, respectively. The obtained chromatogram and report of GC analysis for each sample were analyzed to calculate the percentage of main components of volatile oil.

3-Characterization of parsley at Molecular Level:

Random Amplified Fragment DNA (RAPD-PCR) Analysis:

In order to obtain clear reproducible amplification products, different preliminary experiments were carried out in which a number of factors were optimized. These factors included PCR temperature cycle profile and concentration of each of the template DNA, primer, MgCl2 and Taq polymerase. A total of twenty random DNA oligonucleotide primers were independently used according to **Williams et al. (1990)** in the PCR reaction. Only five primers succeeded to generate reproducible polymorphic DNA products. The PCR amplification was performed in a 25 µl reaction volume containing the following: 2.5 µl of dNTPs (2.5 mM), 1.5µl of Mg Cl2 (25 mM), 2.5 µl of 10x buffer, 2.0 µl of primer (2.5 µM), 2.0 µl of template DNA (50 ng/µl), 0.3 µl of Taq polymerase (5 U/µl) and 14.7 µl of sterile ddH2O. The reaction mixtures were overlaid with a drop of light mineral oil per sample. Amplification carried out in Techni TC-512 PCR System. The reaction was subjected to one cycle at 95ºC for 5 minutes, followed by 35 cycles at 96 ºC for 30 seconds, 37 ºC for 30 seconds, and 72 ºC for 30 seconds, then a final cycle of 72 ºC for 5 minutes. PCR products were run at 100 V for one hour on 1.5 % agarose gels to detect polymorphism between the

two parsley varieties under study. Only five primers succeeded to generate reproducible polymorphic DNA products. Table (3) lists the base sequences of these DNA primers that produced informative polymorphic bands. The PCR products were separated on a 1.5 % agarose gels and fragments sizes were estimated with the 100bp ladder marker (5000, 4000, 3000, 2500, 2000, 1500, 1000,750,500 and 250 bp).

Inter Simple Sequence Repeat (ISSR-PCR) Analysis:

ISSR-PCR reactions were conducted by using five primers. Amplification was conducted in 25 µl reaction volume containing the following reagents: 2.5 µl of dNTPs (2.5 mM), 2.5 µl MgCl2 (2.5 mM), and 2.5 µl of 10 x buffer, 3.0 µl of Primer (10 pmol), 3.0 µl of template DNA (25 ng/µl), 1 µl of Taq polymerase (1U/ µl) and 12.5 µl of sterile dd H2O. the PCRs were programmed for one cycle at 94º C for 4 min. followed by 45 cycles of 1 min. at 94 ºC, 1 min. at 57 ºC, and 2 min at 72 ºC the reaction was finally stored at 72 ºC for 10 min. The PCR products separated on a 1.5 % agarose gels and fragments sizes estimated with the 100bp ladder marker. Only five primers succeeded to generate reproducible polymorphic DNA products. Table (3) lists the base sequences of these DNA primers that produced informative polymorphic bands.

Table (3): List of the primer names and their nucleotide sequences of RAPD and ISSR procedures used in the study.

ISSR Primer		RAPD Primer	
Primer Name	**Sequence**	**Primer Name**	**Sequence**
HB-8	5`GAG AGA GAG AGA GG 3`	OP-A01	5´CAGGCCCTTC 3´
HB-9	5`GTG TGT GTG TGT GC 3`	OP-A07	5´GAAACGGGTG 3´
HB-10	5`GAG AGA GAG AGA CC 3`	OP-B07	5´GGTGACGCAG 3´
HB-11	5`GTG TGT GTG TGT TGT CC 3`	OP-B11	5`GTAGACCCGT 3`
HB-12	5`CAC CACCAC GC 3`	OP-C12	5` TGTCATCCCC 3`

Statistical analysis:

A randomized complete block design (RCBD) was adopted for the present trial data and statistically analyzed by the factorial method according to **(Snedecor and Cochran, 1990)** where L.S.D test was used for comparison between means. The DNA bands generated by each primer were counted and their molecular sizes were compared with those of the DNA markers. The bands scored from DNA profiles generated by each primer were pooled together. Then the presence or absence of each DNA band was treated as a binary character in a data matrix (coded 1 and 0, respectively) to calculate genetic similarity and to construct dendrogram tree among the studied apricot strains. Calculation was achieved using Dice similarity coefficients **(Dice, 1945)** as implemented in the computer program SPSS-10.

3. Results and Discussion

Vegetative growth

Table (4) shows plant height of the two cultivars grown under Egyptian conditions. Two cultivars showed significant variation in plant height in the three vegetative cuts of both growing seasons. Plain leaf parsley gave the highest plants at all cuts and in both growing seasons. The same direction was observed in the case of branches number, fresh and dry weights per plant. In prevalent increasing the two previous characters; plant height, fresh and dry weights were in the side of plain leaf parsley type which produce the heaviest dry weight/plant, in the three cuts though both seasons. In the second order, curled type came, then the other ones. The fresh and dry herb yields ton/fed of parsley cultivars varied significantly in through the three cuts in both seasons (Table5). Comparing the curly cultivar, the plain leaf cultivar of parsley was superior in producing higher yield of herb than curly cultivar. The results of this research showed the difference betweengrowth parameter of parsley plants. Although **Kmiecik and Lisiewska (1999)** have indicated the significant differences between the productivity of plain and curly leafed hybrids of parsley planted in northern Europe, **Petropoulos *et al.* (2008)** found that the cultivation of such hybrids in Mediterranean basin did not lead to any differences in productivity.

Table (4): Vegetative growth characters of the two parsley cultivars grown under Egyptian condition.

Varieties	First season			Second season		
	first cut	second cut	Third cut	first cut	second cut	Third cut
	plant height (cm)					
Plainleaf	38.03	32.12	33.18	31.11	35.61	36.22
Curley	22.05	24.21	20.11	20.67	25.23	21.56
LSD	3.23					
	Number of branches/plant					
Plainleaf	12.31	15.12	15.10	20.02	20.08	23.17
Curley	8.52	11.05	10.86	8.69	11.23	12.33
LSD	2.33					
	Fresh Weight/plant (g (					
Plainleaf	21.67	41.30	43.26	24.12	27.28	37.19
Curley	11.77	20.32	20.03	11.93	19.34	21.21
LSD	2.85					
	Dry Weight/plant (g (					
Plainleaf	2.91	7.52	7.02	3.89	5.12	5.68
Curley	1.67	3.84	3.12 b	1.71	3.95	4.22
LSD	0.37					

Table (5): Fresh and dry herb yields (ton ha-1) of the two parsley cultivars at the different three cuts.

Varieties	First season			Second season		
	first cut	second cut	Third cut	first cut	second cut	Third cut
	Fresh herb yield ton/fed					
Plainleaf	22.96	27.62	27.85	23.41	33.20	28.10
Curley	4.80	8.45	6.49	5.26	9.28	10.55
LSD	2.68					
	Dry herb yield ton/fed					
Plainleaf	3.59	4.45	4.73	3.80	4.75	4.34
Curley	0.93	1.07	1.03	0.87	1.25	1.56
LSD	1.98					

Chemical analysis:

Table (6) shows significant variation in chemical composition in the three vegetative cuts of both growing seasons. Plain leaf parsley gave the highest chlorophyll a and chlorophyll b contents at all cuts and in both growing seasons the same trend was observed in the case of carotenoids, β-Carotene, phenols and total antioxidant. There was a little bit increasing in Flavonoids and Free radical Scavenging contents in the three cuts in the second season. No significant differences were observed on vitamin C content between curly and plain parsley cultivars in both growing seasons and at all the three cuts (Table 6). Some studies attributed parsley popularity due to its highconcentration of β-carotene and vitamin C (**Rubatzky *et al.*1999**). Vitamin C is one of the antioxidants that suppress the ROS (reactive oxygen species) induced by stress (**Murshed *et al.* 2008**) and plays a key role in cell division and expansion (**Kato and Esaka 1999; Smirnoff 1993**). In our study, the vitamin concentration value less than that reported in previous study for parsley (123–165 mg/100 g. FW) (**Mordy and Atta 1999; Rubatzky *et al.* 1999**). It should be pointed out that plain leafed parsley demonstrated higher quality than curly-leafed.

Table (6): Some Chemical composition (mg/100g) and essential oil (%) of the two varieties of parsley the curly leaf type (sspcrispum) and the plain leaf type (sspneapolitanum, Danert).

	curly leaf type				plain leaf type			
First season								
	1st cut	2nd cut	3rd cut	L.S.D	1st cut	2nd cut	3rd cut	L.S.D
Chlorophyll a	119.78	198.27	166.60	0.21	134.06	210.81	246.57	0.15
Chlorophyll b	44.22	65.08	54.07	0.08	42.72	73.32	78.96	0.17
Carotenoids	82.34	147.92	127.55	0.18	102.69	160.00	175.95	0.13
β-Carotene	49.74	33.40	60.24	7.42	63.08	65.55	109.45	8.91
Flavonoids	291.58	294.94	298.85	0.79	295.20	296.53	307.55	0.87
Phenols	62.46	77.91	72.38	0.86	114.03	88.89	113.39	0.60
Total Antioxidant Capacity	458.22	324.89	220.44	7.92	464.00	585.11	632.00	18.90
Free radical Scavenging activity	85.00	84.45	86.19	0.25	89.74	87.37	86.75	0.27
Vit. C	47.27	47.54	47.51	0.01	47.66	47.70	47.77	0.01
Essential oil %	0.92	1.25	1.52	0.37	1.16	1.35	2.19	0.34
Second season								
Chlorophyll a	122.54	121.07	169.50	0.22	137.15	213.25	249.15	0.16
Chlorophyll b	48.45	69.06	58.21	0.10	45.12	77.25	82.25	0.19
Carotenoids	87.44	152.02	132.65	0.20	107.69	165.19	180.16	0.15
β-Carotene	51.74	35.60	62.54	7.92	65.09	67.55	111.54	9.09
Flavonoids	294.68	297.94	299.95	0.81	298.80	299.83	309.65	0.89
Phenols	67.86	81.96	77.88	0.89	119.06	93.09	118.69	0.67
Total Antioxidant Capacity	460.22	326.98	222.64	8.01	466.05	587.11	634.00	19.80
Free radical Scavenging activity	87.02	86.49	88.39	0.27	91.44	89.37	88.05	0.29
Vit. C	65.27	65.24	64.81	0.01	67.66	67.70	67.77	0.01
Essential oil %	1.72	2.25	2.82	0.37	2.16	2.35	2.99	0.34

Table (6) compiles the data of oil percent (%) per cut and through the two seasons. The data reveal a distinct variation through the two cultivars. Plain leaf parsley produced the highest oil percent through the first season in the three cuts. In the second season, the same pattern of the first one was confirmed The essential oil percent was higher than previous studies reporting essential oil percent between 0.04 to 0.15% on a fresh weight basis **(Simon and Overly, 1986).**

Essential oil composition

The results of the GLC analysis of the volatile oils of the two varieties of parsley in second cut in second season are shown in Table (7). The identified compounds are ranged from 89.48% in (sspcrispum) to 95.93% in (sspneapolitanum, Danert). The majority of compounds are both the Oxygenated monoterpenes and Sesquiterpene hydrocarbons are represented (10 compounds) each and ranged from 41.3% in (sspcrispum) to 48.24% in (sspneapolitanum, Danert) and from 25.19% in (sspcrispum) to 27.52% in (sspneapolitanum, Danert) respectively (Table 8).

According to the Monoterpene hydrocarbons are represented (3 compounds) and ranged from7.72% in (ssp*neapolitanum*, Danert) to 12.27% in (ssp*crispum*) and also Oxygenated Sesquiterpene are represented (3 compounds) and ranged from 9.97% in (sspcrispum) to 11.92% in (sspneapolitanum, Danert). The Phytol and Phytol acetate were the only Oxygenated diterpenes compounds found and ranged from 0.53% in (ssp*neapolitanum,* Danert) to 0.75% in (ssp*crispum*). The two varieties of parsley (curly leaf and plain leaf types) were differed in these contents of monoterpenes and sesquiterpenes, these plants were characterized by high contents of Oxygenated monoterpenes and Sesquiterpene hydrocarbons. The main constituents of curly leaf type (ssp*crispum*) essential oil were Myristicin (15.05%), 2-Allyl-4-methyl phenol (10.15%), Isolongipholene (8.59%) and β-Caryophyllene (6.43%). In the volatile oil of (ssp*neapolitanum*, Danert) the major compounds were Myristicin (12.65%), 2-Allyl-4-methyl phenol (10.77%), Apiol (9.5%) and Isolongipholene (9.45%). The results of the present experiments show that the essential oil composition of parsley varies with the type of parsley, the main compound in the two studied types is Myristicin and that is agree with (**Zheng*et al.*, (1992))** who found that as a major volatile aroma constituent of parsley essential oil, myristicin may be an effective cancer chemo preventive agent.

In addition, the detection of apiole in the leaves of the plain leaf and curly leaf types supports the findings of **(Macleod *et al.*, 1985 and Pino*et al.*, 1997)** whereas the absence of this compound from the aerial organs of the turnip-rooted type, or the presence of β-elemene in the leaves and petioles of the curly leaf type, indicates that a source of difference between results in the literature could be the type of parsley investigated.

Table (7): Volatile oil composition (%) of the two varieties of parsleythe curly leaf type (ssp*crispum*) andthe plain leaf type (ssp*neapolitanum*, Danert) (Second cut and second season).

Peak No.	components	Retention time (min)	2nd cut	
			curly leaf type	plain leaf type
1	β-pinene	7.71	4.25	1.92
2	limonene	8.8	3.89	4.12
3	2-Allyl-4-methyl phenol	9.615	10.15	10.77
4	Myrtenal	9.8	2.75	1.93
5	Pulegone	10.1	2.64	3.4
6	γ-terpineol	10.826	4.07	3.62
7	β-citronellol	13.154	3.63	3.46
8	Methyl cinnamate	13.72	0.28	0.28
9	2,5Dimethyl-p-cymene	13.8	4.13	1.68
10	Myristicin	13.922	15.05	12.65
11	2,5Dimethoxy-p-cymene	14.076	0.15	2.44
12	α-Himachalene	14.17	2.29	3.09
13	β-Elemene	14.287	0.43	0.11
14	β-Caryophyllene	14.42	6.43	4.62
15	α-Copaene	14.49	0.21	3.51
16	α-Elemene	14.55	0.43	0.67
17	β-Sesquiphellandrene	14.75	0.28	0.21
18	α-Ylangene	14.82	2.8	2.87
19	δ-Cadinene	14.983	3.37	2.57
20	Isoledene	15.67	0.36	0.42
21	2-Methyl-4-[2,6,6-trimethylcyclohex-1-en-1-ol]	15.77	0.32	0.19
22	Cedren-13ol,8	16.1	3.53	3.51
23	Isolongipholene	16.363	8.59	9.45
24	Apiol	16.63	2.26	9.5
25	Isocalamendiol	16.1683	4.08	5.89
26	10,13-Octadecadilynoic acid, methyl ester	16.788	2.36	2.52
27	Phytol	17.273	0.33	0.12
28	Phytol, acetate	17.92	0.42	0.41
	Total Identified		**89.48**	**95.93**
	Unidentified		**10.52**	**4.07**

Values are means of three replicates.

Molecular genetic identification

Randomly amplified polymorphic DNA (RAPD) markers:

The five 10-mer arbitrary primers succeeded in amplifying DNA fragments for the five genotypes of two parsley varieties as illustrated in Table (9) and Fig (1). Polymorphism levels differed from one primer to another. OP-A07 and OP-B11 primers exhibited low level of polymorphism (50.00% and 58.82%) respectively. However OP-A01 and OP-B07 primers exhibited moderate levels of polymorphism (64.29% and 66.67%) respectively. On the other hand OP-C12 primer exhibited high levels of polymorphism (84.62%).

Table (8): The classification of volatile oil components (%) of the two varieties of parsley the curly leaf type (ssp*crispum*) and the plain leaf type (ssp*neapolitanum*, Danert) according to terpenoieds type. (Second cut and second season).

components	curly leaf type (ssp*crispum*)	plain leaf type (ssp*neapolitanum*, Danert)
Monoterpene hydrocarbons	12.27	7.72
Oxygenated monoterpenes	41.3	48.24
Sesquiterpene hydrocarbons	25.19	27.52
Oxygenated Sesquiterpene	9.97	11.92
Oxygenated diterpenes	0.75	0.53

Table (9): Species-specific RAPD and ISSR markers for two parsley varieties genotypes:

Primers code	Range of M.S.	TAF	MF	PF	SM	Polymorphism (%)
RAPD primers						
OP-A01	246-2708	14	5	0	**9**(246,1121,1635,2100,2708)-(269,604,1500,2071)bp	64.29
OP-A07	279-3464	14	7	0	**7**(807)-(279,401,1082,1490,1646,3080)bp	50.00
OP-B07	314-3397	18	6	0	**12**(694,925,1123,1261,1409,1518,2070,2903)-(405,555,858,1048)bp	66.67
OP-B11	416-5685	17	7	0	**10**(437,1969,2374)-(416,471,1073,1897,2231,4093,5685) bp	58.82
OP-C12	367-3506	13	2	0	**11**(690,776,2238,2896,3506)-(367,481,646,729,871,1156) bp	84.62
Total RAPD primers		76	27	0	49	
ISSR primers						
HB-08	157-682	12	6	0	**6**(157,345,682)-(276,391,5282)bp	50.00
HB-09	127-807	15	8	0	**7** (237,308,418,635,807)-(127,222)bp	46.67
HB-10	128-581	10	7	0	**3** (317,472,581) bp	30.00
HB-11	252-585	11	8	0	**3** (814)-(302,825) bp	27.27
HB-12	174-804	12	5	0	**7** (316,396,438)-(311,451,582,804)bp	58.33
Total ISSR primers		60	34	0	26	
Total		136	61	0	**75**	

TAF = Total Amplified Fragments, MF= Monomporphic Fragments, PF= Polymorphic Fragments, SM= Specific Markers.

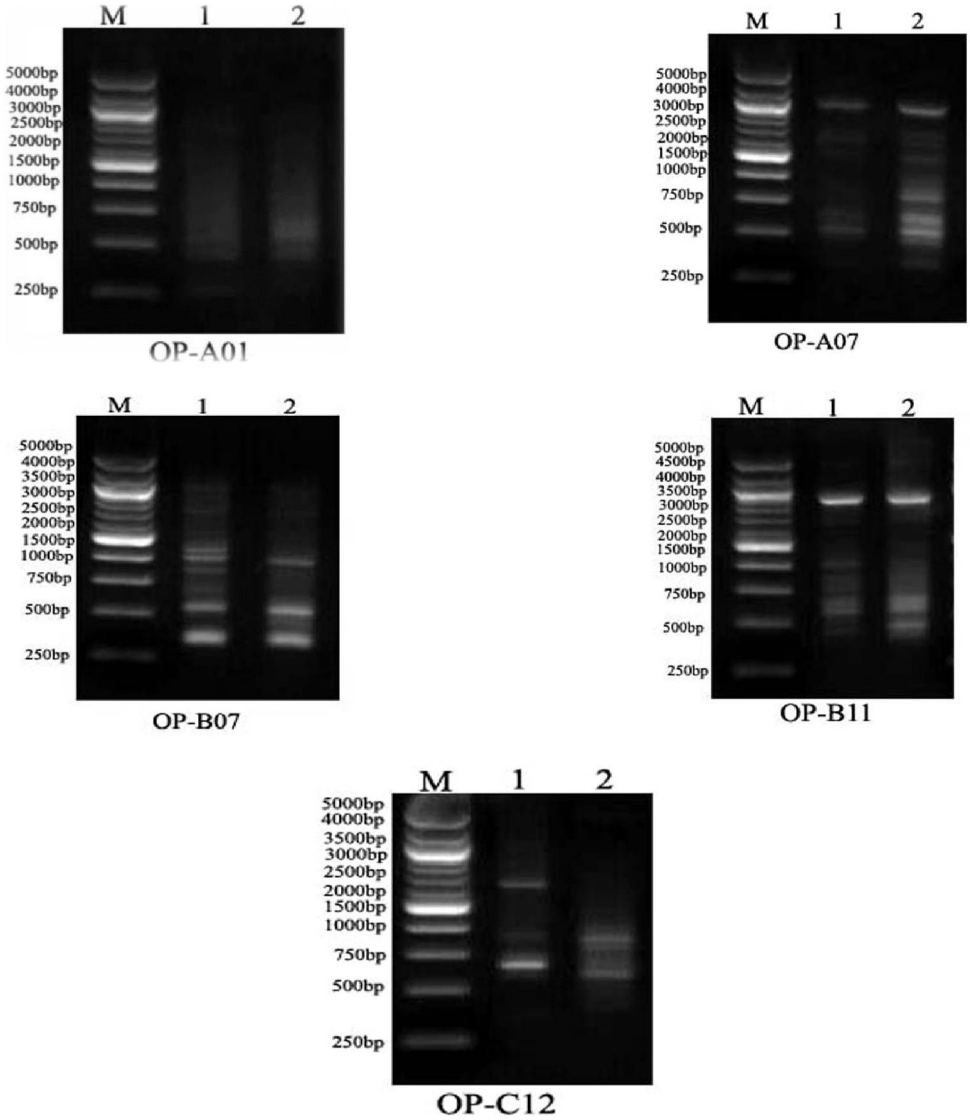

Fig. (1): RAPD-PCR analysis of two parsley varieties genotypes (second season)
1- The curly leaf type (ssp*crispum*). 2- The plain leaf type (ssp*neapolitanum*, Danert)

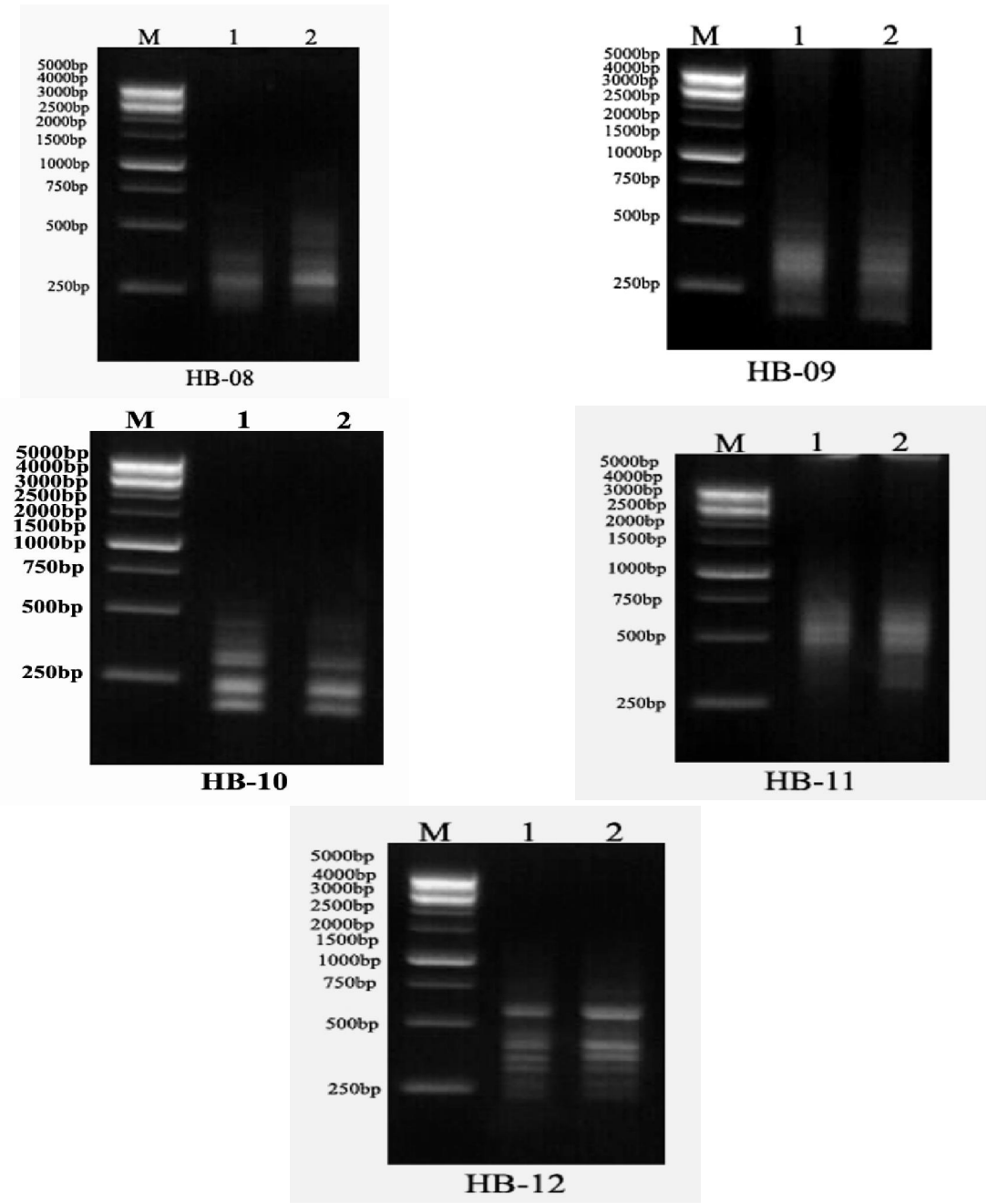

Fig. (2): RAPD-PCR analysis of two parsley varieties genotypes (second season)
1- The curly leaf type (*sspcrispum*). 2- The plain leaf type (*sspneapolitanum, Danert*)

The number of total amplified fragments (TAF), polymorphic fragments (PF), monomorphic fragments (MF) and specific markers (SM) for each sample using the five primers are shown in Table (9). OP-A01 primer produced fourteen fragments with molecular size ranging from246 to 2708bp (Fig.1) with polymorphic percentage (64.29%) nine of them were species - specific markers at (246, 1121, 1635, 2100, 2708) bp for (ssp*crispum*) and (269,604,1500,2071)bp for (ssp*neapolitanum*, Danert), while the other five fragments were present in the two genotypes which are considered as common fragments. OP-A07 primer resulted in fourteen DNA fragments with molecular size ranging from 279 to 3464 bp with polymorphic percentage (50.00%) in which seven of them were species- specific marker at 807bp for (ssp*crispum*) and (279, 401, 1082, 1490, 1646, 3080) bp for (ssp*neapolitanum*, Danert) and the other seven fragments were present in the two genotypes which are considered as common fragments. OP-B07 primer

produced eighteen DNA fragments with molecular size ranging from 314 to 3397bp with polymorphic percentage (66.67 %), and twelve of them were species- specific marker at (694, 925, 1123, 1261, 1409, 1518, 2070, 2903) bp for (ssp*crispum*) and (405,555,858,1048)bp for (ssp*neapolitanum*, Danert), while the other six fragments were presented in the two genotypes which are considered as common fragments. OP-B11 primer resulted in seventeen DNA fragments with molecular size ranging from 416 to 5685bp with fragments were polymorphic (58.82%) and ten of them were species - specific markers at (437, 1969, 2374) bp for (ssp*crispum*) and (416, 471, 1073, 1897, 2231,4093,5685) for (ssp*neapolitanum,* Danert) and the other seven fragments were present in the two genotypes which are considered as common fragments. OP-C12 primer resulted in thirteen DNA fragments with molecular size ranging from 367 to 3506bp with polymorphic percentage (84.62 %) in which eleven of them were species - specific markers at (690,776,2238,2896,3506) bp for (ssp*crispum*) and (367, 481,646,729,871,1156) bp for (ssp*neapolitanum*, Danert), while the other two fragments were presented in all genotypes which are considered as common fragments.

Inter Simple Sequence Repeats (ISSRs) markers:

The five ISSR primers succeeded in amplifying DNA fragments for the two parsley verities genotypes (Fig.2). Polymorphism levels differed from one primer to another, i.e. HB-12 primer recorded the highest level of polymorphism (58.33%), while, HB-08, HB-09 and HB-10 primer exhibited moderate level of polymorphism (50.00, 46.67 and30.00 %) respectively and the lowest level of polymorphism (27.27%) represented by HB-11 as shown in Table (9). The number of total amplified fragments (TAF), polymorphic fragments (PF), monomorphic fragments (MF) and specific markers (SM) for each primer of the five primers are shown in Table (9). HB-08 Primer showed 12 DNA fragments with molecular size ranging from 157 to 682bp (Fig.2 and Table9) with polymorphism (50.00 %), and six of them were positive species- specific markers at 157,345,682bp) for (ssp*crispum*) and (276,391,5282bp) for (*sspneapolitanum*, Danert) genotypes and another six DNA fragmentswere presented in all genotypes which are considered as common fragments..HB-09 primer showed fifteen DNA fragments with molecular sizes ranging from 127 to 807bp, with polymorphism (46.67%), and seven of them were positive species-specific markers at (237,308,418,635,807bp) for (ssp*crispum*) and (127,222 bp) for (ssp*neapolitanum*, Danert) genotypeand another eight DNA fragments were presented in all genotypes which are considered as common fragments. HB-10 primer showed ten DNA fragments with molecular size ranging from 128 to 581bp with polymorphism (30.00 %), and three of them were positive species- specific markers at (317,472,581bp) for (ssp*crispum*) genotype andanother seven DNA fragments were presented in all genotypes which are considered as common fragments. HB-11 primer showed eleven DNA fragments with molecular size ranging from 252 to585p with polymorphism (27.27%), and three of them were positive species- specific markers at (814bp) for (ssp*crispum*) and (302,825bp) for (ssp*neapolitanum*, Danert)

Genotype and another eight DNA fragments were presented in all genotypes which are considered as common fragments. HB-12 primer showed twelve DNA fragments with molecular size ranging from 174 to 804bp with polymorphism (58.33%) and seven of them were positive species- specific markers at (316,396,438bp) for (ssp*crispum*) and (311,451,582,804bp) for (ssp*neapolitanum*, Danert) and the other five fragments were present in all genotypes which are considered as common fragments.

In this work, we compared the applicability of ISSRs and RAPDs as genetic markers to characterize the two parsley varieties genotypes. The results found that RAPD markers were more efficient than the ISSR assay with regard to polymorphism detection, as the highest polymorphism detected 84.62% as compared to 58.33% for ISSR markers. This is in contrast to the results as obtained for several other plant species like in wheat **(Nagaoka and Ogihara, 1997)** and Vigna **(Ajibade *et al*., 2000).** The number of total monomorphic fragments is higher for ISSR than RAPDs. In fact, the ISSRs have a high capacity to reveal polymerphism and offer great potential to determine intra and inter genomic diversity as compared to other arbitrary primers like RAPDs **(Zietkiewicz *et al*., 1994).** A possible explanation for the difference in resolution of RAPDs and ISSRs is that the two-marker techniques target different portions of the genome. The ability to resolve genetic variation among different genotype maybe more directly related to the number of polymorphisms detected with each marker technique rather than a function of which technique is employed. **(Gupta *et al*., 2008**) on *Jatrophacurcas* and **(Mahdy, 2012)** on (*Corchorusolitorius* L. and Lactuca sativa L.) obtained the same conclusion.

Conclusions

In conclusion, the high quality was observed in plain- leafed parsley if compared to curly leafed parsley, which had higher vegetative growth parameter (plant height, number of branches/plan, fresh and dry weight / plant, fresh yield of herb /fed and, dry yield of herb /fed) and phytochemical contents (chlorophyll a,

chlorophyll b, carotenoids, β-Carotene, flavonoids, phenols, total antioxidant capacity, free radical scavenging activity and vit C.) in the two seasons in the three cuts.

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