Original Research Article

Biochemical, Morphological and Molecular Evaluation of Nine Fenugreek Landraces

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 content 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 that there is no significant difference among the genotypes in the germination percentage. Genotype-8 had the highest seedling vigor index, while genotype-10 had the lowest one. Chemical composition such as moisture content, crude protein 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 were polymorphic bands and seven unique markers were found, four of them were positive and the others were negative. RAPD-PCR revealed a total number of 103 DNA bands were detected as generated by 8 random primers, in which 64 were polymorphic bands. Twenty two unique RAPD markers were found, which all of them were 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: fenugreek, *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. Actually, it is being widely cultivated in many countries (Petropoulos, 2002). The fenugreek is a high value but low volume crop with multipurpose applications. It is popularly used as spice and its medicinal value is also highly appreciated for diabetes and heart ailments (Suresh Kumar et al., 2005). Although its cultivation was mostly concentrated in Asia and the Mediterranean region, it is now widely cultivated in northern Africa and central Europe (Petropoulos, 2002; Basu et al., 2014).

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 natural selection and adaptation to occur. Therefore, estimation of the genetic diversity among plants is important for the improvement of any crop and for preserving natural variation for adaptation (Mondini et al., 2009). Genetic diversity can be determined using morphological, biochemical, and molecular markers (Gonçalves et al., 2008). These markers differ from each other with respect to important features such as genomic abundance, level of polymorphism detected, locus specificity, reproducibility, technical requirements, cost, and the type of data that they generate.

Seed storage proteins are deposited in relatively large quantities in mature seeds and typically remain more stable than other plant tissues until they germinate (Mirali et al., 2007). 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 higher levels of genetic polymorphisms on the basis of differences in protein intensity among genotypes (Sinha et al., 2012). Additionally, it is a method commonly used to investigate genetic diversity and to classify plant varieties (Kakaei and Kahrizi, 2011), 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 (Kumar and Tata, 2010; Emre, 2011). The major advantages of this protein marker technique include assessments of codominance, absence of epistatic and pleiotropic effects, ease of use, and a comparatively inexpensive yet powerful method of measuring allele frequencies for specific genes (Mondini et al., 2009). Electrophoretic makers appear to be due to neutral genes which are not linked to any loci that affect the cultivar and value (Vishwanath et al., 2011). Shazia et al. (2011) 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 (Kumar et al., 2009), and involve techniques that provide fast results detailing genetic variation and reflecting underlying genetic diversity (Mamatha, et al., 2017). Furthermore, DNA polymorphisms have become the markers of choice for investigating phylogenetic relationships among various plant varieties (Martosa et al., 2005), genome identification (Plomion et al., 1995), molecular characterization (Singh et al., 2010) and in development of unique molecular signatures (Sudheer-Pamidimarri et al., 2009). RAPD markers are most useful because of low cost, speed and no need of radioactivity (Mohammadi and Prasanna, 2003). It is also used plant population genetic study (Rana and Bhat, 2002), phylogeny, gene tagging, gene mapping (Naghia et al., 2002) assessing genetic variations and identifying hybrids (Jug et al., 2004). Previous studies evaluated genetic diversity among fenugreek accessions using molecular markers such as rapid amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSRs) (Harish et al., 2011; Sundaram and Purwar, 2011; Sharda et al., 2013).

The present study aimed to: i) characterize nine fenugreek landraces at the seedling, chemical, biochemical, and molecular levels, ii) examine the genetic variation and polymorphisms among the landraces understudy using SDS-PAGE and RAPD techniques, and iii) estimate the genetic relationships among these landraces.

2. MATERIAL 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 (1999). 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 transfer into the Petri plates by monitoring seed germination (ISTA, 1999), 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 (1999). Seedling dry weights were determined after drying the plant seedlings to a constant weight in a hot air oven at 85°C (12 h) (Krishnasamy and Seshu, 1990).

2.3 Seed chemical composition analysis

The seed chemical composition content (Moisture, protein, oil, fibers, ash and carbohydrate) of the fenugreek genotypes under investigation was measured according to the proceeding outlined by A.O.A.C.(1990).

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 Laemmli (1970) as modified by Studier (1973).

2.5 DNA extraction

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

2.6 RAPD analysis

RAPD analysis was carried out according to Williams et al., (1990) using 10-mer oligonucleotide primers. Eight primers were selected as potentially useful. The codes and sequences of the used primers are shown in Table (1).

PCR reactions were optimized and mixtures (25 µl total volume) 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). Amplification products (15 µl) were mixed with 3 µl loading buffer and separated on 1.3% agarose gel and 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 according to Dice matrix (Nei and Li 1979). Construction of the dendrogram tree was performed using the unweighted 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 ranged from a low of 96% in genotype10 to a high of 100% in the L3, L7, L8, L11 and L14. Results indicated that the root length of genotype 8 was the highest value (8.8 cm), while L14 gave the lowest value (6.1 cm). Shoot length values of genotypes under study indicated that the highest value was recorded for L11 (5.8 cm), while the lowest value was found for L10 (4.5 cm). The highest fresh weight value was recorded for L13 (173.2 mg), while the lowest fresh weight value was found for L10 (104.2 mg). The dry weight value of the landraces understudy 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 (Naidu et al 2011, Farahbakhsh 2012 and Ritu 2016) on different characteristics 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. The highest moisture content was recorded for L 9 (12.51%), while the lowest moisture content was found for L 3 (11.25%). The results indicated that L 10 had the highest protein content (26.23%), while L 7 gave the lowest value (22.6%). The oil results showed that the highest oil content was found for L 14 (6.53 %), while the lowest oil content was recorded for L 10 (3.46 %). Regarding to the ash content, the results showed that L 11 gave the highest values (7.88 %), while L 10 had the lowest value (5.65 %). Also, the highest fiber content value was recorded for L 8 (7.46 %), while the lowest fiber content value was found for L 7 (4.48 %). Results indicated that the highest value of carbohydrate content was recorded for L 7 (50.52 %), while the lowest value was found for L 11 (42.48 %).

Table 2. Germination and seedling characteristics of fenugreek landraces.

Genotype	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

Many investigators (Singh et al., 2010; Sumayya et al., 2012; Jignesh et al., 2015) have also reported similar results for the same traits of different fenugreek genotypes. As mentioned previously, carbohydrates, proteins, and lipids are the main component of the seeds, and they are mostly responsible for the functional properties that have made them new ingredients in the development of new products. Total crude protein content is also affected by several factors including genetic factors, soil type, climatic conditions, region, and fertilizers (Deshpande and Damodaran 1990).

3.3 SDS-PAGE analysis

Protein banding patterns of the studied fenugreek landraces as revealed by SDS-PAGE for the total seed protein are shown in Tables (4 and 5). The data showed that the total numbers of bands in all of the studied genotypes were 21 bands. The total number of bands among genotypes ranged from 12 for L 8 to 18 for L 13. L5, L7, L9, L11 and L14 gave similar number of bands (16 bands). Meanwhile, L 3 and L10 showed similar number of bands (15 bands).

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

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 characterize them among others. For example, each of L9, L5, L7, and L13 had a unique band, which has molecular weight of 241.7, 154.2, 86.1 and 6.5 kDa, respectively. However, band with MW of about 225.4 kDa is present only in L10, L11, and L13. These obtained results could be considered as positive unique marker (PUM). Meanwhile, bands with MW of about 79.7 and 9.5 kDa were found in all landraces except L8. Similarly, bands with MW of about 28.1 kDa are found in all landraces except L8 and L11. Also, band with MW of about 36.2 kDa is present in all landraces except L7, L8 and L10. This could be considered as negative unique marker (NUM). The data obtained in the present study showed distinct protein polymorphisms in each fenugreek genotype, which may 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. Previous studies (Ahmed et al., 2010; Cheema et al., 2010; Jignesh et al., 2015) found different patterns among fenugreek genotypes using SDS-PAGE.

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		

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 out 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. Hahn et al., (1995) reported that even though RAPD markers are useful for grouping inbred lines with different genetic backgrounds, RFLPs are better for determining the genetic relatedness between lines. Beaumont et al., (1996) 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 a great opportunity and effectiveness in selecting valuable plant genotypes (Young and Cho 2002; Harris, 1999).

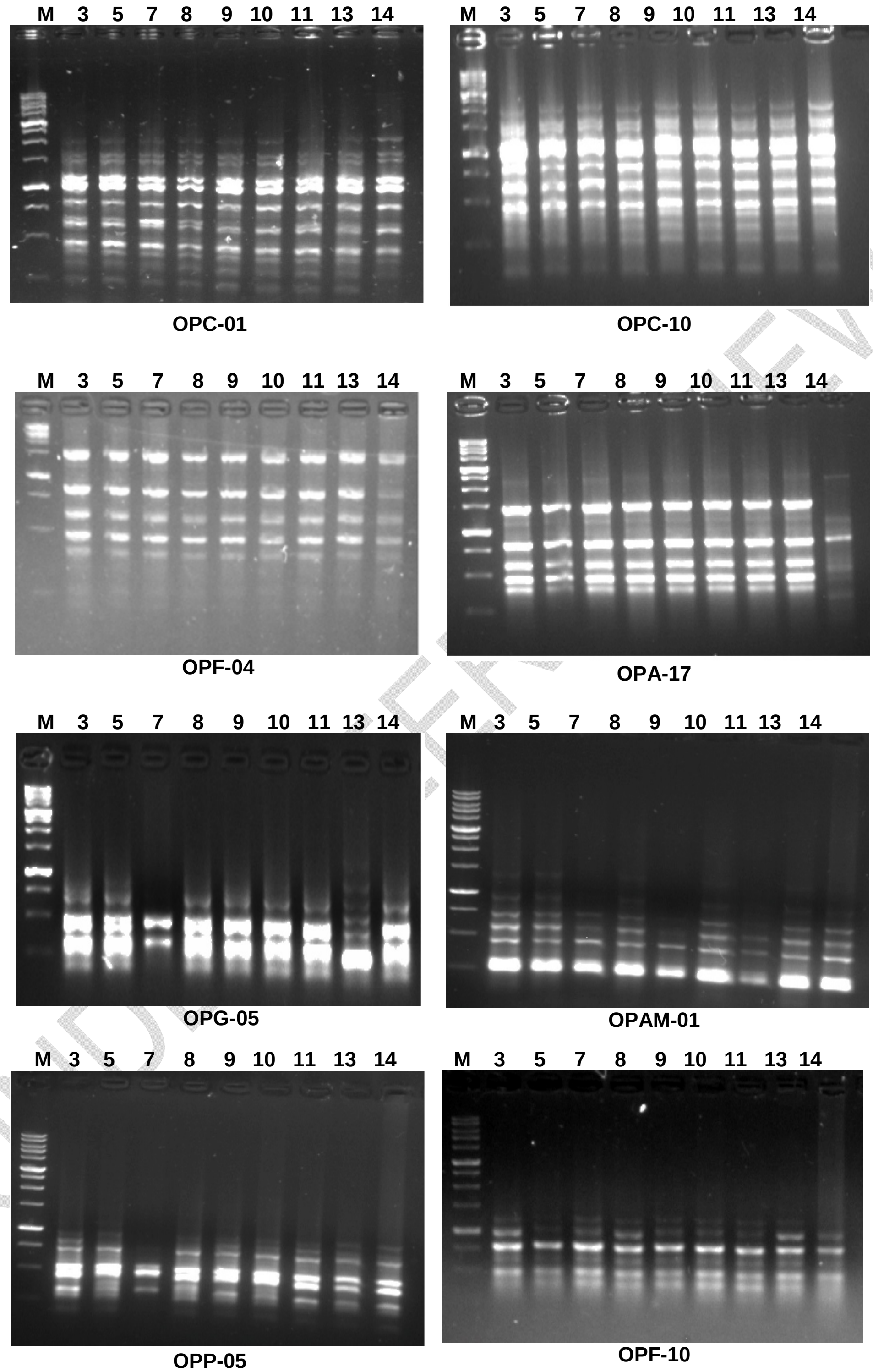


Figure (1): Agarose gel (1.2%) in TAE buffer stained with ethidium bromide showing RAPD-PCR polymorphism of DNA for nine fenugreek landraces (3, 5, 7, 8, 9, 10, 11, 13, and 14, respectively) using eight random primers. M refers to 100 bp DNA Ladder plus.

Although RAPD analysis is quick and well adapted for the efficient non-radioactive DNA fingerprinting of genotypes (Thorman et al., 1994), problems with reproducibility of amplification and with scoring of error data have been reported for RAPDs (Demeke et al., 1997 and Karp et al., 1997). Powell et al., (1996) and Pejic et al., (1998) found the lowest correlations among RAPDs and other marker systems (SSRs, AFLPs, and ISSRs). Pejic et al., (1998) reported that the other DNA markers provide consistent information for germplasm identification and pedigree validation.

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.

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	+	+	+	+	+	+	+	+	+
	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.

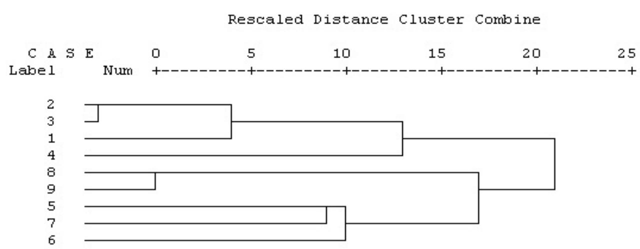
Primer Code	Range size of band (bp)	Total number of bands	Monomorphic bands	Polymorphic bands	Positive marker	Negative marker	Polymorphism %
OPC-01	1399.7-167.7	13	10	3	0	0	23.08%
OPC-10	1449.1-202.6	12	9	3	0	0	25.00%
OPF-04	1676.7-367.4	5	5	0	0	0	0
OPA-17	1278.4-242.7	14	5	9	5	0	64.29%
OPG-05	1481.1-312.4	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		

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.

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.

Figure (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. So, these landraces could be used in the breeding programs for developing the fenugreek.

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