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Biochemical, Morphological and Molecular Evaluation of Nine Fenugreek Landraces

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Authors' contributions

This work was carried out in collaboration between all the authors. All the authors were involved in the design of the study. Author AMAA provide the plant material, author RTB performed the chemical and protein analyses and author SEIE performed the Molecular experimental work, the dendrogram and similarity analysis and wrote the final manuscript. All authors read and approved the final manuscript for publication.

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ABSTRACT

Aim: Identification of plant genotypes is an important process to register the plant cultivars, protect breeder's right, maintain the genotype genetic purity, perform the field inspection as a supportive method to seed analysis and protect seed industry. So, the objective of this work was to distinguish among nine landraces of fenugreek (*Trigonella foenum graecum* L.) at the seedling, chemical, biochemical, and molecular levels.
Methodology: Germination percentage and seedling vigor characteristics were tested using ISTA rules. Seed chemical composition (Moisture, protein, oil, fibers, ash and carbohydrate) was measured. SDS-PAGE and RAPD-PCR methods were used for biochemical and molecular differentiation among the genotypes, respectively.
Results: The results of seedling characteristics revealed no significant difference among the genotypes in the germination percentage. Genotype-8 had the highest seedling vigour index, while genotype-10 had the lowest one. Chemical composition such as moisture content, crude protein

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content, oil content, ash content, crude fiber contents, and carbohydrates were analyzed. SDS-PAGE revealed a total of 21 bands with molecular weight (mw) ranging from 241.7 to 6.5 kDa. Eleven out of 21 was polymorphic bands and seven unique markers were found, four of them were positive and the others were negative. RAPD-PCR revealed a total of 103 DNA bands generated by 8 random primers, in which 64 were polymorphic bands. Twenty two unique RAPD markers were detected and all being positive.

Conclusion: Present investigation provided the information about seed germination, seed characters, biochemical and molecular differences of nine Egyptian fenugreek landraces. The results showed that L8 performed well with respect to seedling vigor index and fiber content, while L10 and L14 performed well with respect to protein and oil content, respectively. So, these landraces could be used in the breeding programs for developing the fenugreek.

Keywords: Trigonella foenum graecum L.; RAPD, SDS-PAGE; Seed vigor; chemical analysis.

1. INTRODUCTION

Fenugreek (*Trigonella foenum graecum* L.) is one of the old legumes used as a food and medicinal plant in the Mediterranean region. It is being widely cultivated in many countries [1]. The fenugreek is a high value but low volume crop with multipurpose applications [1]. It is popularly used as a spice and its medicinal value is highly appreciated for heart ailments and diabetes [2]. Although its cultivation was mostly concentrated in Asia and the Mediterranean region, it is now widely cultivated in northern Africa and central Europe [1,3].

Genetic diversity in plant materials results from variations in DNA sequences and environmental effects. In addition, it is used as a resource for re-vegetation of disturbed sites to allow adaptation and natural selection to occur [4]. Therefore, estimation of the genetic diversity among plants is very important for the improvement of any crop and for preserving natural variation for adaptation [5]. Genetic diversity can be determined by using morphological, biochemical, and molecular markers [4]. These markers differ from each other with respect to important features such as genomic abundance, level of polymorphism detected, locus specificity, technical requirements, reproducibility, cost, and the type of data that they generate [5].

Seed storage proteins are deposited in relatively large quantities in mature seeds and typically remain more stable than other plant tissues until they germinate [6]. Therefore, proteins can be easily extracted from seeds and analyzed with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique leading to separation of seed storage proteins into specific banding patterns, which generates genetic polymorphisms with higher levels on the basis of differences in protein intensity among genotypes [7]. Additionally, it is a method commonly used to investigate genetic diversity and to classify plant varieties [8], as genetic markers for genetic variation, to detect genetic diversity in cultivated and wild plant species, and to provide information on phylogenetic relationships among accessions [9 and10]. The major advantages of this protein marker technique include assessments of codominance, the absence of epistatic and pleiotropic effects, ease of use, and a comparatively inexpensive yet powerful method of measuring allele frequencies for specific genes [5]. Electrophoretic makers appear to be due to neutral genes which are not linked to any loci that affect the cultivar and value [11]. Shazia et al. [12] used SDS-PAGE to analyze seed proteins of 28 fenugreek genotypes. Considerable variation in seed protein composition within most cultivars complicated the use of SDS-PAGE for characterizing cultivars using protein seeds. Even though, there were differences in protein patterns among the genotypes.

Molecular markers, particularly DNA genetic markers, are valuable in that they show genetic differences on a more detailed level without interference from environmental influences [13], and involve techniques that provide fast results detailing genetic variation and reflecting underlying genetic diversity [14]. Furthermore, DNA polymorphisms have become the markers of choice for investigating phylogenetic relationships among various plant varieties [15], genome identification [16], molecular characterization [17] and in development of unique molecular signatures [18]. RAPD markers are most useful because of low cost, speed and no need of radioactivity [19]. It is also used in plant population genetic study [20], phylogeny,

gene tagging, gene mapping [21] assessing genetic variations and identifying hybrids [22]. Previous studies evaluated genetic diversity among fenugreek accessions using molecular markers such as rapid amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSRs) [23,24,25].

The aim of the study was: i) characterizing nine fenugreek landraces at the seedling, chemical, biochemical, and molecular levels, ii) examining the genetic variation and polymer-phisms among the landraces understudy using SDS-PAGE and RAPD techniques, and iii) estimating the genetic relationships among these landraces.

2. MATERIALS AND METHODS

2.1 Plant Material

Seeds of nine Fenugreek (*Trigonella foenum graecum* L.) landraces were provided from the Legume Crops Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. These landraces were collected from Beni Suef (L3 and L7), Menia (L5), Asuit (L8), Sohag (9), Giza (L10, L13, and L14), and Fayoum (L11).

2.2 Seedling Vigor Characteristics

To estimate the germination percentage and seedling characteristics of the fenugreek, 50 randomly seeds of each genotype were tested as recommended by ISTA [26]. All seeds were surface sterilized by immersion in 0.5% sodium hypochlorite (NaOCl) solution for 5 min to prevent fungal infections and then rinsed three times with sterile water to remove any residual from NaOCl. The sterilized seeds were then scattered on the upper surface of two sheets of sterile Whatman No. 1 filter paper that had been pre-moistened with 10 mL of sterile, distilled water and placed in separate sterile Petri plates (150 mm in diameter x 15 mm deep). The plates containing the seeds were placed in a controlled environment chamber at 20 ± 2°C for germination. Seed germination was observed daily with water added to each Petri plate as necessary to maintain moisture levels. Seedling development was measured at 15 days after germinated in the Petri plates by monitoring seed germination [26], by measuring seedling stem and root lengths, and determining seedling fresh and dry weights of ten randomly selected seedlings. Seedling vigor index was calculated following the procedure (seedling length in cm x germination percentage) outlined by ISTA [26]. Seedling dry weights were determined after drying the plant seedlings to a constant weight in a hot air oven at 85°C (12 h) [27].

2.3 Seed Chemical Composition Analysis

The seed chemical composition (Moisture, protein, oil, fibers, ash and carbohydrate) of the fenugreek genotypes under investigation was measured according to the protocol outlined by AOAC [28].

2.4 SDS- Protein Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique was used to characterize the different genotypes by their protein fingerprint. Protein profiling was carried out according to Beaumont et al. [29] as modified by Studier [30].

2.5 DNA Extraction

DNA was extracted from 100 mg of young leaves of each genotype using mi-Plant Genomic DNA Isolation Kit (metabion). The concentration and purity were determined by spectro-photometer.

2.6 RAPD Analysis

RAPD analysis was carried out according to Studier [31] using 10-mer oligonucleotide primers. Eight primers were selected as potentially useful (Table 1).

A total volume of 25 µl PCR reactions were composed of dNTPs (200 µM), Mg Cl2 (1.5 mM), 1x buffer, primer (0.2 µM), DNA (50 ng), and Taq DNA polymerase (2 units). Amplification was carried out in a Thermo Cycler (PTC 200) programmed for 94°C for 3 min (one cycle); followed by 94°C for 30 sec, 36°C for 1 min and 72°C for 2 min (36 cycle); 72°C for 10 min (one cycle), then 4°C (infinitive). 15 µl of each amplification product were mixed with 3 µl loading buffer and separated on 1.3% agarose gel stained with 0.5 µg/ml ethidium bromide, and visualized under ultraviolet light and photographed. DNA fragment sizes were determined by comparisons with the 100 bp DNA Ladder plus.

Table 1. Sequences of the 10-mer RAPD primers (5'-3')

No.	Code name	5'-3' Sequences
1	OPC-1	TTCGAGCCAG
2	OPC-10	TGTCTGGGTG
3	OPF-4	GAATGCGGAG
4	OPF-10	GGGCCACTCA
5	OPA-17	GACCGCTTGT
6	OPG-05	CTGACGTCAC
7	OPAM-01	TCACGTACGG
8	OPP-05	CCCCGGTAAC

2.7 Data Analysis

The results of SDS-PAGE and RAPD analysis were entered in a computer file as binary matrices where 0 stands for the absence of a band and 1 stands for the presence of a band in each individual sample. Similarity coefficients were calculated using Dice matrix [32]. Construction of the dendrogram tree was performed using the unweight pair group method based on arithmetic mean (UPGMA) as implemented in the SPSS program version 10.

3. RESULTS AND DISCUSSION

3.1 Germination and Seedling Characteristics

Variations in seed germination, shoot and radicle length, fresh and dry weights, and seedling vigor among the nine investigated fenugreek landraces are presented in Table 2. Seed germination percentage ranged from a low of 96% in genotype10 to a high of 100% in the genotypes L3, L7, L8, L11 and L14. The root length of genotype 8 was the highest value (8.8 cm), while genotype L14 gave the lowest value (6.1 cm). Shoot length values ranged from the highest value (5.8 cm) for genotype L11 to the lowest value (4.5 cm) for genotype L10. The highest fresh weight value (173.2 mg) was observed for genotype L13, while the lowest fresh weight value (104.2 mg) was found for genotype L10. The dry weight ranged from 10.1 to 13.1 mg for the L3 and L7, respectively. Regarding to seedling vigor index, L8 had the highest value (1440), while L10 had the lowest value (1047). The variations in germination characteristics and chemical composition could be attributed to the genotype of fenugreek and/or the differences in the environmental conditions, the time of harvesting and the storage conditions. Previous studies [33,34,35] for fenugreek characterization have also reported similar results on the same characters.

3.2 Seed Chemical Composition

Results in Table 3 showed the seed chemical composition content of nine fenugreek landraces. L 9 had highest moisture content of 12.51%, while L 3 had lowest moisture content of 11.25%. L 10 had the highest protein content (26.23%), while L 7 gave the lowest value (22.6%). The highest oil content was 6.53% for L 14, while the lowest oil content was 3.46 % for L 10. Regarding to the ash content, L 11 gave the highest values (7.88%), while L 10 had the lowest value (5.65%). L 8 had the highest fiber content value of 7.46%, while L 7 had the lowest fiber content value of 4.48%. L 7 had the highest value of carbohydrate content of 50.52%, while L 11 had least value of 42.48%.

Table 2. Germination and seedling characteristics of fenugreek landraces

	Germination (%)	Radicle length (cm)	Shoot length (cm)	Seedling fresh weight (mg)	Seedling dry weight (mg)	Seedling vigor index
L3	100	6.2	5.0	137.2	10.1	1120
L5	98	6.8	5.3	129.2	10.6	1185
L7	100	6.7	4.8	144.5	13.1	1150
L8	100	8.8	5.6	126.5	11.7	1440
L9	97	6.6	5.2	112.1	11.6	1145
L10	96	6.4	4.5	104.2	10.2	1047
L11	100	6.9	5.8	136.9	11.3	1270
L13	96	7.1	5.5	173.2	12.6	1210
L14	100	6.1	5.6	141.2	12.2	1170

Table 3. Chemical composition analysis of fenugreek seeds

Genotype	Moisture	Protein	Oil	Ash	Fiber	Carbohydrate
L3	11.25	23.86	5.73	6.95	5.53	46.74
L5	12.10	24.04	3.68	6.90	5.52	47.76
L7	12.13	22.60	3.63	6.67	4.48	50.52
L8	12.28	24.19	3.51	7.11	7.46	45.45
L9	12.51	24.71	4.04	7.26	5.95	45.53
L10	12.06	26.23	3.46	5,65	5.88	46.72
L11	12.20	25.26	4.86	7.88	7.32	42.48
L13	12.16	23.81	5.91	6.87	4.72	46.53
L14	11.75	22.74	6.53	6.66	4.61	47.71

Ritu [36], Singh et al. [37], Sumayya et al. [38] have also reported similar results for the same traits of different fenugreek genotypes. It has been reported that carbohydrates, proteins, and lipids make main component of the seeds, which are responsible for the functional properties development of new products. Total crude protein content is also affected by several factors including genetic factors, soil type, climatic conditions, region, and fertilizers [39].

3.3 SDS-PAGE Analysis

Protein banding patterns of the studied fenugreek landraces as revealed by SDS-PAGE are shown in Tables (4 and 5). The data showed 21 bands as a total numbers for all genotypes. The molecular weight (MW) of bands ranged from 241.7 kDa for L9 to 6.5 kDa for L13. Also, there are twelve common bands that were found in all landraces. Some landraces contained specific bands which could be used to identify and characterise them among others. For example, L9, L5, L7, and L13 had unique bands having molecular weights of 241.7, 154.2, 86.1 and 6.5 kDa, respectively. However, band with MW of 225.4 kDa is present only in L10, L11, and L13. These results could be considered as positive unique marker (PUM). Meanwhile, bands with MW of 79.7 and 9.5 kDa were found in most landraces except L8. Similarly, bands with MW of 28.1 kDa are found in most landraces except L8 and L11. Also, band with MW of 36.2 kDa is present in most landraces except L7, L8 and

Table 4. Molecular weight of SDS-PAGE seed storage protein of fenugreek landraces

No. bands	M.W	L3	L5	L7	L8	L9	L10	L11	L13	L14
1	241.7	-	-	-	-	+	-	-	-	-
2	225.4	-	-	-	-	-	+	+	+	-
3	203.6	+	+	+	+	+	+	+	+	+
4	185.4	+	-	+	+	+	+	+	+	+
5	154.2	-	+	-	-	-	-	-	-	-
6	107.5	+	+	+	+	+	+	+	+	+
7	92.9	+	+	+	+	+	+	+	+	+
8	86.1	-	-	+	-	-	-	-	-	-
9	79.7	+	+	+	-	+	+	+	+	+
10	66.7	+	+	+	+	+	+	+	+	+
11	59.9	+	+	+	+	+	+	+	+	+
12	49.7	+	+	+	+	+	+	+	+	+
13	36.2	+	+	-	-	+	-	+	+	+
14	28.1	+	+	+	-	+	+	-	+	+
15	24.9	+	+	+	+	+	+	+	+	+
16	21.8	-	+	+	+	-	-	+	+	+
17	16.6	+	+	+	+	+	+	+	+	+
18	13.7	+	+	+	+	+	+	+	+	+
19	11.7	+	+	+	+	+	+	+	+	+
20	9.5	+	+	+	-	+	+	+	+	+
21	6.5	-	-	-	-	-	-	-	+	-

(+) = band present and (-) = Band absent

L10. This could be considered as a negative unique marker (NUM). The data obtained in the present study showed distinct protein polymorphisms in each fenugreek genotype, which result from base changes in DNA altering protein sites. Therefore, these polymorphisms may serve as genetic markers because they can be highly polymorphic and their variability is generally highly heritable. [40,41,38] founds different patterns among fenugreek genotypes using SDS-PAGE.

3.4 RAPD Analysis

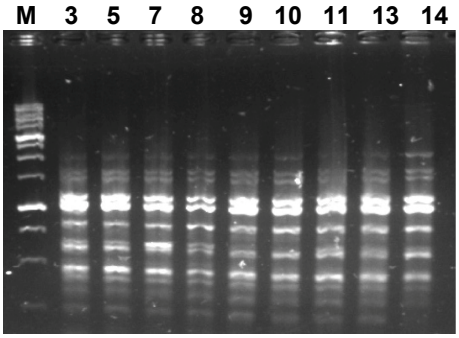
The eight RAPD primers used in this study displayed marked amplification with distinct bands. The RAPD markers generated by these primers revealed characteristic profiles for each genotype in terms of number and position of RAPD bands (Tables 6 and 7, and Fig. 1). A total number of 103 DNA bands were detected as generated by the 8 random primers for the nine landraces used in the present study, in which 64 (62.12%) were polymorphic bands. However, 39 bands were common (monomorphic) for all landraces. Primer OPF-4 gave the lowest number of bands (5 bands) in which all of them were monomorphic bands, while primer OPAM-01 gave the largest number of bands (18 bands) in which 16 of them were polymorphic with percentage 88.89%. The results revealed 22 unique positive markers for all the landraces. Primers OPC-01, OPC-10 and OPF-04 did not show any kind of markers. No negative markers were scored with any primer. These genotype-specific markers can be used in subsequent experiments to detect molecular markers for polymorphic genes with economic importance among these and other genotypes. Cheema et al. [42] reported that even though RAPD markers are useful for grouping inbred lines with different genetic backgrounds, but for determining the genetic relatedness between lines RFLPs are better. Hahn et al. [43] reported that the RAPD technique was found to be a powerful method to provide improved probes coverage on a previously created RFLP map and to locate markers linked to chromosomal regions of interest. RAPD markers have been useful in evaluation of genetic diversity and markers assisted selection offers great opportunities and effectiveness in selecting more valuable plant genotypes [44].

Although RAPD analysis is quick and well adapted for the efficient non-radioactive DNA fingerprinting of genotypes [45], problems with reproducibility of amplification and with scoring of error data have been reported for RAPDs [46,47]. Karp et al. [48], Powell et al. [49] found the lowest correlations among RAPDs and other marker systems (SSRs, AFLPs, and ISSRs). Pejic et al. [50] reported that the other DNA markers provide consistent information for pedigree validation and germplasm identification.

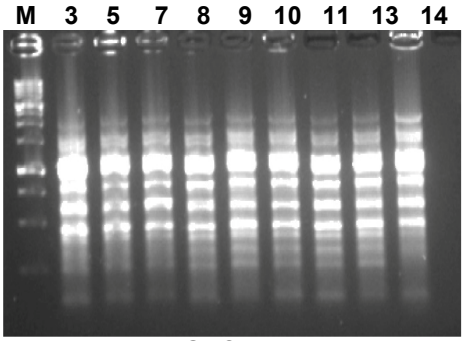
In conclusion, when we use another PCR-based marker technique such as ISSR, SSR, and AFLP, we might obtain higher information content and consequently higher distinguishably among the used genotypes.

3.5 The genetic distance among genotypes

The similarity indices and the dendrogram tree among genotypes utilizing the two methods SDS-PAGE and RAPD are shown in Table 8 and Fig. 2, respectively. The highest percentage of similarity (85%) was scored between L5 and L7, while the lowest percentage of similarity (61%) was scored between L8 and L13. The dendrogram tree divided the nine fenugreek genotypes into two clusters. The first cluster included L3, L5, L7, and L8, while the rest of genotypes were grouped in the second cluster.



OPC-01



OPC-10

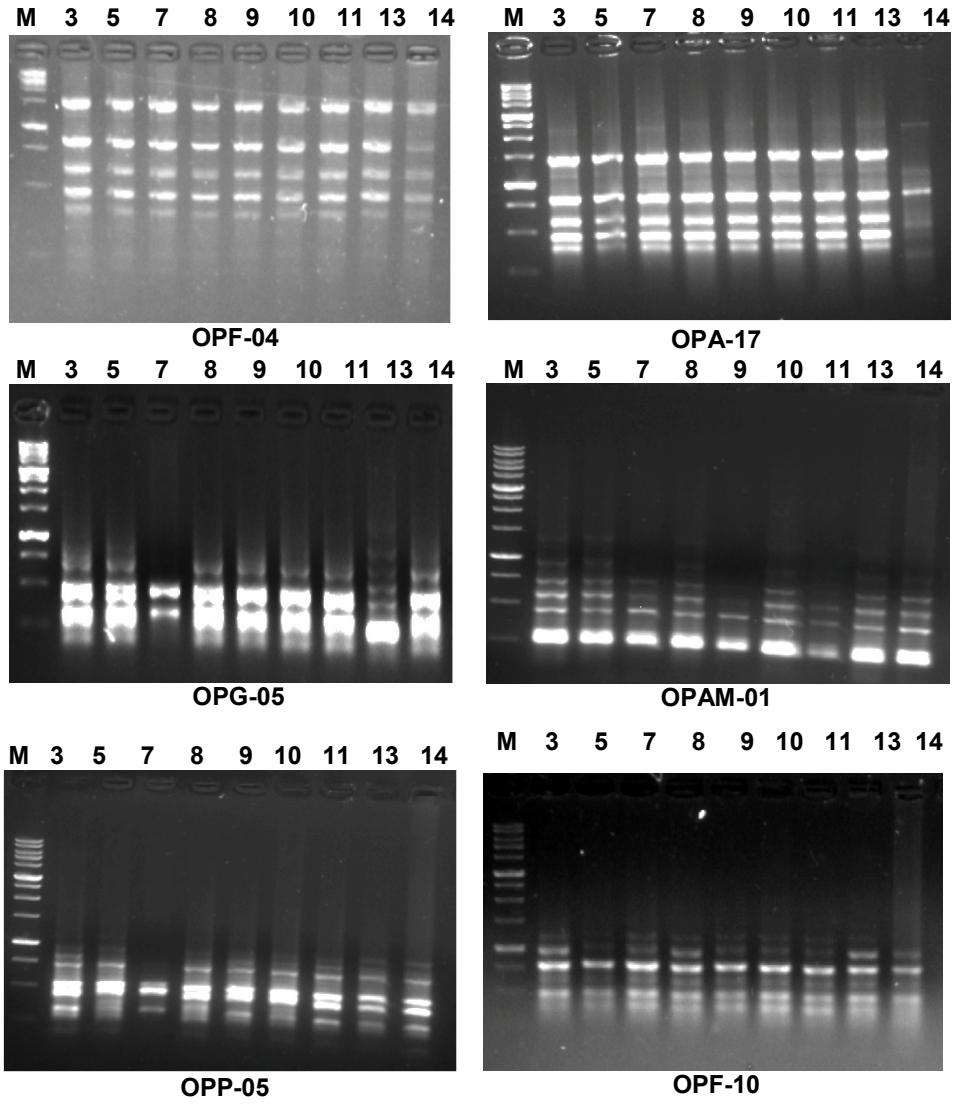


Fig. 1. Agarose gel (1.2%) in TAE buffer stained with ethidium bromide showing RAPD-PCR polymorphism of DNA for nine fenugreek landraces (3, 5, 7, 8, 9, 10, 11, 13, and 14, respectively) using eight random primers. M refers to 100 bp DNA Ladder plus

Table 5. Total number of bands and the MW of the highest and the lowest bands for the SDS-seed proteins in fenugreek landraces

Genotype	High MW (kDa)	Low MW (kDa)	Total bands number	Positive marker	Negative marker
L3	203.6	9.5	15		
L5	203.6	9.5	16	1(154.2)	
L7	203.6	9.5	16	1 (86.1)	
L8	203.6	11.7	12		2 (79.7 and 9.5)
L9	241.7	9.5	16	1 (241.7)	
L10	225.4	9.5	15		
L11	225.4	9.5	16		
L13	225.4	6.5	18	1 (6.5)	
L14	203.6	9.5	16		

Table 6. Molecular weight (bp) of RAPD bands using eight primers

Primer name	MW (bp)	L3	L5	L7	L8	L9	L10	L11	L13	L14
OPC-01	1399.7	+	+	+	+	+	+	+	+	+
	1168.3	+	+	+	+	+	+	+	+	+
	1069.0	+	+	+	+	+	+	+	+	+
	848.1	+	+	+	+	+	+	+	+	+
	756.6	+	+	+	+	+	+	+	+	+
	594.6	+	+	+	+	+	+	+	+	+
	467.2	+	+	+	+	+	+	+	+	+
	333.9	+	+	+	+	+	+	+	+	+
	294.1	+	+	+	+	+	+	+	+	+
	237.8	+	+	+	+	+	+	+	+	+
	209.2	-	-	-	-	+	+	+	+	-
	188.5	+	+	+	+	-	-	-	-	-
	167.7	-	-	-	-	+	+	+	+	-
OPC-10	1449.1	+	+	+	+	+	+	+	+	+
	1297.0	+	+	+	+	+	+	+	+	+
	1221.2	+	+	+	+	+	+	+	+	+
	909.6	+	+	+	+	+	+	+	+	+
	737.9	+	+	+	+	+	+	+	+	+
	569.1	+	+	+	+	+	+	+	+	+
	466.1	+	+	+	+	+	+	+	+	+
	412.0	+	+	+	+	+	+	+	+	+
	370.0	-	-	-	-	-	+	-	+	-
	354.3	+	+	+	+	+	-	+	+	-
	304.2	+	+	+	+	+	+	-	-	-
	202.6	+	+	+	+	+	+	+	+	+
OPF-04	1676.7	+	+	+	+	+	+	+	+	+
	985.0	+	+	+	+	+	+	+	+	+
	653.2	+	+	+	+	+	+	+	+	+
	469.7	+	+	+	+	+	+	+	+	+
	367.4	+	+	+	+	+	+	+	+	+
OPA-17	1278.4	+	+	+	+	+	+	+	+	+
	959.5	-	-	-	-	-	-	-	+	-
	931.1	-	-	-	-	-	+	+	-	-
	915.7	-	-	-	+	-	-	-	-	-
	900.6	-	-	+		+	-	-	-	-
	882.7	-	+	-	-	-	-	-	-	-
	836.8	+	-	-	-	-	-	-	-	-
	703.5	+	+	+	+	+	+	+	+	+
	509.0	+	+	+	+	+	+	+	+	+
	377.0	+	+	+	+	+	+	+	+	+
	318.0	+	+	+	+	+	+	+	+	+
	275.7	-	+	+	-	-	-	+	+	-
	265.5	+	-	-	+	+	+	-	-	-
	242.7	-	-	-	-	-	-	-	-	+
OPG-05	1481.1	+	-	-	-	-	-	-	-	-
	1464.5	-	-	-	+	-	-	-	-	-
	1448.1	-	-	+	-	-	-	-	-	-
	1405.2	-	-	-	-	-	-	+	-	-
	1375.1	-	-	-	-	+	+	-	-	-
	1184.3	+	+	-	-	-	-	-	-	-
	1161.1	-	-	+	+	+	+	-	-	-
	1137.5	-	-	-	-	-	-	-	+	+
	905.7	+	+	+	+	+	+	+	+	+

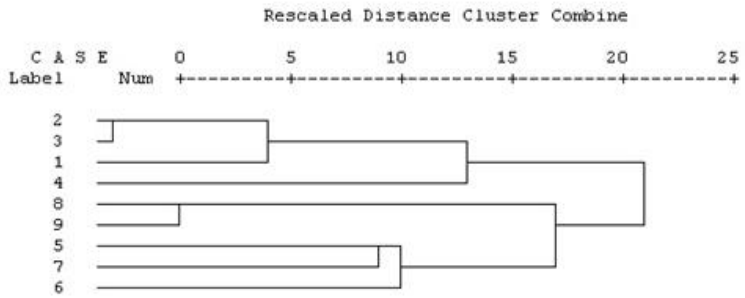
Primer name	MW (bp)	L3	L5	L7	L8	L9	L10	L11	L13	L14
	694.6	+	-	-	+	-	-	-	-	-
	647.3	-	-	-	-	-	+	-	+	-
	631.9	-	-	-	-	+	-	+	-	-
	478.4	+	+	+	+	+	+	+	+	+
	355.7	+	-	-	-	-	-	-	-	-
	335.6	-	-	+	+	-	-	-	-	-
	312.4	-	-	-	-	+	+	+	+	-
OPAM-01	724.5	+	-	-	-	-	-	-	-	-
	687.6	-	+	-	+	-	-	-	-	-
	635.6	-	-	-	-	-	+	-	-	-
	613.5	-	-	-	-	-	-	-	+	-
	528.7	+	+	-	-	-	-	-	-	-
	497.9	-	-	-	+	-	-	-	-	-
	478.8	-	-	-	-	-	+	-	-	-
	428.3	-	-	-	-	-	-	-	+	+
	410.2	+	+	+	-	-	-	-	-	-
	391.4	-	-	-	+	-	-	-	-	-
	360.7	+	-	-	-	-	+	+	-	-
	345.2	-	+	+	-	-	-	-	+	+
	331.6	-	-	-	+	-	-	-	-	-
	311.0	-	-	-	-	+	-	-	-	-
	300.1	-	-	-	-	-	+	+	-	-
	289.7	+	+	+	+	+	+	+	+	+
	279.3	-	-	-	-	-	-	-	+	+
	202.6	+	+	+	+	+	+	+	+	+
OPP-05	477.5	+	+	-	-	-	-	-	-	-
	437.3	+	-	-	+	+	-	-	-	-
	412.3	-	-	-	-	-	+	-	+	-
	397.4	+	+	-	-	+	-	+	-	+
	370.6	-	-	+	+	-	-	-	-	
	359.1	-	-	-	-	+	+	+	-	-
	330.3	-	-	-	-	-	-	+	+	+
	307.2	+	+	+	+	+	+	+	+	+
	281.0	+	+	+	+	+	+	+	+	+
	244.2	-	+	-	-	-	-	-	-	-
	225.3	+	+	+	+	-	-	-	-	-
	205.1	+	+	-	-	+	+	-	-	-
	190.1	-	-	-	+	-	-	+	-	-
	180.3	-	-	-	-	-	-	-	+	+
OPF-10	573.6	-	-	+	-	-	-	-	-	-
	562.8	+	-	-	-	-	-	-	-	-
	547.4	-	-	-	-	+	-	-	+	-
	533.2	-	-	-	+	-	+	+	-	-
	474.0	+	+	+	+	+	+	+	+	+
	389.3	+	+	+	+	+	+	+	+	+
	325.3	+	-	+	-	+	-	-	-	-
	315.1	-	-	-	+	-	-	+	-	-
	304.4	-	-	-	-	-	+	-	+	-
	280.7	+	+	+	+	+	+	+	+	+
	234.2	+	+	+	+	+	+	+	+	+

Table 7. Total number of bands, monomorphic bands, polymorphic bands, positive markers, negative markers and polymorphism % of nine fenugreek landraces using eight RAPD primers

Primer Code	Range size of band (bp)	Total number of bands	Monomorphic bands	Polymorphic bands	Positive marker	Negative marker	Polymorphism %
OPC-01	1399.7-167.7	13	10	3	0	0	23.08%
OPC-10	1449.1-202.6	12	9	3	0	0	25.00%
OPF-04	1676.7-367.4	5	5	0	0	0	0
OPA-17	1278.4-242.7	14	5	9	5	0	64.29%
OPG-05	1481.1-312.4	16	2	14	6	0	87.5%
OPAM-01	724.5-202.6	18	2	16	8	0	88.89%
OPP-05	477.5-180.3	14	2	12	1	0	85.71%
OPF-10	573.6-234.2	11	4	7	2	0	63.64%
Total		103	39	64	22	0	62.12%
Average		12.9	4.9	8	2.8		

Table 8. Similarity matrix among the genotypes based on combined analysis of SDS-PAGE and RAPD

Genotype	L3	L5	L7	L8	L9	L10	L11	L13
L5	.83	-						
L7	.78	.85	-					
L8	.73	.74	.79	-				
L9	.76	.73	.76	.73	-			
L10	.65	.64	.69	.66	.77	-		
L11	.68	.71	.72	.71	.78	.68	-	
L13	.62	.69	.72	.61	.72	.71	.74	-
L14	.73	.78	.79	.72	.77	.68	.77	.83



1= L3, 2 = L7, 3 = L5, 4 = L8, 5 = 9, 6 = L10, 7 = L13, 8 = L14 and 9 = L11.

Fig. 2. Dendrogram of the genetic distances among the nine fenugreek landraces

4. CONCLUSION

Present investigation provided the information about seed germination, seed characters, biochemical and molecular differences of nine Egyptian fenugreek landraces. The results showed that L8 performed well with respect to seedling vigor index and fiber content, while L10 and L14 performed well with respect to protein and oil content, respectively. SDS-PAGE revealed seven unique markers, four of them were positive and the others were negative. RAPD-PCR revealed twenty two unique positive markers. So, these landraces could be used in

the breeding programs for developing the fenugreek.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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