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Elucidation of genetic parameters among some selected genotypes of prickly oil lettuce (*lactuca serriolla* L.) in Egypt, using morpho-agronomic traits and RAPD markers.

Shoaib R.M., Abdel-Samea N.S., Ramadan W.A.*, Ibrahim M.M., and Aboud K.A.

Genetics and cytology Department, Genetic Engineering and Biotechnology Division, National Research Centre. 33 El Buhouth st., Dokki, Cairo, Egypt.

ABSTRACT

Fifteen selected genotypes of prickly oil lettuce were assessed for genetic variation (variability, heritability and genetic advance) and molecular characterization of RAPD markers. Significant variation were detected for all studied characters among genotypes. High phenotypic and genotypic coefficient of variation values were observed for seed weight, oil yield per plant, and number of main branches per plant. Most of the traits exhibited a high heritability value. High genetic advance coupled with high heritability was observed for oil percent, plant height and seed weight. RAPD analyses showed 43 DNA bands scored by the 6 primers used. These bands were identified as 18 polymorphic and 25 monomorphic ones with 41.8% polymorphism. The dendrogram of the 10 selected genotypes were divided into two main groups. The first one comprises 5 selected genotypes, 5, 6, 7, 8 and 9, while the second one includes the other five selected genotypes. These results suggested that, assessment of genetic variation parameters and RAPD markers is helpful for possible distinguishing, identifying, characterizing, and selection processing of genotypes of prickly oil lettuce.

Keywords: Genetic parameters, phenotypic variation, genotypic variation, prickly oil lettuce, RAPD markers

Corresponding author

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INTRODUCTION

Prickly oil lettuce (*lactuca serriolla* L.), family *Asteraceae* is an herbaceous plant. It is native to Siberia, Himalaya and Atlantic areas, also cultivated in temperate lands of Europe, North Africa, and Asia [1]. The bitter taste of the leaves, in this type of lettuce was caused to not eaten as a vegetable, a high percentage of oil (35%) which contains an essential nutrient and vitamin E was characterized in seeds [2]. Cultivation of oil-producing forms, in Egypt has continued to the present time [3]. In traditional folkloric medicines, the plant is used for hypnotic, sedative, expectorant, cough suppressant, antiseptic, purgative, demulcent, diuretic, and antispasmodic [4]. Prickly oil lettuce is considered a direct progenitor of cultivated lettuce (*Lactuca sativa* L.). The progenitors of cultivated crop has genes for resistance to a biotic factors, diseases and pests, as well as genes for physiological and quality characters by their broad genetic variability [5].

El-Esawi [6] reported that genetic variation studies are vital for providing information for propagation, taxonomy, disease resistance, and breeding programs as well as conservation and utilization of Lactuca genetic resources. The effectiveness of selection depends on the magnitude of heritability for the traits being selected. The knowledge of the genotypic coefficient of variation, phenotypic coefficient of variation (GCV, PCV) and heritability enables the breeder to predict the genetic gain under selection which will assist the breeder to formulate the suitable breeding methodology [7]. Seed and oil yield improvement is a major breeding objective of oil seed crop improvement programs. Successful results were obtained in genetic improvement in safflower such as seed yield and oil content and combining the high yield with high oil content [8].

A technique based on DNA has been widely used for authentication of medicinal plant species. RAPD-PCR markers could be used to estimate the systematic relationship between related species. The evaluation of different DNA markers of medicinal plants was studied in classification, identification and breeding [9]. Newer methods in quality control of botanicals are needed for chemical and morphological approaches for authentication. RAPD markers are very useful tools used in medicinal plants for comparing the genetic relationship and diversity patterns identification remains important for botanical drug industry [10]. RAPD markers were used for molecular characterization and evaluate the degree of polymorphism in 36 safflower accessions [11], in sunflower (*Helianthus annuus* L.) genotypes [12], in mustard (*Brassica* spp.) genotypes [13] and among some Egyptian pistachio (*Pistacia vera*) cultivars [14].

Few studies have been conducted on the assessment of genetic variation and RAPD markers among prickly oil lettuce genotypes with regard to molecular and morphological analyses [15, 16]. Hence, the main objective of the present study was to assess of some selected genotypes of prickly oil lettuce through morphological and molecular markers on the basis of genetic parameters (PCV, GCV, Heritability and Genetic advance) and RAPD markers.

MATERIALS AND METHODS

Genetic materials

The plant materials of prickly oil lettuce selected genotypes were obtained from the breeding groups of medicinal and aromatic plants in the National Research Center (NRC). Fifteen genotypes were selected and grown in complete randomized block design experiment with three replicates in the experimental farm of NRC at Nubaria, El-Behira governorate, Egypt, during two successive seasons (2012/2013 and 2013/2014). Seeds of prickly lettuce genotypes were sown in the nursery on November 2012 in both seasons. At fully ripen, five plants of each replicate per each genotype for both seasons were harvested and the plant records were considered as already mentioned.

Plant records

Five quantities studied characters were studied on an individual plant basis. They included:

1-Plant height (cm) per plant.
2-Number of branches per plant
3-Seed yield per plant (gm)
4- Oil percent.
5-Oil yield (gm) per plant.

Oil Content extraction (%)

The oil was extracted on the basis of seed dry weight using Soxhelt apparatus according to (A.O.A.C) [17].

Statistical procedures:

Statistical analyses of the data were conducted using the SPSS ver. 17. The general statistical procedures were practiced according to [18]. The analysis of variance (ANOVA) and the broad sense heritability (h2b) were generally stated according to Steel and Torrie [19], and the genetic advance from the selection ΔGA% was computed according to Johanson *et al*. [20]. Analysis of variance was detected as recorded by Gomez and Gomez [21]. Phenotypic coefficient of variability (P.C.V.) as an index for the variability among plants of any treatment, P.C.V = σ Ph/x.100, Genotypic coefficient of variability (G.C.V.) as an index for genetic variability among individual plants of any population, G.C.V = σ g/x.100 were computed according to Burton [22].

Molecular Analysis:

DNA Isolation

Seeds of the ten selected prickly oil lettuce genotypes were collected and soaked in liquid nitrogen for DNA extraction The DNA was extracted by Cetyl Trimethyl-Ammonium Bromide (CTAB) method [23].

Polymerase chain reaction (PCR) procedure:

A total of 10 random DNA oligonucleotide primers were independently used according to Williams *et al*. [24] in the PCR reaction. Only 6 primers OPA-11, OPB-05, OPC-03, OPC-13, OPD-03 and OPD-05 succeeded to generate reproducible polymorphic DNA products. Table (1) lists the base sequences of the DNA primers that produced informative polymorphic bands. The PCR amplification was performed in a 25 µl reaction volume containing the following: 200 µl of dNTPs (2.5 mM), 1.5 µl of Mg Cl2 (25 mM), 2.5 µl of 10x buffer, 2.0 µl of primer (2.5 µM), 2.0 µl of template DNA (50 ng/µl), 0.3 µl of Taq polymerase (5 U/µl) and 14.7 µl of sterile ddH2O. The reaction mixtures were overlaid with a drop of light mineral oil per sample. Amplification was carried out in Perkin Elmer Gene Amp PCR System 2400. The reaction was subjected to one cycle at 95o C for 5 minutes, followed by 35 cycles at 94 oC for 30 seconds, 55 oC for 30 seconds, and 72oC for 30 seconds, then a final cycle of 72 oC for 5 minutes. PCR products were run at 100 V for one hour on 1.2 % agarose gels which mixed with l x TBE buffer and ethidium bromide was added to the melted gel after the temperature became 55oC. Gels were photographed and scanned with Bio-Rad video densitometer model 620, at a wavelength of 577. The bands detected by photo Capt MW and calculation were achieved using Dice similarity coefficients [25] as implemented in the computer program SPSS version16.

RESULTS AND DISCUSSIONS

Assessment of Genetic variation in morphological characters

Analysis of Variance

The analysis of variance showed that the genotypes differed significantly ($P < 0.01$) for all traits in all studied characters except plant height showed significance at ($p < 0.05$) in the second season only (Table 1). These results confirms the variable response of prickly lettuce genotypes to the environmental conditions and are in line with those obtained by Ibrahim [26] in prickly oil lettuce and Hassan *et al*. [27] in sunflower. The range and analysis of variance indicated potential genetic variation and diversity in the material under consideration. These results determine better scope for genetic improvement by conventional breeding.

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Table 1: ANOVA of five characters studied in two seasons (2012/2013) and (2013/2014) for 15 lines of prickly oil lettuce.

S.O.V	Seasons	Df.	Plant height (cm /plant)	No. of main branches	Seed weight (gm/plant)	Oil percent (%)	Oil yield / plant
Genotypes	I	14	63.54603**	5.450794**	4.321048*	66.59127**	0.372417**
	II		51.46984*	3.952381**	17.262**	19.6127**	0.870052**
Replicates	I	2	5.955556	1.622222	2.018667	4.616222	0.051242*
	II		33.88889*	4.466667	1.652667	3.040222	0.106469
Error	I	28	3.884127	0.836508	0.779143	1.950984	0.030566
	II		17.48413	1.204762	0.581238	2.841651	0.047928

* Significant at 5% probability level; ** Significant at 1% probability level

Genetic parameters for yield characters

Genetic parameters estimate viz., heritability and genetic advance and phenotypic and genotypic coefficients of variation is necessary to the genetic nature of yield and its components. Inheritance of seed or oil yield character is complex and is improved through its component trait. High yield contributing characters which have high heritability coupled with high genetic advance can be achieved by selection. Therefore, the components of variance and heritable components with genetic parameters such as, genetic advance, heritability estimates and genotypic coefficient of variation are important tools to plan a suitable breeding strategy [28, 29]. Range, Mean, Genotypic and phenotypic variability, Heritability and Genetic Advance of the studied genotypes over two seasons for five studied characters are presented in Table 2

Table 2: Genetic parameters estimates of yield and yield components of 15 genotypes of prickly lettuce oil in two seasons

Characters	seasons	Range	Mean	Phenotypic variations (P.C.V.)	Genotypic variation (G.C.V.)	Heritability (H^2_b %)	Genetic Advance (G.A. %)
Plant height (cm /plant).	I	91-110	101.244	8.110654	7.873594	94.23978	15.94148
	II	97-120	111.955	7.417097	6.408122	74.64377	12.76852
Number of main branches/ plant	I	6-13	9.4222	26.61208	24.77859	86.69528	4.478111
	II	9-14	11.333	20.03766	17.5417	76.63897	3.585266
Seed weight (gm/plant)	I	5.7-12	8.906	25.35584	23.33884	84.72326	3.941514
	II	6.3-14.5	10.464	40.36646	39.70355	96.74253	8.418244
Oil percent (%)	I	16-33	25.722	7.738853	7.627919	97.1536	16.56934
	II	22-34	29.388	16.12379	15.06904	87.34476	8.526177
Oil yield / plant	I	1.1-2.6	1.926	32.94477	31.67071	92.41505	1.208518
	II	1.89-3.91	2.743	34.91948	33.99567	94.77893	1.870663

Range and Mean performances

The studied traits possessed higher values in the second growing season compared with the first one (Table 2). Sufficient variation for all the traits were recorded with mean of values. The maximum range of variability was observed for plant height (97.00 to 120.00 cm) followed by oil percent (22 to 34 %) and (9 to14) for number of main branches per plant with mean of values, 101.244, 29.388 and 11.333 in second season, respectively. These results confirm the genetic variation within and among the studied genotypes. These results were agreement with those reported by Rao [30].

A rough estimate about the variation among genotypes in both seasons was presented by the range of mean values. The characters showing the high range of variation have more scope for improvement. High variability in both seasons as evident with mean of values range was exhibited in all the five studied characters. However, the character's seed weight and oil percent having wide range of variation which indicated that, presence of high variability of these characters and thus display greater scope for genotypes selecting desirable. These findings were agreement with the findings of earlier workers in okra [31] and in safflower [32].

Genotypic and phenotypic variability. (GCV and PCV).

The respect of phenotypic and genotypic variability is important for any effective selection method to improve a population because selection of favorable genotypes depends on the amount of variation in the material under investigation. The estimation of genotypic and phenotypic coefficients of variation of various characters of prickly oil lettuce genotypes over the two seasons is presented in Table 2. Data revealed that the phenotypic coefficient of variation (PCV) was slightly higher than the genotypic coefficient of variation (GCV) for all the characters suggesting the presence of environmental effect to some extent in the expression of these characters. Sivasubramanian and Menon [33] were classified the values of PCV and GCV as low (<10.00 %), moderate (10.00- 20.00%) and high (>20.00%).

The PCV assessment (Table 2) were highest for seed weight per plant (40.37 %) followed by oil plant (34.92%) and number of main branches (26.16%), while lowest were for plant height (7.42 %) followed by oil percent (7.74 %). The GCV estimates (Table 2) were highest for seed weight per plant (39.70 %) followed by oil yield (33.99 %) and number of main branches per plant (24.78 %), while lowest values were recorded for plant height (6.41 %) followed by oil percent (7.63 %). Maximum of GCV was shown for seed weight (40.37 %) followed by oil yield/plant (34.91 %) and its difference with PCV was low. Differences between GCV and PCV for other traits were also found to be low indicating that these traits were less affected by environmental fluctuations (Table 2). These results were agreement with Ramazani *et al.* [34] in safflower cultivars, Salmon [35] and Dandrea [36] in Chamomilla recutita genotypes.

The assessment of GCV and PCV are important to use in detecting the variability present in the material. The magnitude of PCV was higher than the corresponding GCV for all the five characters thus, indicating that the apparent variation was not only due to genotype but also due to the favorable effect of environment and selection for these traits. Similar results have been recorded by Sujatha *et al*., [37] and Iqbal, *et al*., [38]. Therefore, a close correspondence between the values of phenotypic and GCV for the majority of the characters were recorded, reflecting the fact that the environment effect is very low. In contrast, the high magnitude of differences between the values of GCV and PCV for the number of main branches per plant shown that this trait was influenced by the environmental effects. Gandhi *et al*., [39] also reported high magnitude in all differences between GCV and PCV for a number of branches

Heritability and Genetic Advance

Heritability and genetic advance have been successfully employed in the breeding programs to reform traits in different seed oil crops. These include Maize [40], Rapeseed [41] and safflower [42]. Therefore, having information regarding the heritability and genetic advance of traits is useful in the breeding programs. Johnson *et al*., [20] classified heritability (>60 %) values as high and suggested the assessment of genetic advance (<10 %), as low, moderate (10 -20%) and high (>20%). The broad sense heritability for all studied characters was high and ranged from (74.64 to 97.15%) for plant height in the second season and oil % in the first season, indicating that genetic factors have higher influence than environment on the expression of these traits. Similar types were reported by Ibrahim *et al*., [43] and Kumar *et al*., [44].

Genetic advance varied between 1.21% for oil yield per plant and 16.57% for oil percent in both seasons (Table 2). Highest estimates of heritability (97.15) and genetic advance (16.57) were recorded for oil percent in the first season. Moderate genetic advance (>10-20%) were shown by oil percent and plant height in both seasons (Table 2). Mokrani *et al*. [45] also found high heritability and moderate to high genetic advance for oil content in safflower plant. High heritability and low genetic advance (<10%) exhibited by number of main branches, seed weight and oil yield. The high heritable character with high or moderate genetic advance could be further improved with individual plant selection. Characters with high heritability and low genetic advance indicated little scope for further improvement through individual plant selection [27].

Molecular Characterization Using RAPD Markers

The amplified fragments of DNA which generated as well as the monomorphic bands, polymorphic bands, primer sequences, unique bands and the percentage of polymorphism in ten selected genotypes are presented in Table (3) and figure (1).

Table 3: Code and sequence of 6 DNA random primers used for identifying the 10 selected genotypes of prickly oil lettuce, as well as number and types of the amplified DNA bands generated by these primers.

Primer code	Sequence	Monomorphic bands	Unique bands	Polymorphic bands	Total bands	Polym-orphism %
OPA-11	5´-CAATCGCCGT-3´	4	-	4	8	50%
OPB-05	5´-TGCGCCCTTC-3´	8	-	2	10	25%
OPC-05	5´-GATGACCGCC-3´	7	-	1	8	14 %
OPC-13	5´-AAGCCTCGTC-3´	2	1	2	5	60 %
OPD-03	5´-GTCGCCGTCA-3´	1	-	7	8	86%
OPD-05	5´-TGAGCGGACA-3´	3	-	1	4	25%
Total		**25**	**1**	**17**	**43**	**41.8%**

A maximum of 43 DNA bands was scored in the RAPD profiles generated by the primers OPA-11, OPB-05, OPC-05, OPC-13, OPD-03 and OPD-05. The size of the amplified bands ranged from about 200 to 1240 bp. These bands were identified as 18 polymorphic bands (41.8%) and 25 monomorphic ones. One unique band was identified in the resulted RAPD-profile generated by the primer OPC-13 (table 3 and fig. 1), was scored at about 240 bp in the selected genotype 3.

The primer OPA-11 generated 4 non-unique polymorphic fragments. The most pronounced is the polymorphic fragments identified at the apparent molecular size of 960, 700, 600 and 560 bp. All selected genotypes have fragment 600 bp. except genotype 7. Genotype 1 has not fragment at about 560 bp., but this fragment was scored with all selected genotypes

Primer OPD-05 generated 2 non-unique polymorphic bands at apparent molecular size 840 and 340 bp. As shown in Table (3) and Figure (1). The fragment with molecular size 840 bp., was exhibited in selected genotypes 5, 7, 9 and 10, while the fragment with molecular size 340 bp., was appeared in selected genotypes 4, 7 and 10.

One non-unique polymorphic band was scored in the RAPD profile generated by the primer OPC-05 at about molecular size 900 bp. was distinguished in selected genotypes 5, 7, 9 and 10.

The results of primer OPC-13 generated 2 non-unique polymorphic bands (Table 3 and Figure 1). The first one was recognized at molecular size 600 bp. which was present in all selected genotypes except genotype 2. The second fragment was exhibited in selected genotypes 3, 6, 7, 8 and 9 with molecular size 440 bp.

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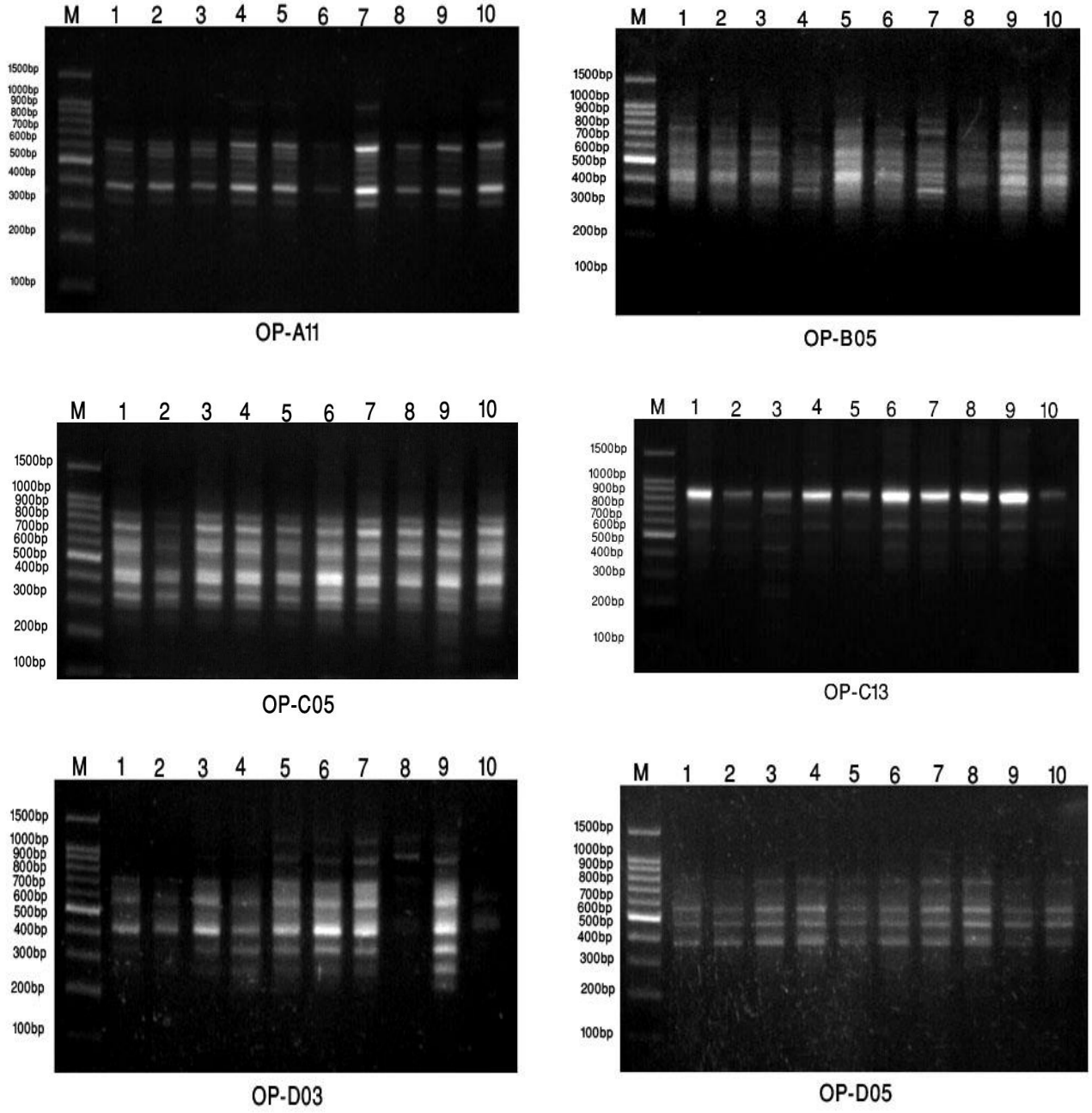

Figure (1): RAPD Profile of 10 selected genotypes of prickly oil lettuce generated (number 1 to 10) by 6 primers, M=DNA Marker

A total of 7 non-unique polymorphic bands were detected by the primer OPD-03 (Table 3 and Figure 1). The fragments with molecular sizes 1240 and 970 bp. were observed in selected genotypes 5, 6, 7, 8 and 9. The band with molecular size 720 bp. was appeared in all selected genotypes except 2 and 10. The fragments with molecular sizes 580 and 340 bp. were observed in all selected genotypes except genotype 8. All selected genotypes have a band at molecular size 260 bp. except genotypes 8 and 10. The fragment with molecular sizes 200 bp. was detected in selected genotypes 4, 5, 6, 7, and 9.

One non-unique polymorphic band exhibited in the RAPD profile generated by the primer OPD-05 at about molecular size 830 bp. was detected in selected genotypes 3, 4, 5, 6, 7, and 8.

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In this investigation, no single primer produced clear unique banding patterns for all selected genotypes under study. However, the combination of all polymorphic bands (unique or non-unique) was enough to discriminate each of the examined selected genotypes by one or more unique bands or a group of combined class patterns.

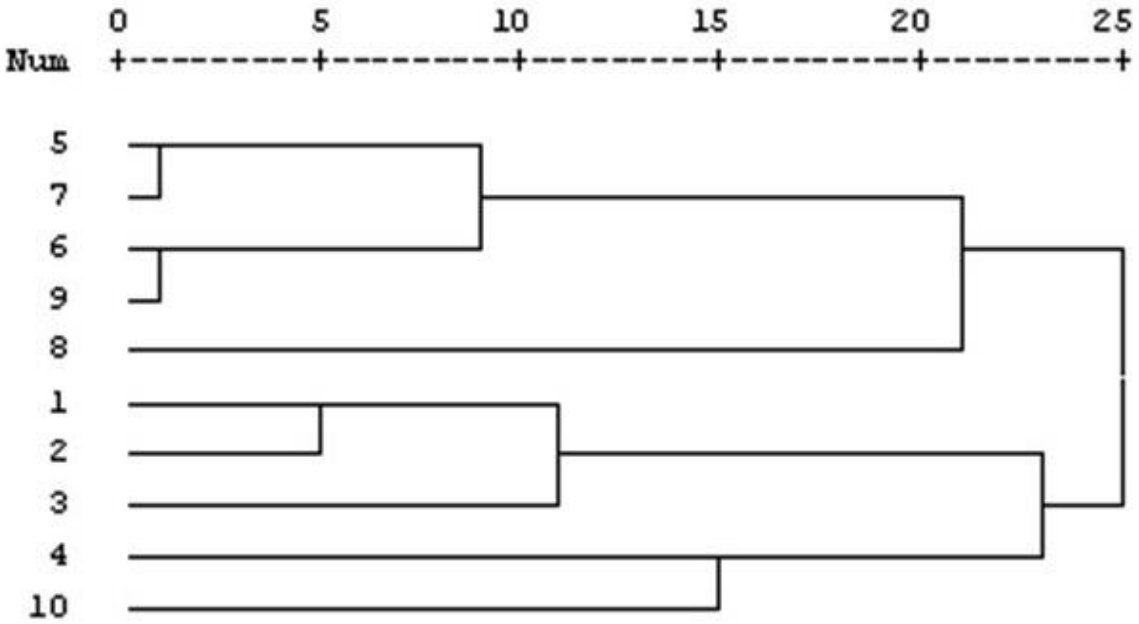

Figure (2): Dendrogram illustrating genetic distance between the10selected genotypes of prickly oil lettuce based on RAPD data.

The results of RAPD analysis were pooled together to generate the dendrogram (Fig. 2). The ten selected genotypes were divided into two main groups. The first comprises the five selected genotypes 5, 6, 7, 8 and 9, and was subdivided into two subgroups; the first includes genotype 8 only. The second subgroup was divided into two classes; the first includes two genotypes 5 and 7. The second class involved selected genotypes 6 and 9. The second group includes the other five, which were subdivided into two subgroups; the first comprises the genotypes 4 and 10. The second subgroup was divided into two classes. The first includes the genotypes 1 and 2, the second class involved only genotype 3. The value of the highest similarity (0.98) was recorded between two genotypes 5 and 10, but the lowest (0.02) was recorded between genotypes 6 and 9.

Similar results were obtained by many authors. Yang *et al*. [46] detect of 216 polymorphic bands from 7 RAPD primers used to elucidate genetic similarity in lettuce cultivars. Okoń and Magdziak [47] assessed the genetic similarity and identification of 7 cultivars and 13 wild species of chamomile using 6 RAPD primers, three of them possessed high resolving power values for analyzed. Esra Maltas et. al. [10] used six RAPD primers were correlated with the performance of *Echinacea* species. Marina *et al*. [48] studied 20 plants of *Astragalus* microcephalus with RAPD primers for detection of the genetic variation in *Astragalus* spp. Sultan *et al*. [49] used three random RAPD primers for the differentiation in *Podophyllum* spp. RAPD primers were a good technical and sufficient variations among mustard (*Brassica* spp.) genotypes [50, 13], in safflower accessions [11], in sunflower genotypes [12] and among some Egyptian pistachio (*Pistacia vera*) cultivars [14]..

CONCLUSION

The analysis of variance showed significant differences among the fifteen selected genotypes of prickly oil lettuce for all characters studied. The genotypic coefficient of variation (GCV) was lesser than the phenotypic coefficient of variation (PCV) for all characters studied. High PCV coupled with high GCV were observed for seed weight per plant (g), oil yield per plant and number of main branches in both seasons,

indicating the presence of wide variability for these traits in the present study. High heritability coupled with high genetic advance was observed for plant height (cm), and seed yield per plant (g) showing the operation of additive gene action in the inheritance of these traits and that improvement in these characters is possible through simple selection and breeding. RAPD results confirmed the possibility of distinguishing, identifying, selection process of genotypes and breeding by using this technique.

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