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Phenotypic and Chemotypic Variation of Four Nigella (*Nigella sativa*) Varieties

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ABSTRACT

The assessment of four *Nigella sativa* varieties from Egypt, Iran, Turkey and Syria indicated highly significant differences in phenotypic and chemotypic characters. Seed yield was the most important character to differentiate all *Nigella* varieties based on high genetic advance and heritability values. In addition, the relative percentage of all detected fatty acids differed (from trace amounts to 63.691%) among varieties although the major saturated and unsaturated fatty acids were palmitic, arachnidic and linoleic acids in all varieties. The relative percentage of 8 essential and 8 nonessential amino acids different in all varieties although the Turkish variety had only 7 nonessential amino acids. Arginine and proline (ranging from 4.94 and 46.07% to 6.40 and 53.70%, respectively) were the major essential and nonessential amino acids, respectively for all varieties. SDS-PAGE electrophoresis revealed a different number of protein bands ranging from 9 bands in the Syrian variety to 18 bands in the Egyptian variety.

Keywords: amino acids, fatty acids, genetic variation, protein electrophoresis

INTRODUCTION

Nigella sativa L. is an important spice of the *Ranunculaceae* family and is commonly known as nigella, black cumin, al-habba al-sawdaa and habbet el baraka. It is widely cultivated in the Mediterranean region (Abou El-Nasr 2005; Ottai *et al.* 2005; Salma *et al.* 2007). *N. sativa* seeds and their oil are consumed for edible purposes and have several therapeutic effects and activities: antihistamic (Chakravarty 1993), antihypertensive (Naik *et al.* 2010), anti-inflammatory and analgesic (Banerjee *et al.* 2010; Woo *et al.* 2012), cancer prevention (Majdalawieh *et al.* 2010; Naik *et al.* 2010; Woo *et al.* 2012), antioxidant (Woo *et al.* 2012), antibacterial (Ali *et al.* 2011), stimulatory effect on the immune system (Salem and Hossain 2000) and anti-tumor (Musa *et al.* 2004; Banerjee *et al.* 2010).

Plant breeders primarily estimate variation in an initial population for its importance so as to choose the most efficient breeding procedure. However, variation provides nature and breeders with an essential tool to develop new cultivars (Elliott 1985). Therefore, many authors have studied genetic variation in the quantitative characters of nigella (Ozguven *et al.* 2001; Salem *et al.* 2001; Banafar *et al.* 2002; D'Antuono *et al.* 2002; Abou El-Nasr 2005; Ottai *et al.* 2005). Salem *et al.* (2001) studied broad sense heritability, variation and correlation coefficients among *N. sativa* characters. D'Antuono *et al.* (2002) reported that the number of seeds/plant was the most important yield component for two *Nigella* species: *N. sativa* and *N. damascena*. Abou El-Nasr (2005), in five *N. sativa* resources, and Ottai *et al.* (2005), in three *N. sativa* cultivars, found highly significant differences among several plant characters: high heritability for capsule length, number of branches and number of capsules/plant and high genetic advance for seed yield as well as number of branches and capsules/plant. Meanwhile, Iqbal *et al.* (2009) found high variation among 34 accessions with two check genotypes of *N. sativs*. Iqbal *et al.* (2010) recorded high genetic variation for plant height, days to first flower, days to 50% flowers, days to maturity, biomass, capsule weight, yield, seed weight and harvest index. Iqbal *et al.* (2011) analyzed the genetic diversity of 36 genotypes of *N. sativa* based on yield traits, nutritional characteristics and mineral nutrients, and found that yield traits contributed 86% of the variability pertaining to nutritional characteristics.

N. sativa seeds contain other ingredients including nutritional components such as carbohydrates, fats, vitamins, mineral elements and proteins (Salma *et al.* 2007; Mohammad *et al.* 2009; El-Naggar *et al.* 2010). However, analyses of the oil content related to its fatty acids (Atta 2003; Ottai *et al.* 2005) and analysis of the protein content related to its amino acids (Al-Gaby 1998) showed different relative percentages in the detected components, providing more insight into the genetic variation among *N. sativa* varieties. SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis) is the most widely used and effective technique for electrophoretic analysis of protein, needing less time and easily separating and examining proteins (Neves and Lourenço 1995).

The present study aimed to evaluate the phenotypic variation (through quantitative characters) and chemotypic variation (of oil fatty acids, protein amino acids and protein electrophoretic analysis) among *N. sativa* varieties to reflect the genetic variation among them.

MATERIALS AND METHODS

Source of materials

Seeds of four varieties (Egyptian, Iranian, Turkish and Syrian; ecovars that are common in these countries without a limit in their origin) of *N. sativa* were obtained from The Group of Genetics and Breeding of Medicinal Plants, Genetics and Cytology Department, National Research Center (NRC), Egypt.

Cultivation methods

The seeds of four *Nigella* varieties were sown at El-Jammal village, Qalubia Governorate (center of the Egyptian delta), Egypt over two successive winter seasons in October to March of 2007/

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2008 and 2008/2009. A completely randomized design with five replications was established. Each replicate had five lines 5 m long and 70 cm wide. The plants were thinned to leave one plant per hill with a 40 cm space. All cultural practices were performed whenever required. At maturity the seeds of each variety were harvested separately. Eight quantitative character was recorded for the second season as follows: 1) Plant height, cm (PH); 2) Number of total branches per plant (TB); 3) Number of mature capsules per plant (MC); 4) Number of immature capsules/plant (IC); 5) Seed yield/plant, g (SY); 6) Capsule length, cm (CL); 7) Capsule diameter, cm (CD); 8) Number of shutters/capsule (SC).

Statistical analysis

Analysis of variance was carried out for each variety separately and then combined analysis was carried out over the four varieties using SPSS software 2001, v. 11). Mean value, standard error and coefficient of variation (CV%) were computed by SPSS software v. 11). The genotypic and phenotypic variances (σ^2_G and σ^2_P, respectively) were estimated according to Shin (1968) while genotypic and phenotypic coefficients of variation (GCV and PCV, respectively) were evaluated according to Burton (1952). Broad sense heritability (H^2_b%) was computed using the formula of Johanson *et al*. (1955) ($H^2_b\% = \sigma^2_G / \sigma^2_P.100$). Genetic advance (GA%) was computed according to Miller *et al.* (1958).

Chemical investigation

1. Fatty acid composition

Seeds of each variety were extracted with petroleum ether at 40-60°C several times (until the extract became clear) in a closed and dark bottle at ambient laboratory temperature to prepare the fatty acid methyl esters (Vogel 1975). Qualitative and quantitative analysis of the fatty acid esters were performed with Gas Liquid Chromatography (GLC) using the following conditions: Pyenicon PRO-GC at the Central Laboratory of the NRC, Egypt using a Sp. 2310 column, 55% cyanopropyl phenyl silicon 1.5 × 4 mm in size. The temperature program was 70°C (initial temperature) at a rate of 8°C/ min to 190°C (final temperature). Injector and detector temperatures were maintained at 250 and 300°C, respectively. Nitrogen was used as the carrier gas at a rate of 30 ml/min. The relative percentage of each compound was determined on the bases of the peak area. The qualitative identification of fatty acids was achieved by comparing the retention time (R_t) of their peaks with those of the authentic samples under the same conditions.

Amino acid composition

The seed amino acid content of each *Nigella* variety was determined as described by Spackman *et al.* (1958) and Moore *et al.* (1958). The analysis was performed in the Central Service Unit of the NRC using an LC 3000 amino acid analyzer (Eppendorf-Biotronik, Germany). The technique was based on the separation of the amino acids using strong cation exchange chromatography followed by a ninhydrine color reaction and photometric detection at 570 nm. Samples were hydrolyzed with 6N HCl at 110°C in Teflon-capped vials for 24 h. After vacuum removal of HCl, the residues were dissolved in a lithium citrate buffer, pH 2.2. 20 µl of the solution was loaded onto a cation exchange column (pre-equilibrated with the same buffer), then four lithium citrate buffers with pH values of 2.2, 2.8, 3.3 and 3.7 were successively applied to the column at flow rate of 0.2 ml/ min. The ninhydrine flow rate was 0.2 ml/min at a pressure of 0-150 bar. The pressure of the buffer ranged from 0 to 50 bar and the reaction temperature was 130°C.

Total soluble protein

Protein electrophoresis analysis was carried out for the seeds of each *Nigella* variety. SDS-PAGE was performed according to Laemmli (1970). One gram of seeds was defatted with petroleum ether. Sample preparation and the extraction of total soluble protein were performed according to Stegemann (1980). Gels were scanned and analyzed using a Gel Doc Bio-Rad System.

RESULTS

Analysis of variance

Analysis of variance for eight quantitative characters of four *Nigella sativa* varieties (Egyptian, Iranian, Turkish and Syrian) was carried out among the individual plants within each variety (separately) and between varieties (combined). The analysis of variance in **Table 1** shows that the individual plants within each variety had highly significant differences for PH. Three traits (TB, MC, SC) showed significant variation only among individuals of the Syrian variety. However, significant variation was also detected in IC for Iranian and Turkish varieties, as well as in SY for Turkish and Syrian varieties. Moreover, CL showed significant differences among the individuals of all varieties except for the Iranian variety, where individuals showed non-significant variation. Variation in CD was non-significant for the individuals within all varieties, when analyzed separately (one way). However, combined analysis (two way) presented highly significant variation between *Nigella* varieties in all studied characters (**Table 1**).

Variation criterions

Data illustrated in **Table 2** show the range, mean and CV% for all the studied characters of *Nigella* varieties. A wide range was detected in the Iranian variety for MC, IC and

Table 1 Analysis of variance (MS) for eight quantitative characters of four *Nigella sativa* varieties (separately and combined).

Character	Separated analysis					Combined analysis	
	Items	Egyptian	Iranian	Turkish	Syrian	Items	MS
PH	Individuals	238.17**	284.77**	714.27**	15.33**	Varieties	1777.2**
	Error	18.57	7.24	10.37	11.68	Error	10.74
TB	Individuals	4.90 ns	9.07 ns	5.60 ns	20.77*	Varieties	208.11**
	Error	10.65	8.82	6.05	4.32	Error	6.55
MC	Individuals	768.90 ns	1711.93 ns	883.90 ns	68.10**	Varieties	10346.87**
	Error	624.35	588.73	320.20	7.70	Error	332.98
IC	Individuals	20.73 ns	60.27*	33.57*	4.90 ns	Varieties	72.40**
	Error	7.53	12.97	7.77	9.70	Error	8.37
SY	Individuals	25.07 ns	24.57 ns	21.00*	3.43**	Varieties	157.24**
	Error	7.82	6.47	4.55	0.23	Error	4.10
CL	Individuals	0.53**	0.07 ns	0.08*	0.08**	Varieties	0.28**
	Error	0.02	0.03	0.02	0.01	Error	0.02
CD	Individuals	0.01 ns	0.01 ns	0.01 ns	0.02 ns	Varieties	0.07**
	Error	0.01	0.01	0.01	0.01	Error	0.01
SC	Individuals	0.23 ns	0.27 ns	0.73 ns	2.00*	Varieties	2.13**
	Error	0.48	0.32	0.23	0.45	Error	0.33

MS = mean square value; * and ** = significant at $P < 0.05$ and 0.01, respectively. PH = plant height, cm; TB = number of total branches/plant; MC = number of mature capsules/plant; IC = number of immature capsules/plant; SY = seed yield/plant, g; CL = capsule length, cm; CD = capsule diameter, cm; SC = number of shutters/capsule

Table 2 Range, mean and coefficient of variability (C.V. %) for eight quantitative characters of four *Nigella sativa* varieties.

Varieties	Items	PH	TB	MC	IC	SY	CL	CD	SC
Egyptian	Range	54.0 – 90.0	12.0 – 21.0	51.0 – 124.0	4.0 – 17.0	5.0 – 16.0	1.2 – 1.8	0.9 – 1.2	5.0 – 7.0
	Mean	70.0 ± 2.39	15.73 ± 0.72	93.73 ± 6.24	6.73 ± 0.84	10.40 ± 0.23	1.43 ± 0.01	1.04 ± 0.02	5.73 ± 0.15
	CV%	13.25	17.73	25.77	48.11	33.07	11.38	7.08	10.35
Iranian	Range	46.0 – 80.0	15.0 – 23.0	33.0 – 129.0	1.0 – 20.0	2.0 – 11.0	1.0 – 1.8	0.9 – 1.3	6.0 – 7.0
	Mean	59.53 ± 2.40	18.27 ± 0.72	58.53 ± 7.43	7.53 ± 1.28	5.07 ± 0.85	1.24 ± 0.05	1.04 ± 0.01	6.53 ± 0.13
	CV%	15.64	15.37	49.17	65.97	64.80	16.07	10.13	7.90
Turkish	Range	24.0 – 67.0	6.0 – 15.0	11.0 – 86.0	5.0 – 18.0	1.0 – 11.0	1.0 – 1.6	0.8 – 1.1	5.0 – 7.0
	Mean	43.53 ± 3.74	9.80 ± 0.61	34.07 ± 5.48	8.93 ± 0.98	3.33 ± 0.76	1.29 ± 0.05	0.92 ± 0.02	5.73 ± 0.15
	CV%	33.31	24.15	62.24	42.62	88.32	14.35	9.37	10.35
Syrian	Range	48.0 – 61.0	11.0 – 21.0	35.0 – 50.0	8.0 – 18.0	2.0 – 5.0	0.8 – 1.3	0.7 – 1.1	5.0 – 8.0
	Mean	57.33 ± 0.87	16.87 ± 0.76	43.13± 1.26	11.73 ± 0.69	3.87 ± 0.27	1.09 ± 0.04	0.93 ± 0.03	6.0 ± 0.24
	CV%	5.85	17.34	11.35	22.88	27.42	15.25	11.15	15.43
General	Range	24.0 – 90.0	6.0 – 23.0	11.0 – 129.0	1.0 – 20.0	1.0 – 16.0	0.8 – 1.8	0.7 – 1.3	5.0 – 8.0
	Mean	57.60 ± 1.75	15.15 ± 0.54	57.37 ± 4.03	8.73 ± 0.54	5.67 ± 0.51	1.26 ± 0.03	0.98 ± 0.01	6.0 ± 0.10
	CV%	23.54	27.69	54.39	47.46	69.89	16.77	11.01	12.27

PH = plant height, cm; TB = number of total branches/plant; MC = number of mature capsules/plant; IC = number of immature capsules/plant; SY = seed yield/plant, g; CL = capsule length, cm; CD = capsule diameter, cm; SC = number of shutters/capsule

Table 3 Genetic component variability for eight quantitative characters of four *Nigella sativa* varieties.

Component	Varieties	PH	TB	MC	IC	SY	CL	CD	SC
σ^2_G	Egyptian	73.20	1.92	48.18	4.40	5.75	0.17	0.01	0.08
	Iranian	92.51	0.08	374.09	15.77	6.03	0.01	0.01	0.02
	Turkish	234.63	0.15	187.90	8.60	5.48	0.02	0.01	0.17
	Syrian	1.22	5.48	20.13	1.60	1.07	0.03	0.01	0.52
σ^2_P	Egyptian	91.77	8.73	672.53	11.93	13.57	0.19	0.01	0.40
	Iranian	99.75	8.90	962.82	28.74	12.50	0.04	0.12	0.30
	Turkish	245.0	5.90	508.10	16.37	10.03	0.04	0.01	0.40
	Syrian	12.90	9.80	27.83	8.10	1.30	0.03	0.01	0.97
GCV	Egyptian	12.22	8.81	7.41	31.17	23.06	28.83	3.04	4.94
	Iranian	16.16	1.55	33.05	52.74	48.43	8.06	0.04	2.00
	Turkish	35.19	3.95	40.23	32.84	70.30	10.96	0.44	7.20
	Syrian	1.93	13.88	10.40	10.78	26.73	14.51	6.80	12.02
PCV	Egyptian	13.69	18.78	27.67	51.32	35.42	30.48	7.45	11.04
	Iranian	16.78	16.33	53.01	71.36	69.73	16.13	33.31	8.43
	Turkish	35.96	24.79	66.16	45.31	95.11	15.50	10.31	11.04
	Syrian	6.26	18.56	12.23	24.26	29.46	16.41	11.78	16.41
H^2_b%	Egyptian	79.76	21.95	7.16	36.88	42.37	89.47	16.67	21.01
	Iranian	92.74	0.94	38.85	54.87	48.24	33.33	0.56	5.49
	Turkish	95.77	2.54	36.98	52.54	54.65	50.00	7.69	42.02
	Syrian	9.43	55.93	72.34	19.75	82.26	78.35	33.33	53.45
GA%	Egyptian	4.78	3.45	2.61	11.34	16.27	6.88	0.96	1.68
	Iranian	3.22	0.01	12.52	19.46	20.26	2.33	0.05	0.40
	Turkish	5.44	0.47	17.30	11.00	24.12	3.49	0.60	1.99
	Syrian	0.41	5.74	3.80	4.16	7.02	5.03	2.51	5.35

σ^2_G, σ^2_P = genotypic and phenotypic variances, respectively. H^2_b% = Broad sense heritability; GCV, PCV = genotypic and phenotypic coefficient of variability, respectively; GA% = genetic advance

CL (33-129, 1-20 and 1.0-1.8, respectively), the Egyptian variety was characterized by the widest range for SY (5-16 g), the Turkish variety by the widest range in PH (24-67 cm) and the Syrian variety had the widest range for TB (11-21). However, the Syrian variety presented the narrowest range for most traits. On the other hand, the highest mean value was related to the Egyptian variety for PH (70 ± 2.39 cm), MC (93.73 ± 6.24), SY (10.4 ± 0.23 g), CL (1.43 ± 0.01 cm) and CD (1.04 ± 0.02 cm) while the Iranian variety had the highest mean value for TB and SC (18.27 ± 0.72 and 6.53 ± 0.13, respectively), and the highest mean value for IC corresponded to the Syrian variety (11.73 ± 0.69). The lowest mean value was related to the Turkish variety for PH (43.53 ± 3.7 cm), TB (9.8 ± 0.6), MC (34.07 ± 5.48), SY (3.33 ± 0.76 g) and CD (0.92 ± 0.02 cm), the Egyptian variety presented the lowest mean value for IC (6.73 ± 0.84) and SC (5.73 ± 0.15) while the Syrian variety showed the lowest mean value for CL (1.09 ± 0.04 cm). Concerning to the CV% value, IC and SY showed a high CV% value (> 20%) in all varieties. In addition, all varieties had high CV% for MC except for the Syrian variety which had 11.35%. The Turkish variety had a high CV% value for PH and TB (**Table 2**).

Genetic component of variation

Table 3 shows the σ^2_G, σ^2_P, GCV, PCV, H^2_b% and GA% for the 8 studied characters of the 4 *Nigella* varieties studied. σ^2_P was always higher than σ^2_G in all characters for all varieties, but only CD had equal values for σ^2_G and σ^2_P in all varieties, except for the Iranian variety. However, the highest values of GCV, PCV, H^2_b% and GA% corresponded to the Turkish variety for PH (35.19, 35.96, 95.77 and 5.44, respectively), to the Iranian variety for IC (52.74, 71.36, 54.87 and 19.46, respectively), to the Egyptian variety for CL (28.83, 30.48, 89.47 and 6.88, respectively) and to the Syrian variety for SC (12.02, 16.41, 53.45 and 5.35, respectively). The Syrian variety presented the highest values for GCV, H^2_b% and GA% for TB (13.88, 55.93 and 5.74, respectively) and for CD (6.80, 33.33 and 2.51, respectively). Moreover, the highest GCV, PCV and GA% values were related to the Turkish variety for MC (40.23, 66.16 and 17.30, respectively) and SY (70.30, 95.11 and 24.12, respectively). In contrast, the Syrian variety presented the highest H^2_b% value for MC and SY (72.34 and 82.26, respectively), the Turkish variety had the highest PCV for TB (24.79), while the highest PCV value for CD (33.31) was related to the Iranian variety (**Table 3**).

Table 4 GLC analysis of seed fatty acid composition of four *Nigella sativa* varieties.

Component	*C.N	Egyptian	Iranian	Turkish	Syrian	Mean	C.V.%
Caprylic	8:0	0.283	**Trace.	Trace.	Trace.	0.07 ± 0.07	200.00
Myristic	14:0	Trace.	0.959	0.201	0.411	0.39 ± 0.21	105.19
Palmitic	16:0	13.793	13.080	13.369	18.404	14.66 ± 1.26	17.13
Stearic	18:0	Trace.	0.707	Trace.	0.220	0.23 ± 0.17	143.85
Oleic	18:1	21.639	20.157	21.219	23.702	21.68 ± 0.74	6.85
Linoleic	18:2	62.974	61.873	63.691	53.572	60.53 ± 2.35	7.76
Arachidic	20:0	1.311	3.225	1.520	3.690	2.44 ± 0.60	49.14
Total saturated fatty acids		15.387	17.971	15.090	22.725	17.79 ± 1.77	19.86
Total unsaturated fatty acids		84.913	82.030	84.910	77.274	82.21 ± 1.77	4.30
Total fatty acids		100.000	100.001	100.000	99.999		

* C.N = carbon number **trace amounts less than 0.2%

Table 5 Seed amino acids composition of four *Nigella sativa* varieties.

Acids	RRT	Egyptian	Iranian	Turkish	Syrian	MeanA	C.V.%
Essential amino acids							
Threonine	0.547	0.34	0.23	0.13	2.06	0.69 ± 0.46	132.95
Methionine	1.533	1.14	1.17	1.61	0.80	1.18 ± 0.17	28.15
Isoleucine	1.626	1.92	2.27	2.32	2.15	2.17 ± 0.09	8.23
Leucine	1.672	3.37	4.09	4.18	3.77	3.85 ± 0.18	9.52
Phenylalanine	1.857	2.46	2.99	2.78	2.63	2.72 ± 0.11	8.29
Histidine	2.197	2.42	3.07	2.89	2.19	2.64 ± 0.20	15.42
Lysine	2.345	2.33	2.92	2.68	2.02	2.49 ± 0.20	15.87
Arginine	2.724	5.62	6.40	6.35	4.94	5.83 ± 0.35	11.85
Non-essential amino acids							
Aspartic	0.441	4.73	5.29	5.44	4.45	4.98 ± 0.23	9.36
Serine	0.596	1.78	1.55	1.52	2.20	1.76 ± 0.16	17.81
Glutamic	0.653	12.98	1.48	---	11.19	6.41 ± 3.31	103.21
Glycine	0.997	1.98	2.21	2.31	1.88	2.10 ± 0.10	9.50
Proline	1.000	46.07	51.56	53.70	47.40	49.68 ± 1.78	7.16
Alanine	1.031	2.75	3.35	3.20	2.72	3.01 ± 0.16	10.58
Cystine	1.268	7.23	8.21	8.09	6.66	7.55 ± 0.37	9.74
Tyrosine	1.792	2.88	3.21	2.80	2.94	2.96 ± 0.09	6.01
Total essential amino acids		19.60	23.14	22.94	20.56	21.56 ± 0.88	8.14
Total non-essential amino acids		80.40	76.86	77.06	79.44	78.44 ± 0.88	2.34
Total amino acids		100.00	100.00	100.00	100.00		

RRT = relative retention time; C.V. % = coefficient of variation percentage; EAA = essential amino acid; NEAA = non-essential amino acid

Chemical investigations

1. Fatty acid composition

Qualitative and quantitative analysis of the esters fatty acids of the four *Nigella* varieties were carried out with GLC. The relative percentage of the detected methyl esters of fatty acids is illustrated in **Table 4**. Seven fatty acids were detected in all *Nigella* varieties: 5 of them were saturated fatty acids while the others were unsaturated fatty acids. Linoleic acid was the major fatty acid in all varieties with 62.974, 61.873, 63.691 and 53.572% (relative percentage) for Egyptian, Iranian, Turkish and Syrian varieties, respectively with a inter-variety mean of 60.53 ± 2.35%. The second major fatty acid was oleic acid with a relative percentage of 21.639, 20.157, 21.219 and 23.702, respectively and 21.68 ± 0.74% as the mean value. Palmitic acid was also detected as one of the major fatty acids in the four *Nigella* varieties with a relative percentage of 13.793, 13.080, 13.369 and 18.404%, respectively and a mean inter-variety value of 14.66 ± 1.26%. Moreover, the total relative percentage of saturated fatty acids ranged from 15.09% in the Turkish variety to 22.725% in the Syrian variety with a mean inter-variety value of 17.79 ± 1.77%. The total relative percentage of unsaturated fatty acids ranged from 77.274% in the Syrian variety to 84.913% in the Egyptian variety with the general mean value of 82.21 ± 1.77%. Arachidic acid was found in amounts that ranged from 1.311 to 3.69% in Egyptian and Syrian varieties with general mean of 2.44 ± 0.6%. Myristic, stearic and caprylic acids were also detected but in minor amounts for all varieties with mean values of 0.39 ± 0.21, 0.23 ± 0.17 and 0.07 ± 0.07%, respectively. The unsaturated fatty acids (oleic and linoleic) had the lowest CV% (6.85 and 7.76%, respectively). Only palmitic acid had a low CV% (< 20%) among all saturated fatty acids, while the others presented a high CV% ranging from 49.14% for arachidic to 200% for caprylic acid (**Table 4**).

2. Amino acid composition

Seed amino acid composition of the four studied *Nigella* varieties was analyzed by an amino acid analyzer. The relative percentages of the detected amino acids are shown in **Table 5**. Sixteen amino acids were detected in all *Nigella* varieties except for the Turkish variety which had 15 (glutamic acid was absent). Proline acid was the major amino acid in all four varieties with an absolute percentage of 46.07, 51.56, 53.70 and 47.40% for Egyptian, Iranian, Turkish and Syrian varieties, respectively. The second major amino acid was cystine acid, followed by arginine and aspartic acid in the Iranian, Turkish and Syrian varieties, respectively while the second major amino acid in the Egyptian variety was glutamic acid (12.98%, relative percentage), followed by cystine and arginine acid. The relative percentage of cystine was 7.23, 8.21, 8.09 and 6.66% for Egyptian, Iranian, Turkish and Syrian varieties, respectively. Arginine acid was one of the major amino acids in all four varieties: 5.62, 6.40, 6.35 and 4.94% for Egyptian, Iranian, Turkish and Syrian varieties, respectively. All detected amino acids had a different relative percentage among *Nigella* varieties and had a CV% value of <20% except for methionine, glutamic and therionine acids (CV% = 28.15, 103.21 and 132.95%, respectively). The total percentage of essential amino acids ranged from 19.6% in the Egyptian variety to 23.14% in the Iranian variety with general value of 21.56 ± 0.88 and 8.14% as the CV% value while the total percentage of nonessential amino acids ranged from 76.86% in the Iranian variety to 80.40% in the Egyptian variety with the general mean value of 78.44 ± 0.88% and a low CV% (2.34%) (**Table 5**).

Table 6 Total soluble densitometry profiles of four *Nigella sativa* varieties.

No. of bands	MW (KDa)	Egyptian	Iranian	Turkish	Syrian
1	190.0	+	+	-	-
2	179.0	+	+	+	+
3	64.0	+	+	+	-
4	52.7	+	-	-	-
5	45.0	+	+	-	-
6	39.0	+	+	-	-
7	28.0	+	+	-	-
8	25.5	+	+	+	-
9	23.6	+	+	+	+
10	23.4	+	+	+	+
11	23.0	-	-	-	+
12	22.8	+	+	+	+
13	22.6	+	+	+	-
14	21.5	+	+	+	-
15	21.0	+	+	+	+
16	15.5	+	-	-	-
17	14.0	+	+	+	+
18	11.5	+	+	+	+
19	10.0	+	+	+	+
Total of bands		18	16	12	9

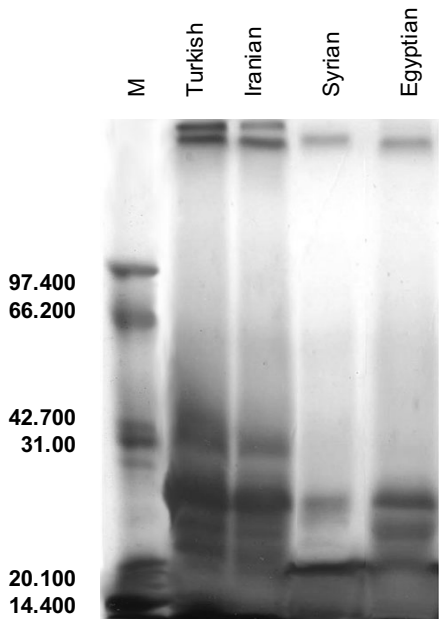

Fig. 1 SDS-PAGE of total soluble protein extracted from the seeds of four *Nigella sativa* varieties. M = marker.

SDS-PAGE analysis of total soluble proteins

The electrophoretic profile of the total soluble protein within the seeds of the four *Nigella* varieties was compared (**Fig. 1; Table 6**). A maximum of 18, 16, 12 and 9 bands were observed in Egyptian, Iranian, Turkish and Syrian varieties, respectively with different molecular weights ranging from 10 to 190 KDa. Eight bands of 10, 11.5, 14, 21, 22.8, 23.4, 23.6 and 179 KDa were detected in all varieties. The Egyptian variety was distinguished by two bands (15.5 and 52.7 KDa) and the Syrian variety by only one band (23 KDa). Four bands of 21.5, 22.6, 25.5 and 64 KDa appeared in all varieties except for the Syrian variety. Also, four bands (28, 39, 45 and 190 KDa) were found in the Egyptian and Iranian varieties only. Based on the protein bands, the four varieties could be divided into two groups. The first group contained Egyptian and Iranian varieties which were identical for all bands except for only two bands (15.5 and 52.7 KDa) which were found in the Egyptian variety but absent in the Iranian variety. The second group contained Turkish and Syrian varieties which differed by only 5 bands, four of them (21.5, 22.6, 25.5 and 64 KDa) only being found in the Turkish variety; in contrast, one band (23 KDa) was found only in the Syrian variety (**Table 6**).

DISCUSSION

The quantitative characters studied revealed high genetic variation among the four *Nigella sativa* varieties. Many authors found similar genetic variation among *Nigella* varieties (Srivastava and Tripathi 2000; Banafar *et al.* 2002; D'Antuono *et al.* 2002; Abou El-Nasr 2005; Ottai *et al*. 2005; Iqbal *et al*. 2010). Egyptian variety had the highest value of PH, MC, SY, CL and CD as well as the lowest value of IC. Also, its individual plants presented non significant variation for all studied characters except only for PH and CL, in addition to the low CV% value (less than 20%) for the most characters. All these items proved that the Egyptian variety was the best stable and homogenous variety confirming the results of Ottai *et al*. (2005) who found the same result when they evaluated the variation of three Egyptian cultivars of *N. sativa* among their plant characters, insecticidal activity and lipid composition. Hence, a hybrid program using the Egyptian variety is expected to be a potential means of improving *N. sativa*. Iqbal *et al*. (2010) studied the genetic variation among 31 genotypes collected in Pakistan and found that three, MP23, MP111 and MP120, were better for more than one character, so they recommended that the use of a better accession is an important factor for improving *N. sativa*. On the other hand, SY had the highest genetic advance with considerable value of heritability for all varieties to show that it the most important character of *N. sativa*. Reddy *et al*. (2004) noted that high genetic advance with high heritability for any plant character indicates that this character is controlled by additive gene effects and additive × additive epistatic interactions. Kavani *et al*. (2001) reported that this case (the trait which characterized with high heritability and genetic advance) indicates scope for the improvement of the character through a selection program.

Analysis of fatty acid composition indicated that *N. sativa* varieties had different percentage values for all detected fatty acids. Linoleic followed by oleic were the major unsaturated fatty acids and palmitic followed by arachidic were the major saturated fatty acids for all *Nigella* varieties. The same result was found by Ozguven *et al*. (2001), Atta (2003), Ottai *et al*. (2005) and Salma *et al*. (2007). Meanwhile, 16 (eight essential and eight nonessential) amino acids were detected in all varieties except Turkish variety had eight essential and seven non-essential amino acids confirming the result of Omar *et al*. (1999) who detected eight essential amino acids in *N. sativa*. While the proline was the major non-essential, arginine was the major essential amino acid for all studied varieties. Contradicted with El-Naggar *et al*. (2010) who reported that glycine (1943.38 pmoles/mg) is the most abundant amino acid followed gamma amino butyric acid, glutamic and aspartic acid (396.24, 345.26, 234.64 pmoles/mg, respectively). Moreover, SDS-PAGE electrophoresis analysis revealed different number of protein bands among varieties ranged from 9 to 18 bands to confirm the different genetic background for *Nigella* varieties. This result was less than the result of Abou El-Nasr (2005) who detected a total of 19 bands for the proteins of five *N. sativa* resources, 17 of them detected in 'Rajab', 'Haraz' and 'Khider', in addition to 16 and 15 bands that were detected in 'Tolba' and 'Luxor', respectively.

In conclusion, Egyptian, Iranian, Turkish and Syrian varieties of *N. sativa* differed at the phenotypic level (quantitative characters) and at the chemotypic level (fatty acids, amino acids and protein electrophoresis), clearly presenting genetic variation among and between them.

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