

**GENETIC ANALYSIS AND EVALUATION  
OF SOME EGYPTIAN LANDRACES OF ROCKET  
(*ERUCA SATIVA* MILL.) FOR HIGH SEED OIL YIELD**

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**ABSTRACT**

*This study was conducted during the period from 2014 to 2016 at Kaha Vegetable Research Farm, Qalioubiya Governorate, ARC, Egypt to assess genetic variability and heritability for fresh yield, seed yield, seed oil yield and related traits in seventeen Egyptian rocket landraces collected from different regions of Egypt. Results showed that estimated broad-sense heritability showed high values and the large portion of phenotypic variance ( $\sigma_p^2$ ) was due to the genetic variance ( $\sigma_g^2$ ) for the traits leaf area, fresh yield, seed yield, seed oil percentage, seed oil yield, antioxidant activity and total phenols content. Therefore, these characters can be improved through selection based on phenotypic observations in early segregating generations in rocket germplasm. From this study, it can be concluded that L<sub>4</sub> and L<sub>13</sub> were the best landraces with respect to fresh yield, seed yield, seed oil yield and reasonable oil characters. AFLP (The Amplified Fragment Length Polymorphism) fingerprinting was applied to a group of rocket landraces to find if there is any geographical differentiation in rocket diversity from Egypt. The high level of polymorphism within rocket landraces and the high number of landraces-specific bands suggest that AFLPs are powerful markers for diversity analysis in rocket landraces. Region-specific AFLP markers were found (present in landraces from the same origin and absent in others). AFLPs generated data show that the highest genetic similarity value to be 99.8% and the lowest value to be 82.7% with an average of 91.25%. Based on AFLP analysis, the highest similarity percentage (99.8%) detected by the AFLP assay was between L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub>, L<sub>9</sub>, L<sub>11</sub>, L<sub>12</sub> and L<sub>13</sub> all of them share the same genetic background and geographical region since they were collected from the nearby regions in Egypt. While the lowest percentage of similarity (82.7%) was detected between L<sub>8</sub> and L<sub>17</sub> landraces. The UPGMA dendrogram revealed two distinct clusters corresponding almost to the geographical origin of the landraces in Egypt.*

Key words: *Eruca sativa*, Landrace, PCV, GCV, Heritability, AFLP.

**INTRODUCTION**

Rocket (*Eruca sativa* Mill.) is an important vegetable crop during winter season belonging to family Brassicaceae. Besides its popular uses as vegetables, rocket is also considered a medicinal plant. Common names include rocket salad, garden rocket, arugula and roquette where it is generally grown for young leaves that are eaten as green salad or seeds that are produced for oil. Rocket salad is an ancient, under-utilized crop, which is either gathered from the wild or cultivated mainly as a leaf vegetable and oil crop in several European and Near East countries. Ancient Egyptians and Romans both have considered the leaves in salad to be an aphrodisiac. The economic potential of rocket salad is steadily increasing in recent years.

Besides increasing yield, improving quality of the oil seed crop is also very essential. The oil seed will be considered to be of good quality if it

has high protein, oil, oleic acid and low linolenic acid, glucosinolates and erucic acid (CFIA 1999). The oil obtained from the seed is applied to the body as antimicrobial and anti-lice agent. One gram seeds as an early morning dose, before breakfast, cure bleeding piles. The oil slightly warmed over fire, if poured into ears, relieves pain. Although rocket oil is not edible because of its chemical composition, it is generally mixed with mustard oil to give more pungency to the later a trait preferred in mustard oil. Sharma (1991) estimated the composition of oil in rocket as 46.3% for erucic acid, 28.7% for oleic acid, 12.4% for linoleic acid and 10.5% for saturated fatty acids. The medicinal effect of the plant is ascribed to its possession of antioxidant compounds. Phenolic compounds have shown to be effective antioxidant constituents, due to their ability to scavenge free radicals which is the cause of many chronic diseases (Arouma 1998).

In plant breeding, information about variability and heritability of fresh yield, seed oil yield, seed yield and quality characters is important for an efficient breeding program. Efforts are in progress to create rocket genotypes with improved quality characters but limited information is available on the nature of variability and magnitude of heritability of *E. sativa* genotypes, in spite of largely underutilized in most countries. In breeding programs, wide range of variability and high heritability of traits could be an excellent tool for improving or selection a genotype (Akbar *et al* 2003).

Numerous examples are available for the presence of great genetic variation for both qualitative and quantitative traits in rocket germplasm. Duhoon and Koppa (1998) found a wide range of phenotypic variation among native rocket population in India. Yadava *et al* (2006) evaluated *E. sativa* accessions and great variations were assessed in oil content and fatty acid composition. Furthermore, Sastry and Meena (2003) assessed the variability and heritability and found that the characters seed yield, fresh yield and oil content showed highly significant variability. Also, they reported high heritability (broad sense) value for oil content (72.2%), moderate heritability for seed yield (43.4%), whereas low heritability was observed for fresh yield (20.4%). Bozokalfa *et al* (2010) revealed that there is a genetic variability for the traits leaf length, plant weight and number of leaves among *Eruca* spp. genotypes and showed effectiveness of selection for different agronomic traits of *Eruca* genotypes for further breeding. They found that these traits influenced more by genetic factors than non-genetic. Also, the differences between G.C.V and P.C.V were narrow, meanwhile, the heritability estimated as 97.15% for leaf length, 99.73% for plant weight and 89.29% for number of leaves, indicating that environmental factors did not greatly affect phenotypic variation of such characters. On winter rapeseed, high genetic variability was recorded for oil yield and seed yield which demonstrated the low effect of environment for the inheritance of

these characters (Ahmad *et al* 2013). Furthermore, estimated broad sense heritability showed high values (98.6% and 85.2%) in oil yield and seed yield traits, respectively, indicating that the observed significant phenotypic differences among the studied genotypes are of genetic nature and there are small environmental effects on the phenotypic variation. Also, the data of both characters revealed that large portion of phenotypic variance ( $\sigma^2_p$ ) was due to the genetic variance ( $\sigma^2_g$ ). Therefore, these characters can be improved through selection based on phenotypic observations in early segregating generations. Jat *et al* (2014) indicated that variance due to genotypes was highly significant for the traits seed yield and oil content %. Also, broad sense heritability estimates were low to moderate for both characters. Aytaç *et al* (2008) found that large portion of phenotypic variance ( $\sigma^2_p$ ) was due to the genetic variance ( $\sigma^2_g$ ) for seed yield character. Giessen *et al* (2003) and Akbar *et al* (2007) on rocket observed slight differences between P.C.V and G.C.V in the traits seed yield and oil yield. Also, they found high heritability for these traits indicating the importance of the genetic effect in controlling the inheritance of these traits. However, the low variation was found in number of leaves (Ziaullah Ghazali *et al* 2014).

Three types of markers have been generally used in the assessment of genetic diversity in rocket (*Eruca sativa* Mill.), morphological markers, biochemical markers and molecular markers.

In the last decade, molecular markers played a major role in studying of crop genetic diversity in different species and germplasm collections, fingerprinting, genetic characterization and improvement of many crop species. Moreover, they contributed in expanding our abilities to assess biodiversity, reconstruct accurate phylogenetic relationships and understand the structure and evolution of plant populations. Using molecular marker technology in rocket represents a high efficiency tool for indirect selection and would enhance the efficiency and accuracy of screening for oil production. Furthermore, quantitative analysis has proven useful for locating genes controlling complex traits and provides a more accurate estimation of gene location than qualitative analysis because of its lower sensitivity to even modest numbers of phenotypic mis-scores (Wright 1998). The Amplified Fragment Length Polymorphism (AFLP) has been widely used to rapidly generate molecular markers among various organisms from bacteria to plants (Vos *et al* 1995). The AFLP analysis combines the reliability of restriction enzyme digestion with the utility of the polymerase chain reaction. AFLP fragments are inherited in a Mendelian fashion as dominant or codominant markers, making the techniques amenable to tracking inheritance of genetic loci in a segregating population. AFLP detects the presence of point mutations, insertions, deletions and other genetic rearrangements; a very reproductive and reliable but with higher

multiplex ratio (Vuylsteke *et al* 2007). The advantages of this technique include the large number of loci analyzed, high polymorphism levels, high reproducibility without prior sequence knowledge, and genome-wide marker distribution (Wu *et al* 2004).

AFLP analyses have proven able to resolve genetic variations when other genetic markers have reached their limits (Ojuedrie *et al* 2014) and so have the potential to reveal genetic diversity and differentiate between populations. AFLP has already been used successfully with a number of crops such as rice (Mackill *et al* 1996), tea (Powell *et al* 1997), almond (Sorkheh *et al* 2007), barley (Russel *et al* 1997), *Cynodon* species (Wu *et al* 2005), and has been shown to reveal significant levels of DNA polymorphism in plants (Menz *et al* 2004).

The present investigation was carried out to assess genetic variability and heritability for fresh yield, seed yield, seed oil yield and related traits in seventeen Egyptian rocket germplasm. Also, to investigate molecular profiles among the landraces using AFLP molecular marker technique.

#### **MATERIALS AND METHODS**

This study was conducted during the period from 2014 to 2016 at Kaha Vegetable Research Farm, Qalioubiya Governorate, ARC, Egypt. Seventeen genotypes/landraces were collected from different regions of Egyptian governorates for use as materials for this study as shown in Table (1) and Fig. 1. These landraces were chosen based on their morphological characters and grown during two successive winter seasons in 2014/15 and 2015/16.

The sowing dates were 20<sup>th</sup> October and 5<sup>th</sup> November in 2014 and 2015, respectively. All landraces were evaluated in a randomized complete block design (RCBD) with three replications. Plot size was 2.0 m x 3.0 m. Rocket seeds were sown directly in the field. The plant to plant distance was maintained at 10.0 cm by thinning at 25 days after sowing. Leaves were harvested twice (six and eleven weeks after sowing date). Leaves were harvested from each plant with stems at least 1.0 cm above the cotyledons to avoid damaging the apex. Leaf samples were harvested by hand with a knife in the afternoon, due to the leaves showing a much lower concentration of nitrates compared to the morning harvest (Pimpini and Enzo 1996).

**Table 1. List of *Eruca* accessions and origins used in study.**

Accession	Governorate	Origin	Accession	Governorate	Origin
L <sub>1</sub>	Giza	Abo El-Nomrous	L <sub>10</sub>	Matrouh	Siwa
L <sub>2</sub>	Qalioubiya	Benha	L <sub>11</sub>	Fayoum	Fayoum
L <sub>3</sub>	Menoufiya	Ashmoun	L <sub>12</sub>	Fayoum	Ibshway
L <sub>4</sub>	Menoufiya	Quesna	L <sub>13</sub>	Fayoum	Tamiya
L <sub>5</sub>	Sharkiya	Menia Al-Kamh	L <sub>14</sub>	Beni suef	Beni suef
L <sub>6</sub>	Behiera	Abou-Homous	L <sub>15</sub>	Assiut	Assiut
L <sub>7</sub>	Kafr El-Sheikh	Al-Borolus	L <sub>16</sub>	Sohag	Sohag
L <sub>8</sub>	Dakahleya	Dekernis	L <sub>17</sub>	Aswan	Aswan
L <sub>9</sub>	Dakahleya	El-Mansoura			



**Fig. 1. Distribution of collected *Eruca* landraces from Egypt.**

Ten competitive plants were randomly selected at week 6 after planting (at first cutting) from each plot to record the following observations: leaf length (cm), leaf area (cm<sup>2</sup>), No. of leaves per plant, fresh leaves weight/plant (g) and total chlorophyll content (mg/100 fresh weight). Also, antioxidant activity (%) and total phenols content (mg /g dry weight) were estimated. At the marketable stage (first and second cutting), plants of each experimental plot were cut and weighted, and then fresh yield (ton/fed) was calculated. At the time of maturity, seed yield (kg/fed), seed oil percentage (%) and seed oil yield (kg/fed) were estimated. Oil percentage content of the seeds was estimated according to A.O.C.S. (1964).

#### **Gas chromatography analysis for fatty acids**

Fatty acids were extracted from rocket seeds oil (after saponification and acidification) according to the official method of A.O.A.C. (2000). The methyl esters of fatty acids were prepared using (methanol: concentrated sulphuric acid at 99:1 v:v) and fatty acids methyl esters were extracted with hexane, according to the method described by El-Kholy (1996).

#### **Identification of fatty acids methyl esters by GLC**

Fatty acids methyl esters were analyzed by Hewlett Packard gas chromatograph 5890 equipped with flame ionization detector. The chromatograph was fitted with FFAP 30 m x 0.32 mm x 0.25 mm capillary column coated with polyethylene glycol-TPA modified. The column oven temperature was programmed at 7 °C/min from 150 °C to 240 °C where it was kept for 30 min. Injector and detector temperatures were 260 and 280 °C, respectively. Gases flow rates were 33, 30, 330 ml/min for N<sub>2</sub>, H<sub>2</sub> and air, respectively and split ratio was 100:1. Peak identification was performed by comparison of the relative retention time (RRT) for each peak with those of standard fatty acids. The relative retention time of oleic acid was given a value of 1.0. The peaks areas were measured using Chemstation Program, and relative areas of the identified fatty acids were recorded.

**Total phenols content:** Total phenol content was determined by the Folin-Ciocalteu method. Aliquots of 0.5 ml of sample or standard solution (0-500 mg/l) were added to a ten-fold diluted Folin-Ciocalteau' s reagent (2.5 ml) and 2 ml of 7.5% sodium carbonate solution. The mixture was incubated for 30 min at room temperature and measured spectrophotometrically at 765 nm. The total amount of phenolic compounds was calculated using gallic acid calibration curve. The results were expressed as mg gallic acid equivalents per gram of dry weight (Singleton and Rossi 1965).

#### **DPPH radical scavenging assay (Antioxidant activity %)**

**Preparation of leaves extract.** Rocket leaves (2 g) were extracted with 50 ml of methanol (80%) for 48 hour. The extracts were then evaporated to dryness under vaccum at 40 °C using a rotary evaporator. The obtained extracts were stored at 4°C until further use. The antioxidant

activity was measured using the stable free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Briefly, a 3.0 ml of methanolic solution of Rocket extract (50µg/ml) was added to a 1.0 ml solution of DPPH (0.1 mM) in methanol. After incubation at room temperature for 30 min, the absorbance was spectrophotometrically determined at 517 nm, and the radical scavenging activity was expressed as percentage inhibition of free radical DPPH.

$$\% \text{Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where  $A_{\text{control}}$  is the absorbance of the control reaction (containing all reagents except the test compound), and  $A_{\text{sample}}$  is the absorbance of the test compound, according to the method described by Oktay *et al* (2003).

#### **Amplified fragment length polymorphism (AFLP) technique**

Genomic DNA was extracted from youngest leaves using DNeasy® Plant mini Kit for DNA isolation from plant tissue with modifications by adding 5 mg/ml PVP to the extraction buffer.

AFLP analysis was performed using the Invitrogen AFLP® Analysis system I, and AFLP Starter primer kit (Cat # 10544-013 and 10483-014, respectively, Invitrogen Corporation, Carlsbad, California, USA) as indicated in Table 2.

**Table 2. A list of AFLP primers and adapters sequence.**

<b>Name</b>	<b>Sequence (5'-3')</b>
<b><i>MseI</i> forward adapter</b>	<b>GACGATGAGTCCTGAG</b>
<b><i>MseI</i> reverse adapter</b>	<b>TACTCAGGACTCAT</b>
<b><i>EcoRI</i> forward adapter</b>	<b>CTCGTAGACTGCGTACC</b>
<b><i>EcoRI</i> reverse adapter</b>	<b>AATTGGTACGCAGTCTAC</b>
<b><i>MseI</i> primer core region (M)</b>	<b>GATGAGTCCTGAGTAA</b>
<b><i>MseI</i> pre-selective primer (M+1)</b>	<b>M+C</b>
<b><i>MseI</i> selective primers (M+3)</b>	
<b>M+CTA</b>	<b>GATGAGTCCTGAGTAACTA</b>
<b>M+CTC</b>	<b>GATGAGTCCTGAGTAACTC</b>
<b>M+CTG</b>	<b>GATGAGTCCTGAGTAACTG</b>
<b>M+CTT</b>	<b>GATGAGTCCTGAGTAACTT</b>
<b><i>EcoRI</i> primer core region (E)</b>	<b>GACTGCGTACCAATTC</b>
<b><i>EcoRI</i> pre-selective primer (E+1)</b>	<b>E+A</b>
<b><i>EcoRI</i> selective primers (E+3)</b>	
<b>E+ACA</b>	<b>GACTGCGTACCAATTCACA</b>
<b>E+ACC</b>	<b>GACTGCGTACCAATTCACC</b>
<b>E+ACG</b>	<b>GACTGCGTACCAATTCACG</b>
<b>E+ACT</b>	<b>GACTGCGTACCAATTCACT</b>

Amplicon template preparation, pre-amplification and selective amplification were conducted according to manufacturer's protocol of the BRL AFLP analysis system I kit (Gibco, USA) for small genome using a total of 200 ng of genomic DNA in EcoRI(E)/MseI(M) digestion.

Four sets of selective primer combinations were used ( $M_{CTA} \times E_{ACA}$ ,  $M_{CTC} \times E_{ACC}$ ,  $M_{CTG} \times E_{ACG}$  and  $M_{CTT} \times E_{ACT}$ ) The DNA concentration was adjusted to 200 ng/ $\mu$ L, and DNA was digested in 50  $\mu$ L of restriction-reaction mixture containing 4 U of EcoRI and 4 U of MseI in T4 ligase buffer. It was then incubated for 2 h at 37 °C. The success of the AFLP technique is dependent upon complete restriction digestion of DNA, therefore, much care should be taken in consideration to isolate high quality (purity) genomic DNA, intact without contaminating nucleases or inhibitors. For the ligation reaction, a mixture containing EcoRI adapter (25 pmol/ $\mu$ L), MseI adapter (25 pmol/ $\mu$ L), 2 U of T4 DNA ligase, and 1X T4 DNA ligase buffer was added to the restriction reaction and incubated for 2 h at 37 °C. Preamplification was carried out in 25- $\mu$ L volumes. The reactions contained 1X PCR buffer, 1.5 mM MgCl<sub>2</sub>, 1 mM dNTP, 15pmol/ $\mu$ L of each preselective primer EcoRI-A and MseI-C, 0.2 U of Taq polymerase (promega), and 200 ng/ $\mu$ L template DNA. Preamplification with primers having a single selective nucleotide were performed using a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, California, USA) with following cycle profile: 4 min of DNA denaturation step at 94 °C, followed by 28 cycles of 50 s at 94 °C, 1 min at 56 °C, and 1 min at 72 °C, with final elongation at 72 °C for 10 min. The reaction mixtures were diluted 50-fold for selective PCR. Selective amplification was conducted with 4 combinations of selective primers using the following nucleotides:  $M_{CTA} \times E_{ACA}$ ,  $M_{CTC} \times E_{ACC}$ ,  $M_{CTG} \times E_{ACG}$  and  $M_{CTT} \times E_{ACT}$ ). Reactions were conducted in a 25- $\mu$ L volume containing 1X PCR buffer, 0.75 mM MgCl<sub>2</sub>, 1 mM dNTP, 0.25  $\mu$ M of each selective primer EcoRI-ANN and MseI-CNN, and 0.2 U of Taq polymerase (promega). PCR was performed for 35 cycles with the following cycle profile: a 50-s DNA denaturation step at 94 °C, a 30-s annealing step, and a 1-min extension step at 72 °C. The annealing temperature was set at 65 °C for the first cycle, gradually reduced by 0.7 °C for each of the next 13 cycles, and kept at 56 °C for the remaining 23 cycles.

Amplification products were electrophoresed in 6% (w/v) denaturating polyacrylamide sequencing gels for 2h at 2000W, and DNA bands were visualized silver staining according to methods of (Vos *et al* 1995).

#### **Data analysis**

Amplified products for AFLP markers were visually examined for each primer or each primer combination. Also, Gel Documentation System (Gel-Doc 2000, with Diversity Database Fingerprinting Software, version



2.1, Bio-Rad Laboratories, Hercules, California, USA) was used for gel analysis, scoring, data handling, cluster analysis and construction of dendrograms.

Pair-wise comparisons of Rocket landraces were used to determine similarity coefficients according to Dice's similarity coefficient (DSC's). The similarity coefficient was then used to construct dendrogram using the Un-weighted Pair Group Method with Arithmetic Averages (UPGMA) employing.

#### **Statistical analysis**

An analysis of variance (ANOVA) for all characters was carried out to determine the significant differences between evaluated genotypes and mean comparisons were based on the LSD test (Steel and Torri 1981). Also, the Bartlett's test of the variance of error for genotypes in both seasons (2014/15 and 2015/16) was homogeneous for all traits. So, the combined analysis of variance for the two seasons was computed for all traits according to Snedecor and Cochran (1989). Genotypic and phenotypic coefficients of variation were determined in accordance with Singh and Chaudhary (1995) method. Heritability estimates were calculated by using the formulae of Allard (1960).

### **RESULTS AND DISCUSSION**

#### **Performance of the collected landraces**

Means of the evaluated rocket landraces in the 2014/15 and 2015/16 winter plantings and combined analysis of both seasons are presented in Tables 3, 4, 5 and 6. Significant differences were observed among the landraces for all studied traits.

Combined analysis of both seasons showed significant differences for leaf length character among the evaluated landraces (Table 3). Leaf length ranged from 26.43 cm to 30.95 cm. The maximum length value was recorded by the landrace L<sub>14</sub> (30.95 cm) followed by L<sub>2</sub> (30.67 cm) without significant differences between them, while the shortest leaves (26.43 cm) were found in the landrace L<sub>16</sub>.

Concerning leaf area trait, significant differences were observed among the evaluated landraces (Table 3). Mean area of leaf of the evaluated landraces ranged from 29.11 cm<sup>2</sup> to 57.01 cm<sup>2</sup>. The highest value of leaf area was shown by the landrace L<sub>16</sub> (57.01 cm<sup>2</sup>) followed by landrace L<sub>2</sub> (55.93 cm<sup>2</sup>) without significant differences between them. The lowest value of leaf area was exhibited by landrace L<sub>10</sub> (29.11 cm<sup>2</sup>).

Regarding no. of leaves per plant trait (Table 3), the landrace L<sub>12</sub> showed the highest number of leaves per plant (10.08) followed by landrace L<sub>11</sub> (9.73) without significant differences between them, meanwhile, the lowest number were recorded in landrace L<sub>4</sub> (6.90).

**Table 3. Mean performances of the evaluated rocket landraces for leaf length (cm), leaf area (cm<sup>2</sup>) and number of leaves/plant characters.**

Characters Landraces	Leaf length (cm)			Leaf area (cm <sup>2</sup> )			No. leaves/plant		
	2014/ 15	2015/ 16	Comb.	2014/ 15	2015/ 16	Comb.	2014/ 15	2015/ 16	Comb.
L <sub>1</sub>	28.63	29.60	29.12	43.75	55.84	49.79	6.80	7.10	6.95
L <sub>2</sub>	29.27	32.07	30.67	53.72	58.13	55.93	8.73	7.03	7.88
L <sub>3</sub>	29.43	30.67	30.05	45.59	59.83	52.71	9.40	8.20	8.80
L <sub>4</sub>	25.67	32.93	29.30	51.72	54.15	52.94	7.47	6.33	6.90
L <sub>5</sub>	26.20	33.07	29.63	43.53	55.70	49.61	6.27	8.33	7.30
L <sub>6</sub>	25.97	30.77	28.37	42.03	40.04	41.04	10.60	8.50	9.55
L <sub>7</sub>	29.53	27.93	28.73	51.30	53.77	52.54	9.93	8.20	9.07
L <sub>8</sub>	25.87	29.67	27.77	29.79	43.54	36.67	10.40	7.77	9.08
L <sub>9</sub>	26.27	29.43	27.85	51.53	57.04	54.28	8.80	10.00	9.40
L <sub>10</sub>	26.50	30.97	28.73	26.07	32.14	29.11	8.07	7.93	8.00
L <sub>11</sub>	26.07	27.67	26.87	33.15	32.45	32.80	11.60	7.87	9.73
L <sub>12</sub>	25.00	29.47	27.23	32.13	44.66	38.39	11.87	8.30	10.08
L <sub>13</sub>	24.90	30.17	27.53	50.47	50.62	50.55	8.00	9.43	8.72
L <sub>14</sub>	29.67	32.23	30.95	41.31	41.36	41.34	8.60	7.00	7.80
L <sub>15</sub>	30.20	25.73	27.97	37.58	42.36	39.97	9.67	8.37	9.02
L <sub>16</sub>	25.83	27.03	26.43	55.09	58.92	57.01	7.87	8.90	8.38
L <sub>17</sub>	25.03	32.03	28.53	35.38	43.32	39.35	10.53	7.67	9.10
LSD at 5%	1.65	2.16	2.30	1.75	4.11	3.94	0.62	0.51	1.17

**Table 4. Mean performances of the evaluated rocket landraces for fresh leaves weight/plant (g), total chlorophyll (mg/100 g fresh weight) and antioxidant activity (%) characters.**

Characters Landraces	Fresh leaves weight/plant (g)			Total chlorophyll content (mg/100 g fresh weight)			Antioxidant activity%		
	2014/15	2015/16	Comb.	2014/15	2015/16	Comb.	2014/15	2015/16	Comb.
L <sub>1</sub>	13.13	16.60	14.87	31.60	32.83	32.22	27.38	27.47	27.42
L <sub>2</sub>	12.60	14.00	13.30	28.37	31.53	29.95	29.15	29.84	29.50
L <sub>3</sub>	14.13	13.60	13.87	35.37	34.77	35.07	38.36	37.19	37.78
L <sub>4</sub>	11.73	15.30	13.52	34.10	31.63	32.87	38.98	36.20	37.59
L <sub>5</sub>	10.67	19.87	15.27	34.80	34.60	34.70	37.72	37.11	37.42
L <sub>6</sub>	10.17	15.00	12.58	33.60	33.37	33.48	37.51	35.81	36.66
L <sub>7</sub>	11.77	12.30	12.03	36.07	37.60	36.83	29.12	30.13	29.63
L <sub>8</sub>	12.00	12.60	12.30	36.90	38.17	37.53	28.42	30.72	29.57
L <sub>9</sub>	13.27	15.33	14.30	31.70	34.67	33.18	27.36	27.72	27.54
L <sub>10</sub>	10.33	12.73	11.53	32.93	35.27	34.10	25.85	25.11	25.48
L <sub>11</sub>	11.33	11.27	11.30	35.47	38.03	36.75	26.05	25.16	25.60
L <sub>12</sub>	10.30	12.70	11.50	38.50	36.23	37.37	25.00	24.60	24.80
L <sub>13</sub>	12.00	15.17	13.58	38.37	35.63	37.00	23.85	25.39	24.62
L <sub>14</sub>	11.07	13.33	12.20	30.10	34.07	32.08	26.09	27.62	26.85
L <sub>15</sub>	12.93	13.43	13.18	31.10	34.27	32.68	27.41	28.47	27.94
L <sub>16</sub>	10.80	15.10	12.95	35.20	33.67	34.43	24.09	24.59	24.34
L <sub>17</sub>	9.50	12.40	10.95	35.60	35.77	35.68	25.43	24.74	25.09
LSD at 5%	0.97	0.97	1.58	3.75	0.73	2.19	1.12	0.93	1.05

**Table 5. Mean performances of the evaluated rocket landraces for total phenols content (mg/g dw), fresh yield (ton/fed) and seed yield (kg/fed) characters.**

Characters Landraces	Total phenols content			Fresh yield (ton/fed)			Seed yield (Kg/fed)		
	2014/ 15	2015/ 16	Comb.	2014/ 15	2015/ 16	Comb.	2014/ 15	2015/ 16	Comb.
L <sub>1</sub>	5.13	4.89	5.01	12.426	9.667	11.047	296.67	290.53	293.60
L <sub>2</sub>	3.43	2.90	3.17	11.536	13.371	12.454	303.00	280.86	291.93
L <sub>3</sub>	3.64	3.10	3.37	15.479	14.412	14.945	286.67	261.54	274.11
L <sub>4</sub>	3.04	2.90	2.97	14.750	14.834	14.792	323.33	293.70	308.52
L <sub>5</sub>	3.16	3.06	3.11	13.503	17.400	15.451	298.33	289.00	293.67
L <sub>6</sub>	3.54	3.27	3.41	9.139	9.975	9.557	231.67	187.23	209.45
L <sub>7</sub>	3.80	3.54	3.67	9.497	7.396	8.447	276.67	263.40	270.03
L <sub>8</sub>	3.63	3.44	3.53	11.541	10.954	11.248	210.00	214.07	212.03
L <sub>9</sub>	4.85	4.49	4.67	9.925	10.850	10.387	296.67	282.36	289.51
L <sub>10</sub>	4.11	3.97	4.04	9.449	14.876	12.162	301.67	317.84	309.75
L <sub>11</sub>	3.67	3.35	3.51	10.234	7.955	9.094	253.33	252.15	252.74
L <sub>12</sub>	4.16	4.06	4.11	10.124	8.748	9.436	210.00	190.93	200.46
L <sub>13</sub>	4.19	3.96	4.08	15.768	13.782	14.775	400.00	394.97	397.48
L <sub>14</sub>	3.76	3.63	3.69	13.122	10.658	11.890	360.00	285.14	322.57
L <sub>15</sub>	3.05	2.92	2.98	11.495	8.324	9.910	250.00	252.13	251.06
L <sub>16</sub>	3.02	2.81	2.92	13.723	14.150	13.937	216.67	217.23	216.95
L <sub>17</sub>	2.90	2.92	2.91	10.743	9.632	10.188	226.67	222.04	224.35
LSD at 5%	0.18	0.28	0.18	1.172	1.088	1.661	20.25	22.38	19.04

**Table 6. Mean performances of the evaluated rocket landraces for seed oil percentage (%) and seed oil yield (kg/fed) characters.**

Characters Landraces	Seed oil percentage (%)			Seed oil yield (Kg/fed)		
	2014/15	2015/16	Comb.	2014/15	2015/16	Comb.
L <sub>1</sub>	24.79	24.37	24.58	73.550	70.780	72.165
L <sub>2</sub>	24.01	24.68	24.35	72.740	69.473	71.107
L <sub>3</sub>	25.27	24.42	24.85	72.395	63.635	68.015
L <sub>4</sub>	31.85	30.46	31.16	103.023	89.458	96.241
L <sub>5</sub>	21.70	20.85	21.27	64.747	60.268	62.507
L <sub>6</sub>	27.35	25.04	26.20	63.396	46.926	55.161
L <sub>7</sub>	26.76	25.38	26.07	73.957	66.707	70.332
L <sub>8</sub>	24.09	27.42	25.76	50.572	58.861	54.716
L <sub>9</sub>	25.39	24.47	24.93	75.342	69.057	72.199
L <sub>10</sub>	20.26	18.86	19.56	61.140	59.962	60.551
L <sub>11</sub>	24.91	24.13	24.52	63.129	60.764	61.947
L <sub>12</sub>	24.18	23.86	24.02	50.920	45.534	48.227
L <sub>13</sub>	24.50	23.97	24.24	98.013	94.796	96.405
L <sub>14</sub>	22.95	22.13	22.54	82.749	63.105	72.927
L <sub>15</sub>	27.93	27.23	27.58	69.831	68.649	69.240
L <sub>16</sub>	28.15	26.35	27.25	60.970	57.262	59.116
L <sub>17</sub>	26.42	26.10	26.26	59.751	57.985	58.868
LSD at 5%	1.52	1.33	1.87	7.249	6.696	6.025

Significant differences were observed among the evaluated landraces for fresh leaves weight/plant trait (Table 4). The recorded values ranged from 10.95 to 15.27 g for the landraces L<sub>17</sub> and L<sub>5</sub>, respectively.

Total chlorophyll content trait reflected also a great variation among the evaluated landraces (Table 4). The collected landraces had mean of total chlorophyll content ranging from 29.95 to 37.53 mg/100 g fresh weight. The highest value was shown by the landrace L<sub>8</sub> (37.53 mg/100 g fresh weight) followed by landraces L<sub>12</sub> and L<sub>13</sub> (37.37 and 37.00 mg/100 g fresh weight, respectively) without significant differences between them.

Concerning antioxidant activity trait, significant differences were observed among the evaluated landraces (Table 4). Mean value of antioxidant activity of the evaluated landraces ranged from 24.34 to 37.78 %. The highest value of antioxidant activity was shown by the landrace L<sub>3</sub> (37.78 %) followed by landraces L<sub>4</sub> (37.59 %) and L<sub>5</sub> (37.42 %) without significant differences between them. The lowest value of antioxidant activity was exhibited by landrace L<sub>16</sub> (24.34 %).

Data obtained on total phenols content of collected rocket landraces evaluated in the 2014/15 and 2015/16 winter plantings are presented in Table 5. Combined analysis of both seasons showed significant differences for this trait among the evaluated landraces. Total phenols content of the evaluated landraces ranged from 2.91 to 5.01 mg /g dw. The landrace L<sub>1</sub> produced the highest significant total phenols content (5.01 mg /g dw) followed by the landrace L<sub>9</sub> (4.67 mg /g dw) with significant differences between them. The lowest total phenols content was detected in the landrace L<sub>17</sub> (2.91 mg /g dw).

Data obtained on fresh yield/feddan of collected rocket landraces evaluated in the 2014/15 and 2015/16 winter plantings are presented in Table 5. Combined analysis of both seasons showed significant differences for this trait among the evaluated landraces. Fresh yield of the evaluated landraces ranged from 8.447 to 15.451 tons/fed. The landrace L<sub>5</sub> produced the highest significant fresh yield (15.451 tons/fed) followed by the landrace L<sub>3</sub> (14.945 tons/fed) without significant differences between them. The lowest fresh yield was detected in the landrace L<sub>7</sub> (8.447 tons/fed).

Regarding seed yield trait (Table 5), the landrace L<sub>13</sub> showed the highest value (397.48 kg/fed), meanwhile, the lowest value was recorded in the landrace L<sub>12</sub> (200.46 kg/fed).

Concerning seed oil percentage trait, significant differences were observed among the evaluated landraces (Table 6). Mean oil percentage of the evaluated landraces ranged from 19.56 to 31.16 %. The highest value of oil percentage was shown by the landrace L<sub>4</sub> (31.16 %) followed by landrace L<sub>15</sub> (27.58 %) with significant differences between them. The lowest value of seed oil percentage was exhibited by landrace L<sub>10</sub> (19.56 %).

Data obtained on seed oil yield/feddan of collected rocket landraces evaluated in the 2014/15 and 2015/16 winter plantings are presented in Table 6. Combined analysis of both seasons showed significant differences

for this trait among the evaluated landraces. Seed oil yield of the evaluated landraces ranged from 48.227 to 96.405 kg/fed. The landrace L<sub>13</sub> produced the highest significant seed oil yield (96.405 kg/fed) followed by the landrace L<sub>4</sub> (96.241 kg/fed) without significant differences between them. The lowest seed oil yield was detected in the landrace L<sub>12</sub> (48.227 kg/fed).

#### **Fatty acid composition of rocket landraces seed oil**

Gas liquid chromatography technique was employed to study the composition of rocket landraces seed oil fatty acids. Data of the relative percentage of the identified fatty acids are shown in Table 7.

**Table 7. Fatty acids composition of seeds oils of the evaluated rocket landraces.**

<b>Laundresses</b>	<b>L<sub>1</sub></b>	<b>L<sub>2</sub></b>	<b>L<sub>3</sub></b>	<b>L<sub>4</sub></b>	<b>L<sub>5</sub></b>	<b>L<sub>6</sub></b>	<b>L<sub>7</sub></b>	<b>L<sub>8</sub></b>
<b>Fatty acid composition</b>								
<b>Myristic acid (C14:0)</b>	<b>0.09</b>	<b>0.22</b>	<b>0.39</b>	<b>0.09</b>	<b>1.91</b>	<b>0.14</b>	<b>0.09</b>	<b>0.11</b>
<b>Tetraenoic acid (C14:1)</b>	<b>ND</b>	<b>0.07</b>	<b>0.17</b>	<b>ND</b>	<b>1.04</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>Pentadecanoic acid (C15:0)</b>	<b>ND</b>	<b>ND</b>	<b>0.19</b>	<b>ND</b>	<b>1.17</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>Palmitic acid (C16:0)</b>	<b>3.94</b>	<b>4.00</b>	<b>3.67</b>	<b>3.80</b>	<b>4.21</b>	<b>4.09</b>	<b>3.73</b>	<b>4.18</b>
<b>Palmitoleic acid (C16:1)</b>	<b>0.24</b>	<b>0.27</b>	<b>0.27</b>	<b>0.27</b>	<b>0.26</b>	<b>0.22</b>	<b>0.22</b>	<b>0.24</b>
<b>Heptadecanoic acid (C17:0)</b>	<b>0.05</b>	<b>0.05</b>	<b>0.05</b>	<b>0.06</b>	<b>0.04</b>	<b>0.05</b>	<b>0.04</b>	<b>0.04</b>
<b>Heptadecenoic acid methyl ester (C17:1)</b>	<b>0.07</b>	<b>0.07</b>	<b>0.08</b>	<b>0.07</b>	<b>0.02</b>	<b>0.07</b>	<b>0.07</b>	<b>0.08</b>
<b>Stearic acid (C18:0)</b>	<b>0.99</b>	<b>1.03</b>	<b>0.98</b>	<b>1.00</b>	<b>1.13</b>	<b>1.03</b>	<b>0.97</b>	<b>1.09</b>
<b>Oleic acid (C18:1)</b>	<b>15.03</b>	<b>15.85</b>	<b>15.18</b>	<b>15.00</b>	<b>15.23</b>	<b>12.71</b>	<b>13.59</b>	<b>14.59</b>
<b>Linoleic acid (C18:2)</b>	<b>9.40</b>	<b>9.34</b>	<b>9.70</b>	<b>9.52</b>	<b>6.09</b>	<b>8.98</b>	<b>9.14</b>	<b>9.10</b>
<b>γ- linolenic (C18:3n6)</b>	<b>0.04</b>	<b>0.04</b>	<b>0.04</b>	<b>0.04</b>	<b>0.10</b>	<b>0.04</b>	<b>0.04</b>	<b>0.33</b>
<b>Linolenic (C18:3n3)</b>	<b>7.14</b>	<b>9.72</b>	<b>10.04</b>	<b>9.86</b>	<b>4.66</b>	<b>10.50</b>	<b>10.32</b>	<b>9.40</b>
<b>Eicosanoic acid (C20:0)</b>	<b>0.71</b>	<b>0.75</b>	<b>0.75</b>	<b>0.72</b>	<b>0.85</b>	<b>0.77</b>	<b>0.72</b>	<b>0.71</b>
<b>Eicosanoic acid (C20:1)</b>	<b>8.86</b>	<b>9.06</b>	<b>8.61</b>	<b>9.19</b>	<b>9.32</b>	<b>8.67</b>	<b>8.98</b>	<b>8.87</b>
<b>Eicosanoic acid (C20:2)</b>	<b>0.38</b>	<b>0.36</b>	<b>0.36</b>	<b>0.39</b>	<b>0.25</b>	<b>0.41</b>	<b>0.41</b>	<b>0.37</b>
<b>Eicosanoic acid (C20:4)</b>	<b>0.10</b>	<b>0.10</b>	<b>0.09</b>	<b>0.10</b>	<b>0.05</b>	<b>0.11</b>	<b>0.11</b>	<b>0.10</b>
<b>Docosanoic acid (C22:0)</b>	<b>0.92</b>	<b>0.97</b>	<b>0.96</b>	<b>0.94</b>	<b>1.01</b>	<b>0.87</b>	<b>0.91</b>	<b>1.02</b>
<b>Erucic acid (C22:1)</b>	<b>45.93</b>	<b>44.78</b>	<b>44.76</b>	<b>45.81</b>	<b>46.87</b>	<b>48.19</b>	<b>47.35</b>	<b>46.21</b>
<b>Lignoric acid (C24:0)</b>	<b>0.45</b>	<b>0.48</b>	<b>0.47</b>	<b>0.46</b>	<b>0.53</b>	<b>0.45</b>	<b>0.48</b>	<b>0.49</b>
<b>Nervonic acid (C24:1)</b>	<b>2.10</b>	<b>2.07</b>	<b>2.02</b>	<b>2.12</b>	<b>1.85</b>	<b>2.04</b>	<b>2.16</b>	<b>2.45</b>
<b>Saturated fatty acid</b>	<b>7.15</b>	<b>7.50</b>	<b>7.46</b>	<b>7.07</b>	<b>10.85</b>	<b>7.40</b>	<b>6.94</b>	<b>7.64</b>
<b>Unsaturated fatty acid</b>	<b>89.29</b>	<b>91.73</b>	<b>91.32</b>	<b>92.37</b>	<b>85.64</b>	<b>91.94</b>	<b>92.39</b>	<b>91.74</b>
<b>Total anon</b>	<b>3.56</b>	<b>0.77</b>	<b>1.22</b>	<b>0.56</b>	<b>3.51</b>	<b>066</b>	<b>0.67</b>	<b>0.62</b>

**Table 7. Cont.**

Laundresses	L <sub>9</sub>	L <sub>10</sub>	L <sub>11</sub>	L <sub>12</sub>	L <sub>13</sub>	L <sub>14</sub>	L <sub>15</sub>	L <sub>16</sub>	L <sub>17</sub>
<b>Fatty acid composition</b>									
Myristic acid (C14:0)	0.11	1.84	1.95	0.09	1.07	0.10	0.28	0.17	0.34
Tetraenoic acid (C14:1)	ND	0.99	1.16	ND	0.57	ND	0.12	0.04	0.14
Pentadecanoic acid (C15:0)	ND	1.05	1.17	ND	0.61	ND	0.14	ND	0.16
Palmitic acid (C16:0)	4.18	4.26	4.54	4.13	4.31	3.76	3.57	4.00	3.78
Palmitoleic acid (C16:1)	0.24	0.28	0.26	0.25	0.25	0.23	0.22	0.29	0.24
Heptadecanoic acid (C17:0)	0.04	0.06	0.06	0.05	0.06	0.04	0.04	0.06	0.06
Heptadecenoic acid methyl ester (C17:1)	0.08	0.05	0.02	0.08	0.05	0.07	0.06	0.07	0.07
Stearic acid (C18:0)	1.09	1.06	1.19	1.14	1.13	1.03	0.99	1.03	1.06
Oleic acid (C18:1)	14.59	14.65	14.76	15.10	15.74	13.63	13.16	15.93	14.34
Linoleic acid (C18:2)	9.10	7.57	4.77	9.41	7.36	9.17	8.61	9.12	9.75
$\gamma$ -linolenic (C18:3n6)	0.33	0.07	ND	0.04	0.07	0.05	0.04	0.04	0.04
Linolenic (C18:3n3)	9.40	6.95	2.27	10.41	6.23	10.67	9.55	9.43	10.96
Eicosanoic acid (C20:0)	0.71	0.72	0.99	0.77	0.78	0.76	0.76	0.74	0.75
Eicosanoic acid (C20:1)	8.87	8.75	9.48	8.94	9.23	8.44	8.88	9.15	8.80
Eicosanoic acid (C20:2)	0.37	0.30	0.19	0.38	0.29	0.40	0.39	0.35	0.40
Eicosanoic acid (C20:4)	0.10	0.07	0.08	0.10	0.06	0.12	0.11	0.10	0.12
Docosanoic acid (C22:0)	1.02	0.89	1.31	0.97	1.01	0.97	0.97	0.98	0.88
Erucic acid (C22:1)	46.21	44.60	48.27	45.16	46.20	47.22	48.50	45.34	44.56
Lignoric acid (C24:0)	0.49	0.46	0.63	0.48	0.30	0.49	0.50	0.49	0.43
Nervonic acid (C24:1)	2.45	1.73	2.48	1.93	2.23	2.16	2.14	2.01	2.00
Saturated fatty acid	7.64	10.34	11.84	7.63	9.27	7.15	7.25	7.47	7.46
Unsaturated fatty acid	91.74	86.01	83.74	91.80	88.28	92.16	91.78	91.87	91.42
Total anion	0.62	3.65	4.42	0.57	2.45	0.69	0.97	0.66	1.12

From the data presented in Table 7, it could be noticed that palmitic acid was the major saturated fatty acid in rocket oil and it ranged from 3.57 to 4.54% in landraces L<sub>15</sub> and L<sub>11</sub>, respectively. While, erucic acid was the major unsaturated fatty acid and it ranged from 44.56 to 48.50 % in landraces L<sub>17</sub> and L<sub>15</sub>, respectively.

Results also revealed that, the total saturated fatty acids (SFA) ranged from 6.94 to 11.84 % in L<sub>7</sub> and L<sub>11</sub> landraces, respectively. While, unsaturated fatty acids (USFA) ranged from 85.64 to 92.37 % in L<sub>5</sub> and L<sub>7</sub> landraces, respectively. Meanwhile, the ratio of USFA/SFA was 10.42:1. These results are in agreement with those reported by Wanasundara *et al* (1997), Khan and Shahidi (2001), Erdemoglu *et al* (2004) and Kotnik *et al* (2006).

#### Components of variances

Estimates of components of variance, *i.e.*, environmental ( $\sigma^2_e$ ), genetic ( $\sigma^2_g$ ) and phenotypic ( $\sigma^2_p$ ) variance, genotypic (GCV) and



phenotypic (PCA) coefficient of variation, GCV/PCV ratio and broad-sense heritability (BSH) for the studied traits are presented in Table 8. The studied traits leaf area, fresh yield, antioxidant activity, total phenols content seed yield, seed oil percentage and seed oil yield, showed low difference between phenotypic and genetic variance, indicating that the large portion of the phenotypic variance ( $\sigma_p^2$ ) was due to the genetic variance ( $\sigma_g^2$ ) and the significant differences among the rocket landraces are of genetic nature.

**Table 8. Components of variance ( $\sigma_e^2$ ,  $\sigma_g^2$  and  $\sigma_p^2$ ), genotypic and phenotypic coefficient of variation (GCV% and PCV%) and broad sense heritability (BSH) for studied traits.**

Characters Components	Leaf length	Leaf area	No. leaves/plant	Fresh leaves weight/plant	Total chlorophyll content	Antioxidant activity
$\sigma_e^2$	3.96	11.64	1.03	1.87	3.59	0.83
$\sigma_g^2$	0.97	73.07	0.75	1.27	4.19	24.12
$\sigma_p^2$	4.94	84.71	1.78	3.15	7.77	24.95
BSH	0.20	0.86	0.42	0.40	0.54	0.97
GCV%	3.46	18.77	10.13	8.74	5.94	16.77
PCV%	7.78	20.22	15.57	13.75	8.09	17.06
GCV/PCV	0.44	0.93	0.65	0.64	0.73	0.98
Characters Components	Total phenols content	Fresh yield	Seed yield	Seed oil percentage	Seed oil yield	
$\sigma_e^2$	0.02	2.07	271.83	1.06	27.22	
$\sigma_g^2$	0.38	4.93	2544.99	6.47	163.89	
$\sigma_p^2$	0.40	7.00	2816.82	7.52	191.11	
BSH	0.94	0.70	0.90	0.86	0.86	
GCV%	17.03	18.89	18.57	10.17	18.93	
PCV%	17.55	22.51	19.54	10.97	20.44	
GCV/PCV	0.97	0.84	0.95	0.93	0.93	

However, the trait leaf length trait showed low difference between phenotypic and environment variance indicating that the large portion of the phenotypic variance ( $\sigma_p^2$ ) was due to the environmental variance ( $\sigma_e^2$ ) and the significant differences among the rocket landraces are of environmental nature. Meanwhile, the data indicated that fresh leaves weight/plant and total chlorophyll content traits were affected by both of genetic and environmental factors.

Estimates of GCV% and PCV%, respectively, for the studied traits were 3.46 and 7.78% for leaf length, 18.77 and 20.22% for leaf area, 10.13

and 15.57% for no. of leaves/plant, 8.74 and 13.75% for fresh leaves weight/plant, 5.94 and 8.09% for total chlorophyll content, 16.77 and 17.06% for antioxidant activity, 17.03 and 17.55% for total phenols content, 18.89 and 22.51% for fresh yield, 18.57 and 19.54% for seed yield, 10.17 and 10.97% for seed oil percentage, and 18.93 and 20.44% for seed oil yield (Table 8). Also, the GCV/PCV ratio for the studied traits ranged from 0.44 (leaf length) to 0.98 (antioxidant activity). Obtained broad sense heritability values for the studied traits ranged from 20% to 97%, suggesting high values of heritability in the characters leaf area, total chlorophyll content, antioxidant activity, total phenols content, fresh yield, seed yield, seed oil percentage and seed oil yield,. However, it was low in leaf length, meanwhile; it was moderate in fresh leaves weight/plant and no. of leaves/plant traits.

In general, we can notice that the differences between phenotypic and genotypic variance for leaf area, antioxidant activity, total phenols content, fresh yield, seed yield, seed oil percentage and seed oil yield traits were low, since the estimated GCV/PCV ratios were high (0.84 to 0.98). In other words, the large portion of phenotypic variance ( $\sigma_p^2$ ) was due to the genetic variance ( $\sigma_g^2$ ). Consequently, estimated broad-sense heritability showed high values for these traits, indicating that the observed significant phenotypic differences among the studied landraces are of genetic nature and there are small environmental effects on the phenotypic variation. Therefore, these characters can be improved through selection based on phenotypic observations in early segregating generations in rocket germplasm. These results confirm findings of Giessen *et al* (2003), Sastry and Meena (2003), Akbar *et al* (2007), Aytac *et al* (2008) and Ahmad *et al* (2013) who indicated that oil content, fresh yield and seed yield characters were influenced by genetic more than non-genetic factors and the differences between GCV and PCV were narrow with respect to genetic advance. Also, Bozokalfa *et al* (2010) found that broad sense heritability ranged from moderate to high for leaf length, plant weight and number of leaves characters indicating that environmental factors did not greatly affect phenotypic variation and suggested selection for improving these traits. However, the low variation was found in number of leaves (Ziaullah Ghazali *et al* 2014). Also, Jat *et al* (2014) indicated that broad sense heritability estimates were low to moderate for seed yield and oil content characters.

#### **Identification of Amplified Fragment Length Polymorphism (AFLP) markers**

Four MseI and EcoRI selective primer combinations were used for fragment amplification. All AFLP polymorphisms were scored as dominant marker.

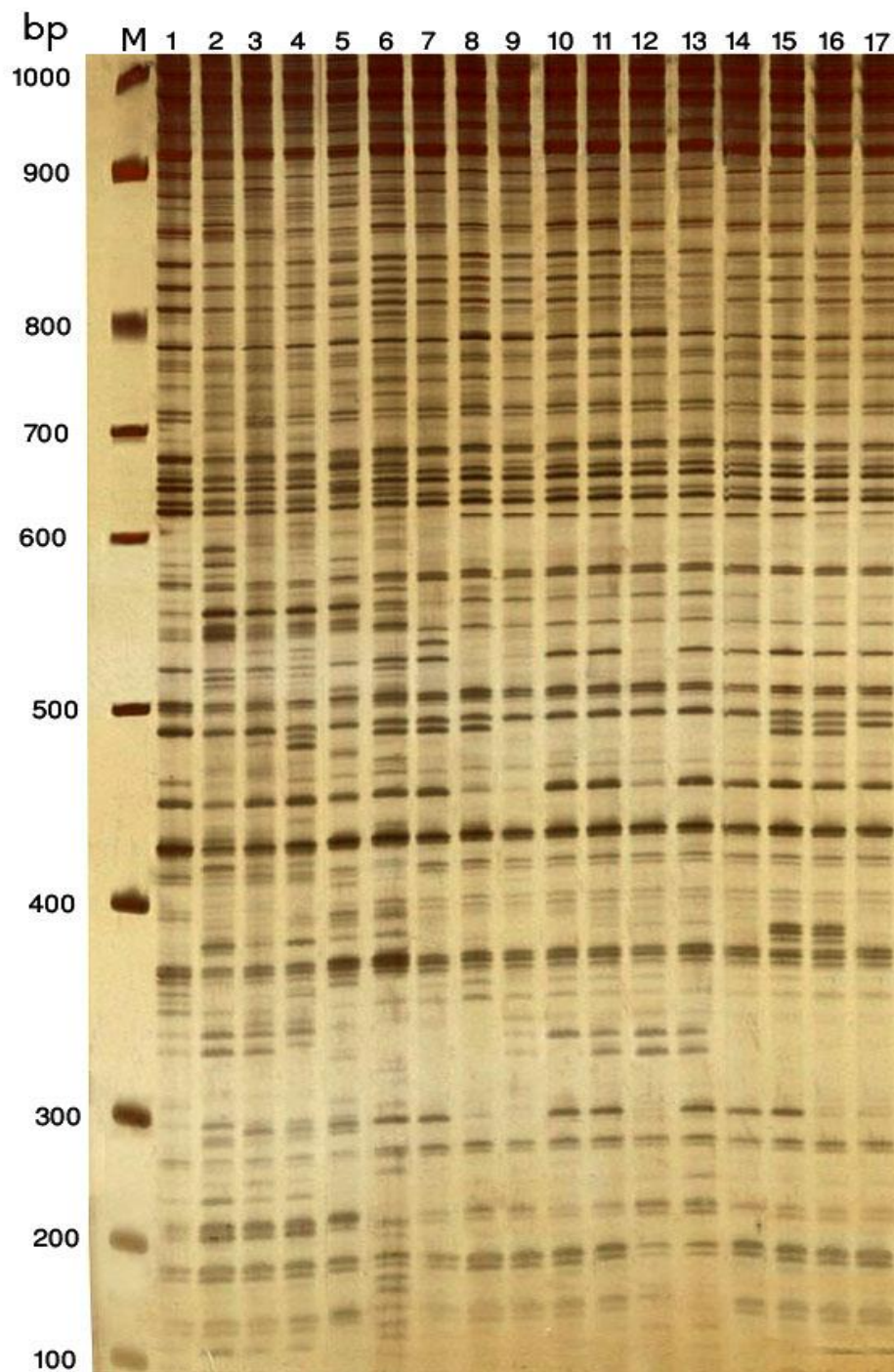
The AFLP primer combinations used generated 230 of selectively amplified DNA fragments and the identification of 63 (27.3%) polymorphic markers as shown in Table 9. The percentage of polymorphism ranged from 23 to 30.5%. The highest percentage of polymorphism (30.5%) was obtained with primer combination  $M_{CTA} \times E_{ACA}$ , while, the lowest percentage (23%) was obtained with the primer combination  $M_{CTG} \times E_{ACG}$ . However, the maximum number of bands per primer combination was found to be 72 which was generated with primer combination  $M_{CTA} \times E_{ACA}$ , whereas, the minimum number of bands per primer combination was 39 obtained with primer combination  $M_{CTG} \times E_{ACG}$ , while the rest of primer combinations came between them, thus confirming the high multiplex ratio produced by AFLP markers. The size of selectively amplified DNA fragments ranged in size from 110 to 620 bp as shown in Figure 2.

**Table 9. Levels of polymorphism, total number of bands, monomorphic bands, polymorphic bands and percentage of polymorphism as revealed by AFLP marker among the seventeen rocket landraces.**

<b>Primer Combinations</b>	<b>Total number of bands</b>	<b>Monomorphic bands</b>	<b>Polymorphic bands</b>	<b>% of polymorphism</b>
$M_{CTA} \times E_{ACA}$	72	50	22	30.5
$M_{CTC} \times E_{ACC}$	61	44	17	27.8
$M_{CTG} \times E_{ACG}$	39	30	9	23.0
$M_{CTT} \times E_{ACT}$	58	43	15	25.8
<b>Total</b>	<b>230</b>	<b>167</b>	<b>63</b>	<b>27.4</b>

All the AFLP primer combinations used detected unique markers even positive or negative. They reveal a total of 22 unique markers comprising (7 negative and 15 positive markers). The highest number of unique markers (6 markers) is obtained with primer combinations of  $M_{CTA} \times E_{ACA}$  and  $M_{CTC} \times E_{ACC}$ , whereas, the lowest number (2 markers) is obtained with  $M_{CTG} \times E_{ACG}$  with molecular weight ranged from 110 to 600 bp. These unique markers can be used to identify three landraces ( $L_4$ ,  $L_{15}$  and  $L_{16}$ ) which gave high seed oil percentage out of the studied seventeen rocket landraces.

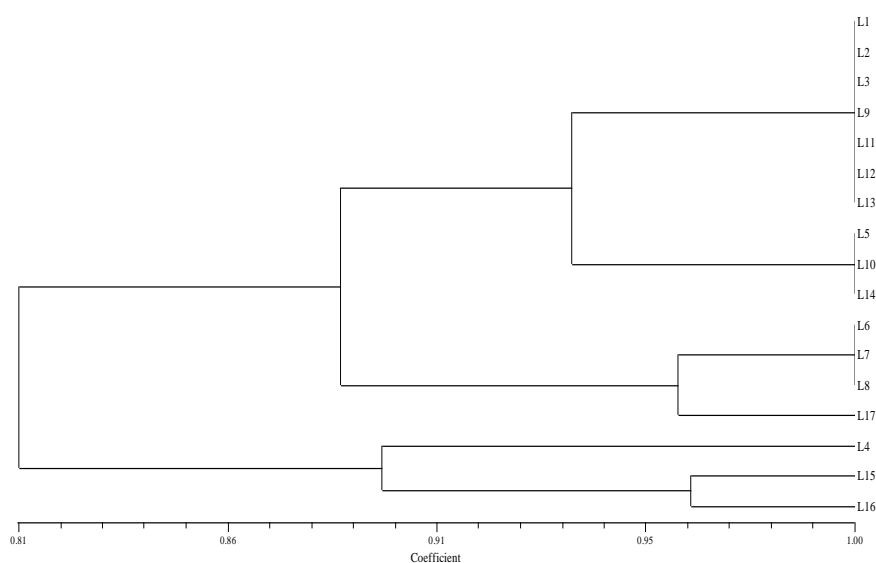
The AFLP scored data (1 for presence and 0 for absence) resulting from the four primer combinations were used to calculate the similarity matrices through Dice's genetic similarities coefficient (DSC's) between 17 rocket landraces. AFLPs generated data show that the highest genetic similarity value to be 99.8% and the lowest value to be 82.7% with an average of 91.25. .



**Fig. 2. AFLP profiles of seventeen rocket landraces (1-17) as revealed by AFLP primer combination ( $M_{CTA}$  X  $E_{ACA}$ ). (M) DNA molecular weight standards 100 bp DNA ladder.**

Based on AFLP analysis, the highest similarity percentage (99.8%) detected by the AFLP assay was between L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub>, L<sub>9</sub>, L<sub>11</sub>, L<sub>12</sub> and L<sub>13</sub>. All of them share the same genetic background and geographical region since they were collected from the nearby regions of Egypt. While, the lowest percentage of similarity (82.7%) was detected between L<sub>8</sub> and L<sub>17</sub> landraces. The most surprising result of this analysis was the very high genetic diversity observed between L<sub>4</sub> landrace and other studied landraces and clearly observed in AFLP electrophoretic patterns (Figure 2).

A constructed dendrogram separated seventeen rocket landraces into two major clusters, according to the geographic region and to classical botanical classification of rocket. The first cluster was divided into two sub clusters, first sub cluster contained L<sub>15</sub> and L<sub>16</sub> which collected from Asuit and Sohag, respectively, and second sub cluster contained L<sub>4</sub> only. While, the second cluster was divided into two sub clusters. First sub cluster contained landraces L<sub>6</sub>, L<sub>7</sub>, L<sub>8</sub> and L<sub>17</sub>. On the other hand, the second sub cluster could be divided into two sub sub clusters, first sub sub cluster contained L<sub>5</sub>, L<sub>10</sub> and L<sub>14</sub>, while, landraces L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub>, L<sub>9</sub>, L<sub>11</sub>, L<sub>12</sub> and L<sub>13</sub> which collected from the same or nearby regions of Egypt are grouped together in the second sub sub cluster as shown in Fig. 3.



**Fig. 3. Dendrogram for the seventeen rocket landraces constructed from the AFLPs generated data using UPGMA method and similarity matrices computed according to DSC's.**

The obtained findings are in good accordance with Singh *et al* (1999) who reported that several rare and accession specific bands were identified, such specific bands could be effectively used to distinguish the different landraces of neem tree from different eco-geographic regions. Srivastava *et al* (2001) used 21 AFLP primer combinations to assess the genetic diversity among 30 varieties and lines of *Brassica juncea*, In addition, Garcia *et al* (2004) proposed that data generated from PCR-based markers, *i.e.*, RAPD, RFLP, AFLP and SSR markers, indicated that AFLP seemed to be the best-suited molecular assay for fingerprinting and assessing genetic relationships among tropical maize inbred lines with high accuracy. In the same regard, Gruijter *et al* (2005) studied genetic variation among *Oesophagostomum bifurcum* (Nematoda) from human and non-human primates from Ghana. While, Marinoni *et al* (2013) studied the genetic and morphological diversity among various local populations of Italian chestnut (Piedmont) and reported that genetic intra-cultivar homogeneity was observed for some of the most valuable cultivars. Khierallah *et al* (2013) stated that AFLPs are highly efficient in detecting genetic relationship among olive cultivars, while, the co-dominant nature of SSRs markers makes them very suitable for segregation and genome mapping in olive trees. In another side, low variability between regions was also reported by Fei *et al* (2012) who showed that phylogenetic analysis of chloroplast DNA sequence data when an origin for chestnut in East Asia and migration to North America from Europe.

#### **CONCLUSION**

From this study, it can be concluded that L<sub>4</sub> and L<sub>13</sub> were the best landraces with respect to fresh yield, seed yield, seed oil yield and reasonable oil characters. These results have been confirmed through molecular genetics study, where a genetic variation region has been identified which may be responsible for oil production as well as has been identified unique positive marker which clearly appeared in these landraces (L<sub>4</sub> and L<sub>13</sub>). This unique positive marker will be isolated and sequenced in an attempt to isolate the full length of the oil production genes in following studies. This study also showed information about the visual, biochemical and genetic diversity of rocket germplasm in Egypt which is important for variety identification and to enhance the classification of germplasm collections and exploit them in breeding programs and for the development and improvement of varieties to increase seed oil yield.

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## التحليل الوراثي وتقييم بعض السلالات المحلية من الجرجير للمحصول العالى من زيت البذرة

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أجريت هذه الدراسة بمزرعة بحوث الخضر بقها بمحافظة القليوبية التابعة لمركز البحوث الزراعية- مصر خلال الفترة من ٢٠١٤ إلى ٢٠١٦ بهدف تقدير التباين الوراثي وكفاءة التوريث لمحصول الأوراق ومحصول البذور ومحصول زيت البذور بالإضافة لبعض الصفات الأخرى الهامة في الجرجير. تم تجميع سبعة عشر سلالة محلية مختلف من الجرجير من مناطق مختلفة من مصر لإستخدامها في هذه الدراسة. أشارت النتائج إلى أن معظم التباين الكلي يرجع إلى التباين الوراثي، وكذلك كانت كفاءة التوريث عالية لصفات مساحة الورقة ، ومحصول الأوراق، ومحصول البذور ، ونسبة الزيت، ومحصول الزيت، ونشاط مضادات الأكسدة ومحتوى النباتات من الفينولات الكلية مما يدل على أنه يمكن تحسين تلك الصفات عن طريق الانتخاب في الأجيال الإنعزالية المبكرة. وأوضحت الدراسة أن أفضل السلالات بالنسبة لمحصول الأوراق والبذور والزيت كان الطرازين الوراثيين L<sub>4</sub>، L<sub>13</sub>. كما أوضحت نتائج تحليل بيانات AFLP-markers أن نسبة التشابه الوراثي بين التراكيب الوراثية للجرجير تتراوح ما بين ٨٢.٧٪ إلى ٩٩.٨٪ مما يدل على مستوى عالى من التشابه الوراثي بين التراكيب

المنزعة فى المناطق القريبة فى حين وجدت اختلافات وراثية بين التراكيب المنزعة فى المناطق المتباعدة. وقد تم تحليل علاقات درجة القرابة والتشابه الوراثى بناءً على مصفوفات التشابه المأخوذة من الواسمات الجزيئية باستخدام طريقة *UPGMA* لرسم دندروجرامات لتوضيح القرابة الوراثية. وأوضحت نتائج هذه الدراسة أن واسمات الـ *AFLP* تعتبر أداة جيدة لتقدير التباين الوراثى وكذلك درجات القرابة الوراثية بين السلالات المختلفة والمنزعة فى مصر. وكذلك إنتاج بصمة وراثية مميزة لكل مصدر بالإضافة إلى تحديد واسمات فريدة ومميزة قد تكون وثيقة الصلة بصفة إنتاج الزيت. وهذا من الأهمية حتى يمكن تحقيق أقصى استفادة وإستخدام لمجموعات المصادر الوراثية النباتية المحفوظة فى البنوك الوراثية كما أنه يمكن أن يفيد بدرجة كبيرة فى التوصيف والحماية والمحافظة على المصادر الوراثية وأستخدامها فى برامج التربية.

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