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Profiling the Seed Storage Protein Among Different Genotypes of *Trigonella foenum-graecum* L. (Fenugreek)

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ABSTRACT

A qualitative as well as quantitative categorization of seed storage proteins profiles of 23 genotypes of *Trigonella foenum- graecum* L. were performed by using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) for exploring the level of genetic discrepancy at seed storage protein level. Total soluble proteins were resolved on 10% resolving gel. A dendrogram was constructed on the basis of weight of seed storage proteins, which divide total genotypes into two groups further classified into different sub groups containing different genotypes in them. The bands obtained from gel electrophoresis can serve as a potent tool in discrimination of different genotypes on the basis of their protein content. Proteins with molecular weight 66, 43 and 35 kDa were found in all the genotypes except Fgk-76, PR, Rmt-303, PEB and Rmt-361, The 43 kDa protein band was found missing in Fgk-67, AFg-2, AM-2, AFg-4, Fgk-73, although the protein with 35 kDa weight was present in all the genotypes but not in Rmt-303 same as 63 kDa which is not present in Fgk-70 and 55 kDa protein band was found missing in Fgk-67, Afg-4 and Rmt-361.

Key words: Genetic diversity, Genotypes, SDS-PAGE, Seed protein, *Trigonella foenum-graecum*.

Abbreviations: *AM*- Azad methi, *AFg*- Ajmer fenugreek, *BSA*- Bovine serum albumin, *CBB*- Coomassie Brilliant Blue, *EDTA*-Ethylene-Diamine-Tetra-Acetic acid, *Fgk*-Fenugreek, *HS*-Hisar Sonali, *GM-2*-Gujarat methi, *KDa*-Kilo Dalton, *PAGE*-Polyacrylamide gel electrophoresis, *PEB*-Pusa early bunching, *PMSF*- Phenylmethane sulfonyl fluoride, *PR*-Pant Ragini, *Rmt*-Rajasthan methi, *SDS*-Sodium dodecyl sulphate, *UPGMA*- Unweighted Pair Group Method with Arithmetic Mean.

INTRODUCTION

The genus *Trigonella* is the largest genera of the (tribe trifoliate) in the Papilionoideae or Faboideae (Balodi, 1991). Fenugreek (*Trigonella foenum-graecum* L.) is an annual forage leguminous crop commonly known as a “Methi” or “Greek Hay” the name indicating its use as a forage crop native of the Mediterranean region (Petropoulos, 2002). It is diploid (2n=2x=16) and adapted for self-pollination and fairly tolerant to frost and low temperature. India is the largest producer of fenugreek in world with areas of Rajasthan, Uttarakhand, Gujarat, Uttar Pradesh, Madhya Pradesh, Maharashtra, Haryana and Punjab Hora *et al.* (2013). The major bioactive compound of fenugreek seeds are trigonelline and sapogenins. In the Middle East and the Balkans the aerial part of the plant is used for abdominal cramps associated with both menstrual pain and diarrhea or gastroenteritis Snehlata *et al.* (2012) Antitumor, antioxidant Naidu *et al.* (2011), cholesterol lowering activity, antidiabetic, antiplasmodial activity (Palaniswamy, 2007), anti hepatotoxic and nephrotoxic, anti inflammatory Shushma *et al.* (2010), prophylactic activity Laroubi *et al.* (2007) of fenugreek have reported by researchers all over the world.

Fenugreek seed contains 45-60% carbohydrates, mainly mucilaginous fiber (galactomannans), 20-30% proteins high in lysine and tryptophan, 5-10% fixed oils (lipids), pyridine alkaloids, mainly trigonelline (0.2-0.38%), choline (0.5%), gentianine and carpaine, the flavonoids apigenin, luteolin, orientin, quercetin, vitexin and isovitexin, free amino acids, such as 4-hydroxyisoleucine (0.09%), arginine, histidine and lysine, calcium and iron, saponins (0.6 - 1.7%), glycosides yielding steroidal sapogenins on hydrolysis (diosgenin, yamogenin, tigogenin, neotigogenin), cholesterol and sitosterol, vitamins A, B1, C and nicotinic acid and 0.015% volatile oils ‘n-alkanes’ and ‘sesquiterpenes’ (Budavari, 1996; Newall *et al.* 1996) are the major constituent present in seeds.

Protein polymorphism serves as genetic marker as proteins are the direct products of active genes, which are quite polymorphic and generally heritable (Gepts, 1990; Erum *et al.* 2011). The polymorphism observed in the protein profiles of different genotypes reflects the changes occur in the active part of the genome. Though protein polymorphism can be analysed through a variety of techniques, but the polyacrylamide gel electrophoresis is a favourable technique for rapid analysis (Fergusan and Grabe, 1986; Smith and

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Smith, 1986; Raymond *et al.* 1991; Turi *et al.* 2010) due to its validity and simplicity for describing genetic variations occur in different genotypes of plants (Ahmad and Slinkard, 1992).

The present study was undertaken for profiling diversity based on seed storage proteins among 23 different genotypes of *Trigonella foenum-graecum.*

MATERIALS AND METHODS

Plant material

For the present investigation, seeds of different genotypes were procured from different places in India (Rmt-1, Rmt-143, Rmt-303, Rmt-305, Rmt-351, Rmt-361 and Rmt-365) from S.K.N College of Agriculture, Jobner, where Rmt stand for Rajasthan Methi. (AFg or Ajmer fenugreek e.g., AFg-1, AFg-2, AFg-3 and AFg-4) are obtained from National Spice and Seed Research Centre Tabiji Ajmer Rajasthan, (Am-1 and Am-2) Am stand for Azad methi, from Chandra Shekhar Azad University of Agriculture and Technology Kanpur Uttar Pradesh, Pusa early bunching (PEB) from IARI New Delhi, Hisar sonali (HS) from Hisar Agriculture University and Pant Ragini (PR), Gujarat methi (GM-2), Fenugreek; Fgk-67, Fgk-68, Fgk-70, Fgk-73, Fgk-75 and Fgk-76) are collected from the Vegetable Research Centre of G.B.Pant University of Agriculture & Technology Pantnagar Uttarakhand.

Extraction of proteins from seeds

For the protein extraction the seeds were collected and washed with distilled water to remove dust and other extraneous material which were further dried and grinded to make the fine powder. This powdered seed material was used for protein extraction by using the extraction buffer which contains, EDTA 5Mm, NaCl 50Mm, Na phosphate 25mM, PMSF (Stock in 0.1m in absolute alcohol), 4 ml cold extraction buffer added to 1 gm powered seed material in a chilled mortar and pestle and stirred well, PMSF was added to a final concentration of 2mM and the mixture was centrifuged at 10000 rpm for 20 minute, the supernatant was collected which contains crude proteins and stored at 4°C for further experiments.

Determination of protein concentration

Protein quantification

Quantification of protein was done according to the method suggested by Bradford (1976). Bovine serum albumin (BSA) was taken as a standard. The absorbance recorded at 595 gives the standard curve of BSA with varying concentration, amount of protein in the sample was calculated by extrapolating the graph of BSA.

Sample application and gel electrophoresis

Method of Laemmli (1970) was used which incorporates sodium dodecyl sulphate (SDS) into a discontinuous denaturing buffer system. The gels were cast and run using Vertical Midi Gel System (Merck Specialties Private Limited) with a 12% separating gel and a 4% stacking gel. The protein samples were prepared in SDS extraction buffer (SEB). Hi range protein Marker (Biolit) was loaded as a molecular weight

Table 1: Genotypes of *Trigonella foenum graecum* L. (fenugreek).

Genotypes	Sources
AM-1	Chandra Shekhar Azad University Kanpur, U.P
AM-2	Chandra Shekhar Azad University Kanpur, U.P
AFG-1	National Seed Spice Research Centre Ajmer, Rajasthan
AFG-2	National Seed Spice Research Centre Ajmer, Rajasthan
AFG-3	National Seed Spice Research Centre Ajmer, Rajasthan
AFG -4	National Seed Spice Research Centre Ajmer, Rajasthan
HS	Dept. of vegetable Crops, CCS, HAU, Hisar, Haryana
GM-2	G.B Pant University of Agriculture & Technology, Pantnagar, Uttarakhand
PR	G.B Pant University of Agriculture & Technology, Pantnagar, Uttarakhand
PEB	Indian Agriculture Research Institute, New Delhi
RMT-1	S.K.N College of Agriculture, Jobner, Rajasthan
RMT-143	S.K.N College of Agriculture, Jobner, Rajasthan
RMT -303	S.K.N College of Agriculture, Jobner, Rajasthan
RMT -305	S.K.N College of Agriculture, Jobner, Rajasthan
RMT -351	S.K.N College of Agriculture, Jobner, Rajasthan
RMT -365	S.K.N College of Agriculture, Jobner, Rajasthan
RMT-361	S.K.N College of Agriculture, Jobner, Rajasthan
FGK-67	G.B Pant University of Agriculture & Technology, Pantnagar, Uttarakhand
FGK-68	G.B Pant University of Agriculture & Technology, Pantnagar, Uttarakhand
FGK-70	G.B Pant University of Agriculture & Technology, Pantnagar, Uttarakhand
FGK-73	G.B Pant University of Agriculture & Technology, Pantnagar, Uttarakhand
FGK-75	G.B Pant University of Agriculture & Technology, Pantnagar, Uttarakhand
FGK-76	G.B Pant University of Agriculture & Technology, Pantnagar, Uttarakhand

marker. The gel after loading with the protein samples was electrophoresed in the tris glycine buffer at 100V for 8 hr.

Staining of Gel

Preparative gels were visualised by staining with Coomassie Brilliant Blue R-250. Gels were stained for 30 min with 0.25% (w/v) CBB R-250 (Hi media), 45% methanol, and 10% glacial acetic acid with gentle shaking. Gels were destained several times with 30% methanol and 10% acetic acid.

Data analysis

Gels were placed on a white light trans-illuminator and photographed. Bands observed in gels were subjected to careful manual scoring and the presence or absence of bands was scored as '+' and '-', respectively (Table 2). Only prominent bands were scored, molecular weight of the polypeptides determined on the basis of their migration distance. Migration distance of the marker polypeptides measured and there Rf values were calculated, graph was plotted by the Rf values of the marker polypeptides against the logarithm of their molecular weight. Rf values of the sample proteins were calculated and their corresponding log10 molecular weights were interpolated from the calibration plot.

RESULTS AND DISCUSSION

Quantification of total protein

The seed proteins are highly stable and their additive nature makes the seed storage protein electrophoresis a powerful tool in elucidating the origin and evolution of cultivated plants. The present investigation revealed variation in 23 genotypes of *Triogonella foenum-graecum* species, collected from different places of India (Table 1).

The amount of total protein content in different genotypes of fenugreek was measured by Bradford method which shows that the highest amount of protein present in Afg-3, followed by Rmt-303 and Rmt-305, the genotypes from Jobner, Rajasthan also have good amount of protein in them whereas the lowest amount was present in Gm-2 (Fig 1).

SDS PAGE analysis

Analysis of seed storage protein by SDS-PAGE has proven for being an effective method for revealing the differences and relationship between the taxa, species and genotypes. SDS-PAGE of seed proteins was performed in order to investigate genetic diversity among *T. foenum-graecum* genotypes. The genetic similarity pattern of 23 genotypes based on SDS-PAGE using UPGMA method was used to construct a dendrogram by using a computer programme NTSYS-PC, version 2.02i (Fig 3). The results of the cluster analysis are shown in dendrogram on the basis of similarity coefficient (Table 3). The dendrogram revealed the two main groups A and B and HS variety which didn't fall in any group due to maximum dissimilarity or diversity; the group A is further divisible into two sub-clusters A1 and A2, sub cluster

Table 2: Comparative evaluation of protein profiles among different genotypes of *Trigonella foenum-graecum* L.

No.	Molecular marker (kDa)	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23
1	80	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+
2	66	+	+	+	+	+	+	+	+	–	+	+	+	–	+	–	+	–	–	–	+	+	–	–
3	63	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–
4	60	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	55	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+
6	47	+	+	+	+	+	+	+	+	–	+	+	–	+	+	–	+	+	+	–	+	+	–	–
7	45	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	43	–	–	+	+	+	–	+	+	–	+	–	–	–	+	–	+	–	–	–	+	+	–	+
9	40	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–
10	35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+

*L1=FGK-67, L2=AFG-2, L3=FGK-70, L4=FGK-68, L5=AFG-1, L6=AM-2, L7=AFG-3, L8=AM-1, L9=FGK-76, L10=FGK-75, L11=AFG-4, L12=FGK-73, L13=PR, L14=RMT-1, L15=RMT-305, L16=RMT-365, L17=PEB, L18=RMT-361, L19=HS, L20=RMT-143, L21=GM-2, L22=RMT-303 and L23= RMT-351.

A1 contains two genotypes (*i.e*. FGK-67 and AFG-2) were 80% similar to one another. Sub-cluster A2 contains three genotypes (RMT-361, RMT-303 and RMT-351) which was Rmt-361 is 80% similar to Rmt-351 and 70% to Rmt-303. B formed the bigger group with three sub clusters B1, B2 and B3. Sub cluster B1 contained 10 genotypes (Fgk-70, FGK-68, AFG-1, AFG-3, AM-1, GM-2, FGK-75, RMT-143, RMT-1 AND RMT-365) where, FGK-70 was about 80% similar to rest of the genotypes in sub-cluster B1. Genotypes FGK-68, AFG-1, AFG-3, AM-1, GM-2, FGK-75, RMT-143, RMT-1 and RMT-365 were 90% similar to each other or they were less diverse. Sub-cluster B2 grouped three genotypes (AM-2, AFG-4 and FGK-73), was AFG-4 and FGK-73 were 90% similar to each other but AM-2 and was about 80% similar to each one of them. Similarly there were three genotypes in sub-cluster B3 (FGK-76, RMT-303 and PEB) which were 90% similar to each other. Variety HS showed least similarity (34%) with all the genotypes which fell in group A or B.

Polymorphism among seven genotypes of wheat, which showed lower level of heterogeneity is showed 0–50% Nemati *et al.* (2012).Overall genetic polymorphism 44% within the seven genotypes of *Abrus precatorius* L reported by De Britto *et al.* (2012) Using SDS-PAGE. Dong *et al.* (2006) have also demonstrated 0-25% polymorphism among 150 genotypes of japonica rice. In wheat, 25-80% polymorphism was observed in 20 genotypes by Sofahan *et al.* (2012); Erum *et al.* (2011) have listed several crop species, such as *Vicia faba, Linum usitatissimum, Vigna mungo, Mentha, Raaphanus sativus, Pyrus sp and Brassica* species and reference may be made to this paper for detailed references. A comparative study of some *T. foenum-graecum* species from Iran using SDS-PAGE profiling of seed protein have also been reported. During their study, the electrophoretic pattern of seed proteins were compared among different species and showed the significant qualitative and quantitative differences among different

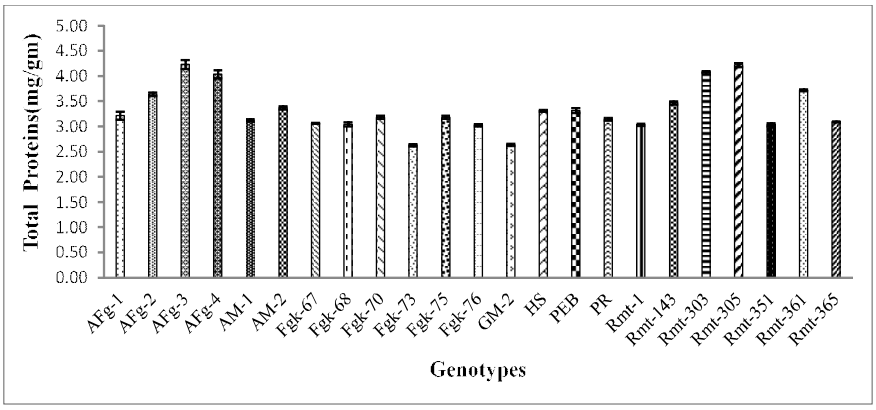


Fig 1: Comparison of total protein content (mg/gm) in seeds of different genotypes of *Trigonella foenum-graecum* L.

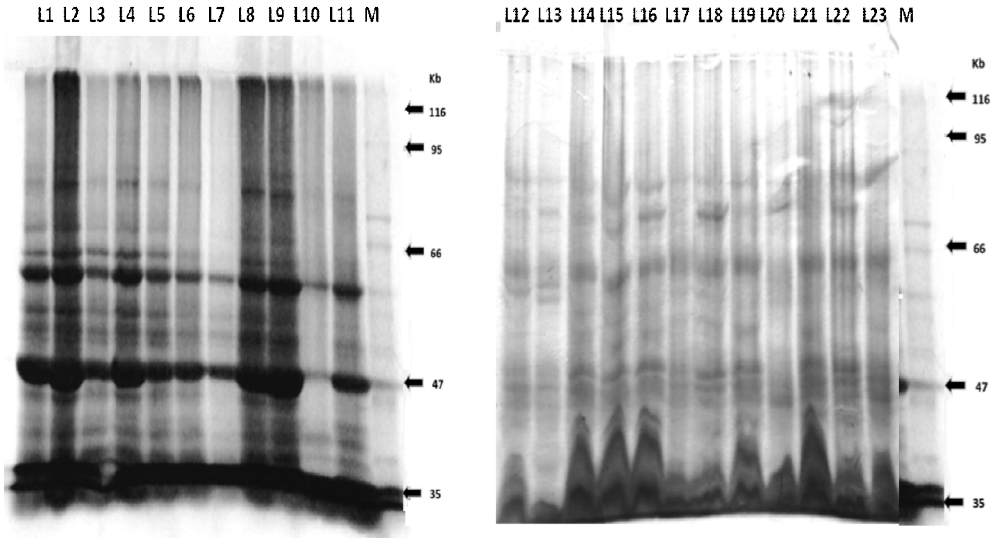


Fig 2: Comparative evaluation of SDS-PAGE protein profile of twenty three genotypes of *Trigonella foenum-graecum*. M=molecular weight marker.

*L1=FGK-67, L2=AFG-2, L3=FGK-70, L4=FGK-68, L5=AFG-1, L6=AM-2, L7=AFG-3, L8=AM-1, L9=FGK-76, L10=FGK-75, L11=AFG-4, L12=FGK-73, L13=PR, L14=RMT-1, L15=RMT-305, L16=RMT-365, L17=PEB, L18=RMT-361, L19=HS, L20=RMT-143, L21=GM-2, L22=RMT-303 and L23= RMT-351.

Table 3: Jaccard similarity coefficient of 23 different genotypes of *Trigonella foenum-graecum* L.

Genotypes	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23
L1	1.0																						
L2	0.8	1.0																					
L3	0.6	0.5	1.0																				
L4	0.7	0.6	0.8	1.0																			
L5	0.7	0.6	0.8	0.9	1.0																		
L6	0.7	0.6	0.6	0.7	0.7	1.0																	
L7	0.7	0.6	0.8	0.9	0.9	0.7	1.0																
L8	0.7	0.6	0.8	0.9	0.9	0.7	0.9	1.0															
L9	0.7	0.6	0.6	0.7	0.7	0.7	0.7	0.7	1.0														
L10	0.7	0.6	0.8	0.9	0.9	0.7	0.9	0.9	0.7	1.0													
L11	0.8	0.7	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.8	1.0												
L12	0.8	0.7	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.9	1.0											
L13	0.6	0.5	0.5	0.6	0.6	0.6	0.6	0.6	0.8	0.6	0.7	0.7	1.0										
L14	0.7	0.6	0.8	0.9	0.9	0.7	0.9	0.9	0.7	0.9	0.8	0.8	0.6	1.0									
L15	0.7	0.6	0.6	0.7	0.7	0.7	0.7	0.7	0.9	0.7	0.8	0.8	0.8	0.7	1.0								
L16	0.7	0.6	0.8	0.9	0.9	0.7	0.9	0.9	0.7	0.9	0.8	0.8	0.6	0.9	0.7	1.0							
L17	0.7	0.6	0.6	0.7	0.7	0.7	0.7	0.7	0.9	0.7	0.8	0.8	0.8	0.7	0.9	0.7	1.0						
L18	0.8	0.7	0.5	0.6	0.6	0.6	0.6	0.6	0.8	0.2	0.7	0.7	0.7	0.6	0.8	0.6	0.8	1.0					
L19	0.7	0.6	0.8	0.9	0.9	0.7	0.9	0.9	0.7	0.9	0.8	0.8	0.6	0.9	0.7	0.9	0.7	0.6	1.0				
L20	0.4	0.5	0.3	0.2	0.2	0.4	0.2	0.2	0.4	0.2	0.3	0.3	0.5	0.2	0.4	0.2	0.4	0.5	0.4	1.0			
L21	0.7	0.6	0.8	0.9	0.9	0.7	0.9	0.9	0.7	0.9	0.8	0.8	0.6	0.9	0.7	0.9	0.7	0.6	0.2	0.9	1.0		
L22	0.6	0.7	0.5	0.4	0.4	0.4	0.4	0.4	0.6	0.4	0.5	0.5	0.5	0.4	0.6	0.4	0.6	0.7	0.7	0.4	0.4	0.1	
L23	0.7	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.7	0.5	0.6	0.6	0.6	0.5	0.7	0.5	0.7	0.8	0.6	0.5	0.5	0.8	1.0

L1=FGK-67, L2=AFG-2, L3=FGK-70, L4=FGK-68, L5=AFG-1, L6=AM-2, L7=AFG-3, L8=AM-1, L9=FGK-76, L10=FGK-75, L11=AFG-4, L12=FGK-73, L13=PR, L14=RMT-1, L15=RMT-305, L16=RMT-365, L17=PEB, L18=RMT-361, L19=HS, L20=RMT-143, L21=GM-2, L22=RMT-303 and L23= RMT-351.

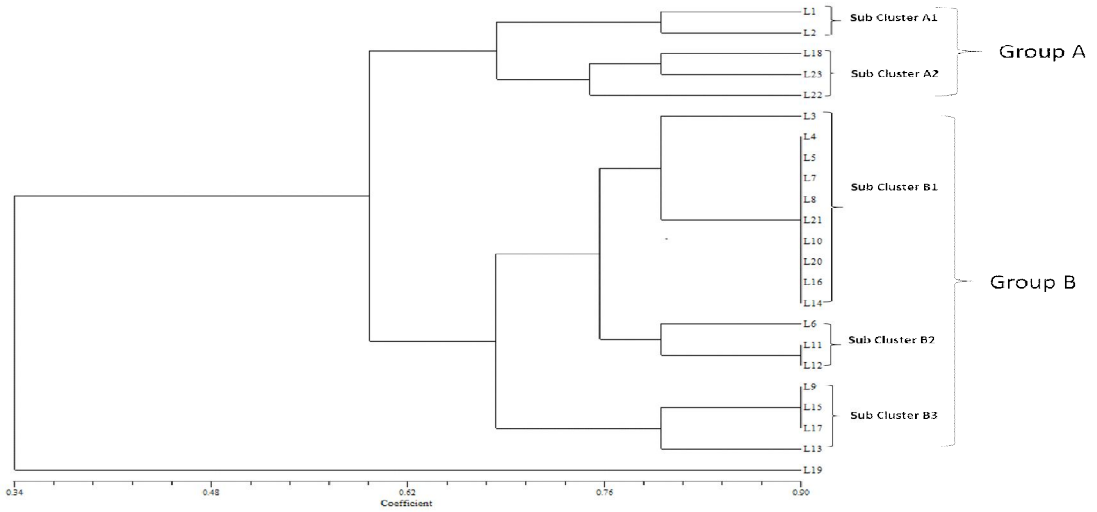


Fig 3: Dendrogram on the basis of UPGMA showing relationship among different genotypes of *Trigonella foenum-graecum* L. L1=FGK-67, L2=AFG-2, L3=FGK-70, L4=FGK-68, L5=AFG-1, L6=AM-2, L7=AFG-3, L8=AM-1, L9=FGK-76, L10=FGK-75, L11=AFG-4, L12=FGK-73, L13=PR, L14=RMT-1, L15=RMT-305, L16=RMT-365, L17=PEB, L18=RMT-361, L19=HS, L20=RMT-143, L21=GM-2, L22=RMT-303 and L23= RMT-351.

species. When the protein content of Rajasthan genotypes were compared with those of Iranian genotypes, some of the Iranian genotypes had high-protein content (% dry matter). Further the Iranian genotypes showed 16-23 prominent protein bands with 70,000–6500 Da in comparison to Rajasthan genotypes reported by Hora *et al.* (2013) with protein bands ranging from 10,000 to 98,000 Da. The differences may be due to the distribution of *Trigonella* species studied by Niknam *et al.* (2004) both cultivated and wild ones. Similarly, protein profiles of 28 genotypes of *T. foenum-graecum* from different ecological zone of Pakistan were compared with Kasuri methi for diversity analysis, which revealed complete distinctions between 28 genotypes of *Trigonella foenum-graecum* L. (common methi) and *Trigonella corniculata* Sibth. & Sm. Kasuri methi. Present studies carried out with 23 genotypes of *Trigonella foenum-graecum and* revealed significant polymorphism. SDS-PAGE analysis provides a strong basis for the discrimination of genotypes on the basis of specific polypeptide-banding pattern.

Haq *et al.* (2014) used SDS–PAGE for analyzing the genetic diversity in various combinations and it was revealed that 12.25 acrylamide gel concentration, 6 micro litre of simple gave the best resolution. Total 22 protein bands were recorded ranging from 10–220 kDa. Many protein subunits of lower molecular weight were also observed but due to inconsistency in reproducibility they were not recorded. Variation is observed in density and sharpness of a few bands but they are not taken in consideration. Only polymorphic band were included in consideration in cluster analysis and construction of dendogram. Total 55 accessions were used to construct dendogram based on SDS-PAGE. On the basis of banding pattern, they divide the gel in to three regions. Region 1 has 6 bands more than 80 kDa MV. Region 2 has 8 bands ranging from 30–80 kDa and region 3 has 7 bands ranging from 10–30 kDa. The result obtained after SDS–PAGE reveal that this method provided a powerful tool for reliable germplasm discrimination based on genetic differences in seed storage protein comparison. Protein with molecular weight 66 kDa is present in all the genotypes except the FGK-76, PR, Rmt-303, PEB and Rmt-361, same variation was found in 43 kDa which is present in all except FGK-67, AFG-2, AM-2, FGK-76, AFG-4, FGK-73, PR, RMT-303, PEB and RMT-361 although the protein with 35 kDa weight is present in all the genotypes but not in RMT-303 same as 63 kDa which is not present in FGK-70. Protein with 55 kDa weight is not present in FGK-67, AFG-4 and RMT-361 (Fig 2).

CONCLUSION

Present studies reveal that there is a significant generic variation in 23 different genotypes of fenugreek which are collected from different places of India they show polymorphism among each other so it can be concluded from the above study that biochemical markers such as seed storage protein provides a strong base for the discrimination of genotypes on the basis of specific polypeptide-banding pattern obtained from the SDS- PAGE.

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Conflicts of interest

Author(s) declares no conflict of interest.

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