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PERFORMANCE AND ASSOCIATIONS OF NIGELLA SATIVA, L. DIFFERENT LANDRACES UNDER SOUTHERN EGYPT CONDITIONS

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ABSTRACT

An assessment was conducted during two successive seasons to study the variation and associations of growth and seed and oil yield of five Egyptian landraces of black Cumin (Nigella sativa L.). The obtained data revealed a significant variation among landraces in all vegetative and flower parameters. Assiut landrace showed the highest value of plant height, stem diameter, fresh and dry weight of herb, and number of capsules per plant. Menia landrace showed the highest flower percentage (65.18%) and fewer days to flower (58.88 days) and to harvest (131.36 days). Aswan, Qena and Assiut produced the highest seed yield (Kg/fed). Nevertheless, Aswan produced the highest volatile oil % and less fixed oil percent followed by Qena and Assiut, respectively. Among the 21 correlation coefficients (r), 15 showed positive values while six had negative (r) values. However, all r values for the total seed yield/feddan with other studied traits were low or negligible. Thus it is suggested that detection of landrace germplasm with array of the desirable traits among those presented here may be possible. The flow cytometry study revealed that the genome size among the five landraces ranged from 21.9 to 25.68 pg. Oil analysis indicated that the seeds of all the landraces of black cumin consisted of four saturated fatty acids (20.9%) and five unsaturated fatty acids (79.1%). Linoleic acid (59.11%), palmitic acid (16.3%) and oleic acid (12.8%) are the major components.

Keywords: *black seed, correlation coefficients, cumin seeds, flow cytometry, habbet el baraka,*

INTRODUCTION

Black cumin (Nigella sativa L.) is an annual medicinal plant belongs to the family Ranunculaceae and its black small seeds used as spice and also in traditional medicine. It is native to Mediterranean regions, Western Asia, and the Middle East. Today, it is considered in modern pharmaceutical and food industries. Seeds or their oil extracts have a lot of medicinal properties like antiviral, antibacterial, antipyretic, carminative and blood sugar lowering effects (Darakhshan et al., 2015). Nigella sativa is grown mainly in Upper Egypt where the climate is dry and warm and local farmers successfully cultivate it by seeds in October and November.

Landraces has been first reported as genetic resources in 1890 (Zeven 1998). Landraces as variable plant populations adapted to local agro-climatic environment with a high capability to tolerate biotic and abiotic stresses producing high yield stability and a transitional yield level under a low input agricultural system (Dwivedi et al. 2016; Kazemi, 2018; Villa et al., 2005).

Landraces have been subject to genetic modification through abiotic, biotic and human exchanges (Dwivedi et al, 2016; Kazemi, 2018; Zeven 1999). Therefore, landraces are ideal genetic resources to explore novel genetic variation that overcomes challenges to crop production and it have played an elementary role in the crops improvement and agricultural production. Cultivation of black cumin in the Upper Egypt region has been started long time ago but few researchers have studied its landraces for the plant improvement. However, limit studies are conducted on black cumin landraces to overcome the drop in its yield (Hassan 2008). The aim of this research was to study the variation of growth and yield related characteristics of five landraces collected from five different regions of Upper Egypt.

Materials and Methods

Plant materials and field experiment:

Seeds of five Nigella sativa landraces were obtained from local farmers in five different Governorates in Southern Egypt (Aswan, Qena. Assiut, Menia and Bani Swief). They were cultivated to study their performance under Assiut environmental conditions. The experiment was conducted at the Floriculture Experimental Farm, Faculty of Agriculture, Assiut University during two successive winter seasons. Seeds of all landraces were planted based on a randomized complete-block design (RCBD) with 3 replications. Each treatment per replicate was presented by three rows 70 cm wide and 3 meters long. Seed sowing was spaced within rows at 30 cm apart.

Measured parameters

The following morphological, yield and yield components parameters were recorded:

1- *Vegetative characteristics*:

Plant height (cm), branch number per plant, stem diameter (cm), root length (cm), fresh and dry weight of herb (g/plant) were measured.

2- *Fruit and flower parameters*:

Number of capsules per plant, capsule diameter (cm), number of seeds per capsule, number of days to flower, flowering percentage after 75 days of sowing, number of days to harvest, seed yield (g) per plant and seed yield in kg per feddan and both volatile and fixed oils yield were studied.

3- *Chemical analysis*

Oil percentage (volatile and fixed oil)

Plant pigments (Chlorophyll a, b and carotenoids) were extracted from the fresh leaves

N, P and K (% in dried leaves)

Flow cytometry and estimation of nuclear genome size

Five landraces were used in this flow cytometry study. Young seedling cotyledons were used as a plant material. The trout red blood cell (TTN) used as an internal control {standard 2C DNA content (CV)}, where 2C= ~ 7 pg. When compare with TTN peak means, we may estimate the 2C value of the sample (s) as follow:

The 2C DNA value of the sample = Peak of the sample (s)/ peak of ttn X Standard 2C DNA content (CV) ~7 pg (Fig. 1)

For example: The genome size of Menia landrace = 331.06/104.54 X 6.94 = 21.9 pg where1pg= 921Mb

Statistical analysis and Correlation coefficients study

All the data were analyzed according to the analysis of variance based on statistical model of randomized complete-block design with three replications. The significant differences between the means were separated using the Least Significant Difference (LSD) test at 5% probability level (Gomes and Gomes 1984). Pearson's correlation coefficients were calculated and tested to examine the associations among some main crop traits.

Results and discusion:

Growth and development

Significant differences among the five landraces are observed over the two seasons of study in the most of the morphological traits (Table 1 and 2). Variation in morphological traits among landraces has been reported by others (Dyulgerov and Dyulgerova, 2013; Hassan, 2008; Kazemi, 2018). The greatest values for plant height, stem diameter, fresh and dry weight are observed in Assiut landrace (Table 1). However, the largest branch number was observed in Asswan landrace followed by Assiut (Table 1) with no significant difference between them in terms of dry weight.

Data presented in Table (2) revealed that significant differences existed among landraces in earliness of flowering and harvest time. The geographical diversity in plants has been proved by others (Cross et al. 2015; Pacifici et al., 2015; Sardans et al., 2017) and may be due to the changes in plant metabolism (Elser et al., 2010)

Menia landrace was the first to start flowering (58.9 days) followed by Qena (59.6 days). Moreover, these two landraces produced the highest percentage of flowered plants (65.18 and 66.08%, respectively) after 75 days of sowing seeds. However, Qena was the first landrace to ripe followed by Menia. The highest capsule number per plant was obtained from Assiut landrace (68.31 capsules) while Menia landraces gave the lowest capsule number per plant (47.89 capsule). On the other hand, Qena produced the highest number of seeds per capsule (113.76 seeds) followed by Assiut (110.57 seeds) and Menia (106.93 seeds).

Seed yield and oil contents

Regarding the seed yield, data presented in Table (3) show a great variation among landraces. Aswan, Qena and Assiut landraces produces the highest seed yield followed by Menia and Bani Sweif. Nimet et al. (2015) showed that yield components of Nigella sativa populations significantly varied according to locations and years.

Variation among landraces has been observed in both volatile and fixed oil yield (Table 3). Aswan and Qena produced the highest yield of volatile oil followed by Assiut while Assiut and Bani Sweif landraces had higher fixed oil yield followed by Qena and Menia. In our samples, it is

indicated that black cumin seeds contain four saturated fatty acids (20.9%) and five unsaturated fatty acids (79.1%). Linoleic acid (60.0%), palmitic acid (16.2%) and oleic acid (12.7%) are the major components (Table 4). The highest volatile oil are *p*-cymene, γ-Terpinene, (+)-α-Longipinene and (+)-Longifolene.

Pigments and elements contents

The highest values of the plant pigments Chlorophyll a and b were detected in Qena landrace followed by Aswan landraces (Table 5). The highest carotenoids content was detected in Qena, Aswan, and Assiut landraces with no significant differences among these landraces. Variation was detected in leaf content of NPK among the five landraces investigated. Higher content of nitrogen was found in Aswan and Menia followed by Qena landrace. Higher content of potassium was detected in Aswan and Qena landraces followed by Assiut and Menia landraces. Higher leaf content of phosphorus was for Qena followed by Menia and Aswan landrace (Table 5).

Flow cytometry and estimation of nuclear genome size

The absolute DNA amount of a sample is calculated based on the values of the TTN peak means (Fig. 1). Absolute DNA amounts are usually reported in pg DNA (Jaroslav Doležel **and** Jan Bartoš 2005**). However, there has been a trend to express DNA amounts in terms of the number of base pairs (bp) and to use the term genome size. The flow cytometry study revealed differences in the genome size among the five landraces. Genome size ranged from 21.90 pg (Menia landrace) to 25.68 pg (Aswan landrace). Many researchers reported intraspecifc variation in genome size in many plant species (**Arumuganathan and Earle, **1991; Michaelson et al., 1991; Rayburn et al., 1997). In sunflower variation was reported as high as 32 % (Michaelson et al. 1991). This may be due to environmental effect or growth conditions. On the other hand, great stability of the nuclear genome has been reported among population of various plant species, pea (Baranyi and Greilhuber, 1995) and Capsicum (Moscone et al., 2003). The aforementioned results, suggesting variability among the used landraces.**

Correlation coefficient study

Among the 21 correlation coefficients (r), 15 showed positive values while six had negative r values (Table 6). Plant height had positive and significant r with both plant dry weight (0.932) and the number of capsules (0.961). The r values were high and positive with branch number/plant (0.831) and days to harvest (0.853). Moderately high negative r value (- 0.370) with the number of seeds/capsule was found for plant height. As a consequence of the aforementioned results, the branch number/plant exhibited a high association with plant dry weight (0.840), number of capsules (0.720) and days to harvest (0.975). Also, plant dry weight positively correlated with days to harvest (0.790) and the number of capsules (0.959) and seed yield (0.368). Additionally, days to harvest positively associated with the number of capsules (0.713) while showing high negative r value with the number of seeds/capsule (-0.762) and seed yield (-0.039).

In general, the negative r values were low or moderately low. Almost all of them were for the number of seeds/capsule with other trait except the total seed yield/fedan. Interestingly, all r values for the total seed yield/fedan with other studied traits were low or negligible. Similar results were reported by Fufa (2016) who detected no significant correlation between seed yield and all the traits studied. The author found that seed yield was positively correlated with number of seed per capsule and dry weight but negatively correlated with days to harvest. The results of our study suggest a possible detection of landrace germplasm with various arrays of the desirable growth and cropping traits among those presented here.

Table (7) shows additional association for the total seed yield/fedan. Great association was clearly existed between the total seed yield/fedan and each of volatile and fixed oil yield and both chlorophyll (a) and carotenoids. However, the total seed yield/fedan showed reduced r value with volatile oil percent (0.640) while having moderate negative association with fixed oil percentage (- 0.467). Also, great negative association was detected between fixed (-0.943*) and volatile oil percent (- 0.943*).

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Table (1) Mean comparisons of vegetative growth characters of *Nigella sativa*, L. landraces

Landraces	Means over two seasons					
	Plant height (cm)	Branch number/plant	Stem diameter (cm)	Root length (cm)	Fresh weight of herb (g/plant)	Dry weight of herb (g/plant)
Aswan	87.32 b	17.85 a	0.94 b	12.74 c	79.67 b	42.52 a
Qena	77.79 d	10.98 d	0.73 c	15.08 a	71.35 c	33.34 c
Assiut	89.36 a	14.52 b	0.98 a	13.83 b	87.44 a	43.66 a
Menia	76.02 d	10.72 d	0.65 d	14.83 a	66.38 d	25.98 d
Bani Sweif	85.10 c	13.25 c	0.94 b	13.76 b	77.40 b	35.24 b

Means followed by the same letter(s) are not significantly different at P ≤ 0.05 using the Least Significant difference Test (LSD).

Table (2) **Mean comparisons of flowering and capsule characteristics of *Nigella sativa*, L. landraces**

Landraces	Means over two seasons					
	Number of days to flower	(%) Flowering 75 days after sowing	Number of days to harvest	Capsule number/ plant	Capsule diameter **(cm)**	Number of seeds/capsule
Aswan	79.63 a	9.38 c	152.00 a	62.37 b	1.05 b	100.53 e
Qena	59.63 d	66.08 a	130.53 d	54.85 c	1.09 a	113.76 a
Assiut	65.50 c	16.97 b	142.00 b	68.31 a	1.05 b	110.57 b
Menia	58.88 e	65.18 a	131.36 c	47.89 d	1.05 b	106.93 c
Bani Sweif	66.25 b	19.59 b	142.38 b	60.31 b	1.03 c	104.79 d

Means followed by the same letter(s) are not significantly different at P ≤ 0.05 using the Least Significant difference Test (LSD)

Table (3) Mean comparisons of seed yield and its component in *Nigella sativa*, L. landraces

Landraces	Means over two seasons					
	Seed yield (g)/plant	Seed yield (kg/feddan)	Volatile oil percentage	Volatile oil yield (ml) per feddan	Fixed oil percentage	Fixed oil yield (kg)/feddan
Aswan	8.46 a	497.58 a	0.37 a	1826.77 a	31.15 c	154.65 c
Qena	8.92 a	524.11 a	0.34 b	1804.55 a	34.13 b	180.00 b
Assiut	8.49 a	499.29 a	0.32 c	1584.68 b	38.90 a	194.76 a
Menia	7.38 b	433.47 b	0.34 b	1468.19 c	34.92 b	147.90 c
Bani Sweif	6.09 c	357.73 c	0.30 d	1084.70 d	38.46 a	137.19 d

Means followed by the same letter(s) are not significantly different at P ≤ 0.05 using the Least Significant difference Test (LSD).

Table (4): Fatty acid composition of the fixed oil of five different landraces of *Nigella sativa*

Compounds		**Assiut**	**Qena**	**Menia**	**Aswan**	**Beni sweif**
	RT	Area %	Area %	Area %	Area %	Area %
Myristic acid	28.58	0.48	0.48	0.48	0.54	0.47
Palmitoleic acid	33.19	0.48	0.49	0.50	0.61	0.49
Palmitic acid	33.89	**16.54**	**15.96**	**16.32**	**16.61**	**16.20**
Linoleic acid	38.13	**58.35**	**59.11**	**59.25**	**59.09**	**59.77**
Oleic acid	38.22	**13.16**	**13.27**	**13.12**	**11.97**	**12.48**
Stearic acid	38.55	4.30	3.95	3.84	3.60	3.99
Linolenic acid	38.73	0.33	0.29	0.26	0.31	0.29
11,13-Eicosadienoic acid	42.14	5.11	5.52	5.28	6.11	5.38
cis-11-Eicosenoic acid	42.19	0.57	0.55	0.57	0.61	0.56
Eicosanoic acid	42.72	0.42	0.38	0.38	0.37	0.39
Total fatty acid		99.74	100	100	99.82	96.43

Table (5) Mean comparisons of chemical composition of *Nigella sativa*, L. landraces

Landraces	Means over two seasons					
	Chlorophyll *(a)*	**Chlorophyll *(b)***	**Leaf content of carotenoids (mg/g FW)**	**Leaf content of nitrogen**	**Leaf content of phosphorus**	**Leaf content of Potassium**
Aswan	1.54 b	0.61 b	0.58 a	2.25 a	0.68 c	1.52 a
Qena	1.75 a	0.72 a	0.56 a	2.07 b	0.73 a	1.53 a
Assiut	1.58 c	0.55 c	0.60 a	1.72 d	0.54 d	1.45 b
Menia	1.59 c	058 c	0.38 b	2.27 a	0.70 b	1.46 b
Bani Sweif	1.27 d	0.55 c	0.37 b	1.86 c	0.53 d	1.43 c

Means followed by the same letter(s) are not significantly different at P ≤ 0.05 using the Least Significant difference Test (LSD).

Table (6): Phenotypic correlation coefficients (r) of the six agronomic traits of black cumin landraces.

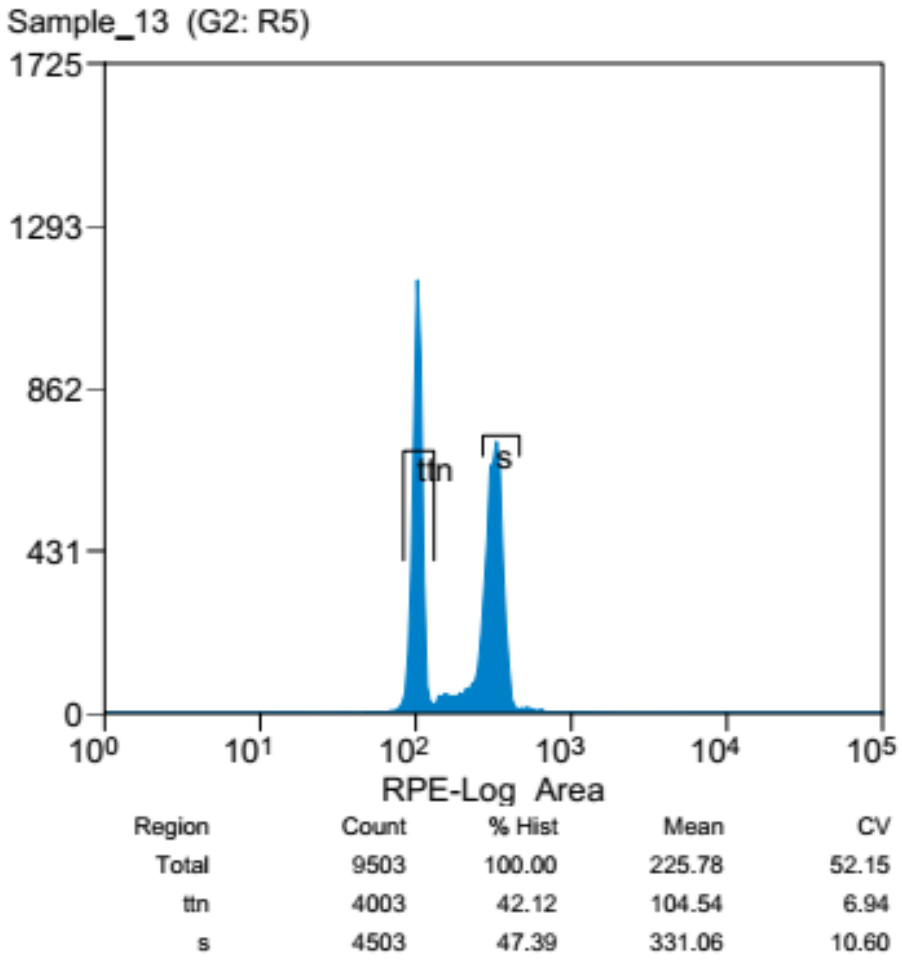
Traits	**Plant height (cm)**	**Branch number/plant**	**DW (gm/plant)**	**Number of Days to Harvest**	**Capsule number/ plant**	**Number of seeds/ capsule**	**Seed yield Kg/fedd**
Plant height (cm)	1						
Branch number/plant	0.831	1					
Dry weigt (g/plant)	0.932*	0.840	1				
Number of Days to Harvest	0.853	0.975**	0.790	1			
Capsule number/plant	0.961**	0.720	0.959**	0.713	1		
Number of seeds/capsule	- 0.370	-0.681	-0.207	-0.762	-0.115	1	
Seed yield kg/fedd	0.030	0.180	0.368	-0.039	0.187	0.402	1

n = 5; * and ** denote significance at P ≤ 0.05 and 0.01, respectively.

Table (7): Phenotypic correlation coefficients (r) of flowering, oil and pigment components traits with seed yield of black cumin landraces.

Traits	**Seed Yield/plant**	**Volatile oil %**	**Fixed oil %**
Days to flower	**0.110**	**0.532**	**-0.442**
Volatile oil %	**0.640**	**-**	**-0.943***
Volatile oil yield	**0.954****	**0.835**	**-0.710**
Fixed oil %	**- 0.467**	**- 0.943***	**-**
Fixed oil yield	**0.789**	**0.058**	**0.174**
Chlorophyll (*a*)	**0.881****	**0.561**	**-0.438**
Chlorophyll (*b*)	**0.115**	**0.371**	**-0.407**
Carotenoids	**0.886****	**0.448**	**-0.250**

n = 5; * and ** denote significance at P ≤ 0.05 and 0.01, respectively.



Region	Count	% Hist	Mean	CV
Total	9503	100.00	225.78	52.15
ttn	4003	42.12	104.54	6.94
s	4503	47.39	331.06	10.60

Fig. 1 Estimation of absolute nuclear DNA amount (genome size) in Menia Landrace (as an example)

أداء وإرتباطات سلالات أرضيه مختلفه من حبه البركه تحت ظروف مصر العليا

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الملخص :

تم إجراء تقييم خلال موسمين متتاليين لدراسة التباين وارتباطات النمو وبذور وإنتاجية الزيت من خمسة أصناف مصرية من الكمون الأسود (حبة البركة السوداء). كشفت البيانات التي تم الحصول عليها تباين كبير بين السلالات في جميع الصفات الخضريه والزهريه. أظهرت السلاله الأرضيه التى جمعت من محافظه أسيوط أعلى قيمة لارتفاع النبات ، قطر الساق ، الوزن الطازج والجاف للعشب ، وعدد الكبسولات للنبات. بينما أظهرت السلاله الأرضيه التى جمعت من محافظه المنيا أعلى نسبة تزهير (٦٥.١٨ ٪) وتبكير فى الأزهار(٥٨.٨٨ أيام) والحصاد (١٣١.٣٦ أيام). السلالات الأرضيه من أسوان وقنا وأسيوط أنتجت أعلى محصول للبذور (كجم / فدان). ومع ذلك ، أنتجت سلاله أسوان أعلى نسبة من الزيت الطيار وأقل نسبة من الزيت الثابت تليها سلالتى قنا وأسيوط على التوالي. من بين ٢١ معامل ارتباط (r) ، أظهر ١٥ معامل قيمة موجبة بينما كانت ست قيم سالبة (r). ومع ذلك ، كانت جميع قيم معامل الارتباط لمحصول البذور / الفدان مع الصفات المدروسة الأخرى منخفضة أو لا تذكر. ومن ثم ، يُحتمل أنه يمكن الكشف عن أصول وراثية أرضية تجمع مختلف توافيق الصفات المرغوب فيها من بين الصفات التي درست هنا. وكشفت الدراسه السيتولوجيه أن حجم الجينوم للخمسة سلالات موضع الدراسه تراوحت بين ٢١.٩ إلى ٢٥.٦٨ جزء من الغرام. أشار تحليل الزيت أن بذور كل سلالات حبه البركه تحتوى أربعة أحماض دهنية مشبعة (٢٠.٩٪) وخمسة أحماض دهنية غير مشبعة (٧٩.١٪). وكان حمض اللينوليك (٥٩.١١٪) ، حمض البالمتيك (١٦.٣٪) وحمض الأوليك (١٢.٨٪) هي المكونات الرئيسية.