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*Original scientific paper*

# ESTIMATION OF SPATIAL GENETIC STRUCTURE IN INTER-REGIONAL POPULATIONS OF *Trigonella foenum-graceum L.* SPECIES THROUGH PHENOTYPIC VARIATION AND SEED PROTEIN PROFILING

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Fenugreek (*Trigonella foenum-graceum* L.) is an important legume crop mainly grown for its pharmacological and nutritional value in Mediterranean region, western Asia, Indian sub-continent and Africa. We evaluated 110 fenugreek accessions from diverse agro-ecological regions i.e. South Asia, Mediterranean, Middle East, Europe and Africa for phenotypic divergence and seed protein based variation. Significant agro-morphological variability was revealed by germplasm viz-a`-viz traits e.g days to flower initiation, days to flower completion, yield plant$^{-1}$, plant habit, vigor, flower colour and plant height. Multivariate approach of Principal Component Analysis and Euclidean distance generated dendrogram distributed all accessions into 6 and 9 distinct groups for morpho-agronomic dissimilarities, respectively. Four principal components (PCs) with Eigen value higher than unity (E>1), represented 65% variability in germplasm. Geographical distribution was evident by scatter plot as germplasm figured in 6 different sub-populations. Iranian accessions were most diverse, showing up in all sub-populations followed by Indian, Turkish, Ethiopian, Pakistani and Egyptian accessions which ranked in 5,4,4,3 and 3 sub-populations, respectively. Electrophoretic pattern of

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seed protein also exhibited considerable polymorphism in the range of 30~100 kDa. Maximum of 16 bands were produced in Turkish PI171872 and Indian PI175321 genotypes. UPGMA based cluster analysis distributed all accessions in 5 groups where accessions from close geographical proximity settled adjacently.

*Key words*: Fenugreek, genetic diversity, GIS, spatial heterogeneity

## INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.*)* is an annual flowering crop of Fabaceae family *Leguminosa*e and genus T*rigonella* which comprises of 36 species. *Trigonella* is a Latin term for little triangle which is attributed to its small triangular shaped flowers. In literature the fenugreek pods are also mentioned as ox-horn like or goat-horn like mainly due to seed pods growth in opposite direction (ERUM *et al*., 2011). VAVILOV (1926) suggested the Mediterranean region of the "Old world" or parts of Asia as its center of origin. Moreover, a recent study advocated Turkey as its origin and main center of diversity (ACHARYA *et al*., 2006). *Trigonella foenum-graecum L.,* are diploids with 2n = 16 chromosomes. However, some species of *Trigonella* are reported with 18, 28, 30, 32 and 44 chromosomes (SNEHLATA and PAYAL, 2012; ZANDI *et al*., 2015). Fenugreek is a suitable crop for short term rotation mainly grown as vegetable, fodder and seed crop for livestock feed such as hay and silage (MCCORMICK *et al*., 2009). Both its leaves and seeds are Edible parts but seed is nutritionally important as it contains 26% protein, 5.8% fat and 44.1% carbohydrate (TALUKDAR, 2013). China, Northern India, Pakistan, Egypt, Turkey, Ethiopia, Ukraine, Greece, Portugal, Spain, United Kingdom, Germany, Australia and Morocco are major growers of fenugreek (GIRIDHAR *et al*., 2016).

Fenugreek is also exploited as an herbal medicine because of its tonic and carminative effects (HALIEM and AL-HUQAIL, 2013; QADIR *et al.,* 2017[a,b]). Medicinal uses of fenugreek include wound healing, development of lactation and sex stimulation (ACHARYA *et al*., 2006). Seed extracts of fenugreek such as galactomannan is used as industrial thickener. According to some recent studies it was revealed that fenugreek can be suggested as a possible remedy for diabetes lowering blood sugar and cholesterol level (AHMAD DAR *et al*., 2015).

Despite the importance of fenugreek in food and medicine, its growth and cultivation is restricted out of their innate habitat (ZANDI *et al.,* 2015). In different regions of the world growers are growing less improved varieties of fenugreek facing lack of improved high yielding, disease resistant varieties which is hindering global production of the crop (JAIN *et al*., 2013; ZANDI *et al*., 2015). Major reason behind this regime is lack of information about genetic diversity and characterization of these species in spite of massive germplasm collections in Gene banks across the world. In order to address this issue, unexploited germplasm should be analyzed in terms of its genetic diversity. Assessment of genetic diversity is very important for conserving natural variation necessary for adaptation and selection of superior genotypes for crop improvement (MONDINI *et al*., 2009). Mutations, recombination in genetic material, and environmental effects are the three main factors responsible for genetic variation in different plant materials (HALIEM and AL-HUQAIL, 2013). Different types of markers i.e. morphological, biochemical and molecular, have been used to evaluate the genetic diversity of plant species (JAN *et al.,* 2017[a,b]; SALEEM *et al.,* 2017; ARIF *et al.,* 2015; HALIEM and AL-HUQAIL, 2013; ERUM *et al*., 2011). Selection of markers mainly depends upon the genomic abundance, level of polymorphism identified, locus specificity, reproducibility, technical necessities, cost and type of data generated (HALIEM and AL-HUQAIL, 2013). Proteins are abundantly stored in seeds where

they remain stable for relatively longer time than other plant tissues until germination (MIRALI *et al*., 2007). Seed storage proteins can be easily isolated from seeds and analyzed through SDS PAGE to separate into different banding configuration which generates high level of genetic polymorphism based on alterations in protein intensities among genotypes (KAKAEI and KAHRIZI, 2011; SINHA *et al*., 2012).

Present study was aimed at investigating agro-morphological and seed protein diversity to examine genetic structure and genetic relatedness among a large set of fenugreek germplasm from different countries.

## MATERIALS AND METHODS

One hundred and ten accessions were obtained from Plant Genetic Resources Institute (PGRI), National Agricultural Research Centre (NARC), Islamabad which were acquired by PGRI from US department of Agriculture (USDA). The germplasm, consisted of 14 different countries and diverse ecologies including Afghanistan 11 accession, Angola 1, Egypt 4, Ethiopia 15, Greece 1, India 25, Iran 27, Italy 1, Jordan 1, Nepal 1, Pakistan 7, Spain 1, Syria 1, Turkey 12, Yemen 1 and one from unknown origin. Experiment was conducted at research field of PGRI, NARC in the last week of October 2014. Eight to ten seeds of each accession were sown at pre-defined points with 1 meter point to point distance. Layout comprised of 2 beds and each bed has 55 accessions. Plants were being irrigated after 3 weeks and recommended agronomic practices were carried out till maturity. For five randomly selected plants, data were recorded against various agronomic traits including 4 qualitative traits (flower color, leaf color, plant habit and vigor) and 9 quantitative traits (days to flower initiation, days to 50% flowering, days to flower completion, pod length, pod width, number of branches plant$^{-1}$, plant height and number of seed pod$^{-1}$, yield plant$^{-1}$). For protein profiling, 10mg of finely ground powder of each accession was mixed with 400 µl of protein extraction buffer (0.05 M Tris-HCl (pH 8.0), 0.2% SDS, 5 M urea, 10% glycerol and 5% 2-mercaptoethanol, bromophenol blue BPB) in eppendorf tube. The samples were vertexed for ten minutes and kept it room temperature for 12 hours. Total seed proteins were recovered by centrifugation at 12000rpm for 10 minutes. 10 µl of each sample proteins were resolved on a slab type mini gel (12.25 % polyacrylamide) in Atto gel assembly AE-6530 following LAEMMLI (1970) buffer system. After electrophoresis, gels were stained with 0.5% coomassie brilliant blue (CBB) G-250 in acetic acid-methanol-water (3:22:25 volume ratio) for two hours and de-stained in acetic acid-methanol-water (5:20:75 volume ratio) for 16 hours until dark blue bands were visible on a clear gel background. Bands size were determined by PageRuler pre-stained protein ladder (10~180kDa) SM0671 (Thermo Fisher Scientific Inc).

### *Data Analysis*

Agronomic data were analyzed in M.S Excel 2010 for descriptive statistics. For examining grouping and genetic relatedness, a multivariate procedure of principal component analysis (PCA) analysis was performed on mean data of all the traits using Statistica 12 package (Stat soft inc. USA). Dendrogram was generated for morphological variation data and protein polymorphism binary data using Euclidean distance and un-weighted pair-group method with arithmetic mean (UPGMA) by NTSys PC 2.2 (Exeter software, USA), respectively. ESRI shape files were utilized to generate diversity map on Arc GIS 10.2.2 (ESRI, USA).

## RESULTS

The agro-morphological characterization of fenugreek population exhibited significant phenotypic variation viz-a`-viz qualitative and quantitative traits. Fifty seven accessions had green leaves followed by 29 brown green, 12 red green, 9 light green and 3 red color leaves, respectively. Among all genotypes, 63 were erect type and remaining 47 had prostrate plant habit. Three types of flower color were reported in the germplasm where 61 accessions had yellow flowers followed by 47 light yellow and 2 violet yellow. A varying degree of plant vigor was reported with 48 accessions having Low, 39 as moderate and 23 had high plant vigor.

Descriptive statistics revealed higher coefficients of variation for agronomic traits i.e. days to 50% flowering, yield plant$^{-1}$, plant height, days to flower initiation and days to flower completion (see Table 1). Pod size, number of branches plant$^{-1}$ and seed pod$^{-1}$ were found as least variable traits. Earliest flowering initiation was observed in Iranian accession PI-141728 after 23 days of germination and Indian accession PI164325 from Tamil Nadu state was late flowering with 115 days to flowering initiation. Average days to 50% flowering and days to flower completion for the germplasm were reported as 107 and 134 days, respectively. Highest seed yield plant$^{-1}$ (93g) was recorded in accession PI180351 followed by PI269993 (70.1g) and PI269992 (64.4g), respectively.

*Table 1. Summary of phenotypic and phonological traits variability in 110 Fenugreek accessions*

| Trait | Mean | Minimum | Maximum | Range | Variance | CV% |
|---|---|---|---|---|---|---|
| PH | 59.0±17.9 | 23.3 | 114.5 | 91.2 | 320.3* | 291.2 |
| DFI | 90.6±15.8 | 71.0 | 127.0 | 56.0 | 249.6* | 226.9 |
| DF50 | 107.4±20.0 | 78.0 | 149.0 | 71.0 | 399.4* | 363.1 |
| DFC | 134.2±12.6 | 109.0 | 164.0 | 55.0 | 159.4* | 144.9 |
| Pod L | 8.6±1.5 | 4.1 | 13.5 | 9.4 | 2.2 | 2.0 |
| Pod W | 3.5±0.4 | 2.4 | 4.3 | 1.9 | 0.1 | 0.1 |
| SP Pod | 11.4±2.6 | 5.3 | 17.7 | 12.3 | 6.8 | 6.2 |
| YPP | 19.4±18.6 | 1.0 | 93.6 | 92.6 | 347.1* | 315.5 |
| NBP | 5.1±1.6 | 1.7 | 12.3 | 10.7 | 2.5 | 2.3 |

### Multivariate Analysis

#### *Morphological*

Euclidean distance-based cluster analysis of morphological traits generated 10 cluster groups including four major and six minor classes on a scale of 1.19 to 7.56 (Figure 1). Grouping pattern was observed at 4.7 unit of coefficient which produced cluster I having 11 accessions with highest number of seed pod$^{-1}$ (14) and average size pod length (8.8 mm). Cluster II had 9 tallest accessions (91.2 cm) showing early flowering initiation (77 days). As a largest group with 32 accessions, cluster III had all erect plants with larger than average sized pod and early flowering completion (126 days) while average grain yield was 11.8g of these accessions which was well below population's mean (19.4 g). 12 less vigorous accessions having average pod width (3.6) and days to flowering completion (136 days) were grouped to gather in cluster IV. Cluster V has 4 accessions two each from Iran and Ethiopia, with medium height (60 cm) and

longest pod length (9.6 mm) and Cluster VI had 5 accessions with minimum flowering duration (122 days) and average pod with (3.4 mm) and number of seed pod$^{-1}$ (11.5). As a major group comprising of 25 accessions, cluster VII was characterized as average performing, small stature prostrate (42 cm) plants mostly from Afghanistan and Iran. Cluster VIII and IX each had two accessions as the former had two Afghanistan origin, above average performing accessions i.e. PI220687 and PI268434 and the latter had low seed yielding (6.4 g) plants with minimum number of seed pod$^{-1}$ (7) and pod length (5.1 mm). In cluster X, 4 accessions with highest seed yield plant$^{-1}$ (58.4 g) were observed which included three from Pakistan i.e. PI269992, PI269993 and PI-269994 and one from Iran i.e. PI222841. Moreover, few accessions did not showed affinity with other accessions and settled individually like PI173136 having minimum days to flower completion (125 days), PI211635 with minimum pod width (2.5 mm) and days to flower completion (124). Similarly, PI250235 with shortest height (28.3cm), largest pod width (4.3 mm) and maximum flowering duration (162 days) and PI218116 with maximum number of branches plant$^{-1}$ (12) and late flowering initiation settled as independent member in dendrogram.

Principal component analysis (PCA) was applied to all 13 morphological traits data to determine their contribution towards overall phenotypic variation in population. The analysis distributed total variation in 13 principal components (PCs) for all the genotypes. Among these components only four had Eigen value more than 1 hence explained most of the variability (65%) in the germplasm (see Table 2). The first component or PC1 has 36.45% variation which was mainly contributed by traits i.e. days to flowering initiation, days to 50% flowering, days to flowering completion and plant habit. The PC2 characterized 10.82% of the variation with positive contribution of plant height, number of branches plant$^{-1}$, seed pod$^{-1}$, yield plant$^{-1}$ and plant vigor. The third principal component exhibited 9.88% variability due to positive effect of flower color, vigor, plant habit and seed pod$^{-1}$. The PC4 was augmented by traits i.e. seed pod$^{-1}$, yield plant$^{-1}$ leaf color, plant habit and vigor hence explained 8.22% of total variation in the population.

Primarily, PCA generated scatter plot dispersed genotypes on plane according to their agro-morphological similarities (Figure 2). However, in most cases the positions of accessions on a plane was consistent with their geographical distances i.e. accessions from neighboring countries clustered to gather to form a phenotypically homogenous sub-population based on morphological affinities and similar ecological conditions (Table 3, Figure 3). First group or population had accessions, mostly from Afghanistan, Iran and Pakistan. Moreover, one accession each from Ethiopia, Nepal and Spain were also present in this group. Group II had accessions from India, Iran and Pakistan. The third group was somewhat geographically diverse as it included phenotypically identical accessions from India, Iran, Turkey, Greece and Ethiopia. However group IV predominantly comprised of Indian and Iranian accessions. Again, group V had accessions from different countries i.e. India, Pakistan, Iran, Turkey, Italy, Egypt, Ethiopia, Angola and Yemen with similar agro-morphological performance in the field. Likewise group VI had accessions from India, Turkey, Egypt, Ethiopia, Syria and Jordan. Interestingly, geographical proximity was evident in the grouping pattern as accessions from immediate neighboring countries were closer as compared to distant neighbors. Therefore, accessions from India-Pakistan-Iran, Iran-Turkey, Turkey-Greece-Italy, Syria-Jordan, Yemen-Ethiopia were in most cases closely depicted on the scatter plot. Besides, country wise presence of accessions in more than one group described the increased level of divergence within country's germplasm. Accession (s) falling with in one or fewer groups represented lesser extent of phenotypic

divergence and vice versa. We observed accessions from Iran falling in all 6 groups, followed by Indian germplasm exhibiting significant diversity by settling in 5 distinct groups (see figure 4). Likewise, Turkish and Ethiopian genotypes dispersed in 4 groups while Egypt and Pakistan origin accessions ensured presence in 3 groups. Moreover, accessions from Italy, Jordan, Nepal, Spain, Syria and Yemen figured only in one group due to smaller sampling from these countries.

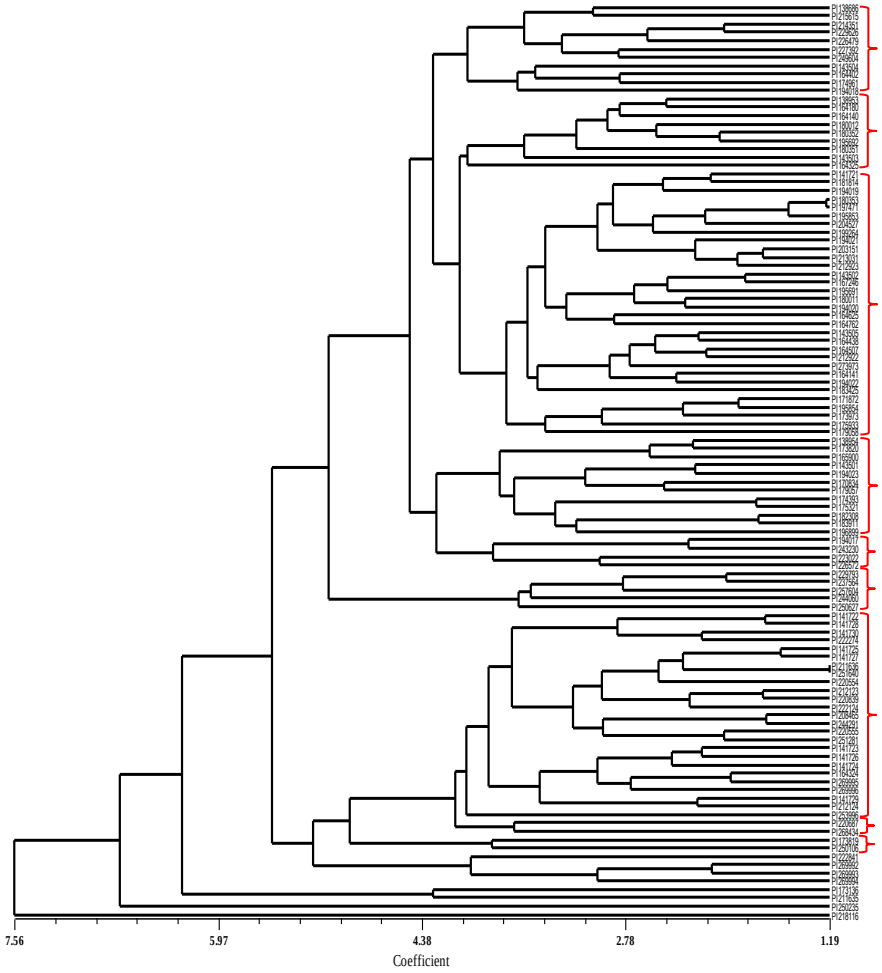


Fig. 1. Distribution of accessions in different clusters based on Euclidean distance dissimilarity coefficient

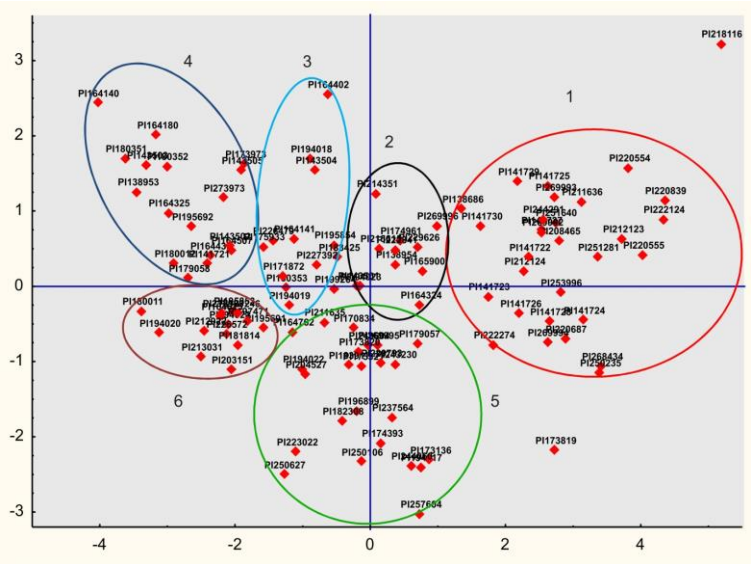


Fig. 2. Euclidean distance based PCA scatter plot distributed 110 fenugreek accessions in 6 different populations

*Table 2 a, b. Principal components and their constituent traits contribution to overall variability among studied germplasm*

a.

| | Eigenvalue | % Total variance | Cumulative Eigenvalue | Cumulative % |
|---|---|---|---|---|
| PC1 | 4.739637 | 36.45875 | 4.73964 | 36.4587 |
| PC2 | 1.406218 | 10.81706 | 6.14585 | 47.2758 |
| PC3 | 1.284986 | 9.88451 | 7.43084 | 57.1603 |
| PC4 | 1.068661 | 8.22047 | 8.49950 | 65.3808 |

b.

| Traits | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| Plant height | -0.79521 | 0.279515 | 0.03095 | 0.076157 |
| Days to flowering initiation | 0.844961 | 0.206528 | -0.07097 | -0.00575 |
| Days to 50% flowering | 0.870359 | 0.17833 | -0.12983 | -0.18973 |
| Days to flowering completion | 0.753447 | 0.090182 | -0.28353 | -0.16685 |
| Pod length | -0.5969 | 0.021184 | -0.30625 | -0.25108 |
| Pod width | -0.40182 | 0.026745 | -0.61294 | -0.28228 |
| Seeds pod$^{-1}$ | 0.010558 | 0.594003 | 0.083259 | 0.447818 |
| Yield Plant$^{-1}$ | -0.46398 | 0.425745 | -0.13389 | 0.270252 |
| Leaf color | 0.18332 | -0.43911 | -0.3418 | 0.573966 |
| Plant habit | 0.711617 | 0.03537 | 0.091023 | 0.376796 |
| Vigor | -0.82658 | 0.16412 | 0.103414 | 0.107141 |
| Flower Color | -0.03106 | -0.18495 | 0.738763 | -0.21261 |
| No. of Branches Plant$^{-1}$ | 0.332821 | 0.674558 | 0.076363 | -0.23366 |

*Table 3. Description of 6 agro-morphologically diverse fenugreek populations revealed by PCA scatter plot*

| Population | Characteristics | No. of Accessions | Accessions Origin |
|---|---|---|---|
| Population1 | Prostrate, Shortest plants, late flowering initiation, small pods and low yield. | 28 | Afghanistan, Ethiopia, Iran India, Pakistan Spain, Nepal |
| Population 2 | Vigorous plants, late flowering initiation, average yield brown-green leaves. | 13 | Afghanistan, Ethiopia, Iran India, Pakistan |
| Population 3 | Taller erect plants, early flowering, medium size pods moderate yield. | 12 | Iran, India, Turkey, Egypt, Ethiopia, Greece |
| Population 4 | Tallest erect plants, earliest flowering initiation, highest number of seeds pod$^{-1}$ and yield. | 17 | Iran, India, Ethiopia |
| Population 5 | Shorter, early flowering plants, medium pods, minimum seed pod$^{-1}$ and yield. | 24 | Angola, Egypt, Italy, Ethiopia Iran, Pakistan, Yemen |
| Population 6 | Taller erect plants, earliest flowering initiation, larger pods, above average yield and brown-green leaves. | 14 | Egypt, Ethiopia, India, Jordan, Syria, Turkey |

*Two Accessions i.e. PI173819 and PI218116 were outlier at the edges of scatter plot due to distinctive traits.

Fig. 3. Clockwise from top left, Population 1 to 6 represented by accessions with discrete morphology.

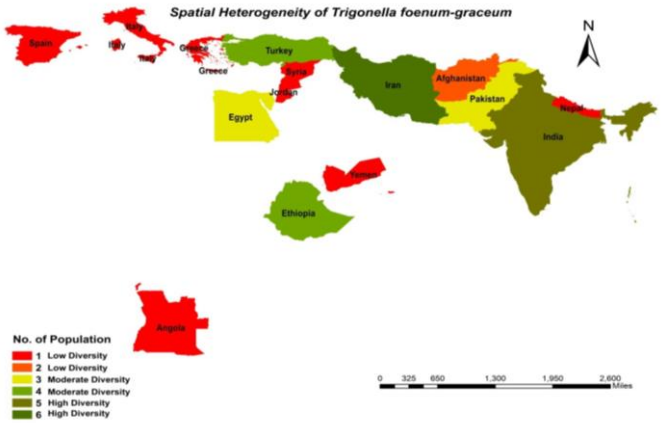


Fig. 4. Inter-regional distribution of phenotypically distinct fenugreek populations and variable level of diversity

*Protein SDS-PAGE*

A total of 106 accessions were analyzed for total seed protein profiling using SDS-PAGE technique. The electrophoresis resolved protein samples from all these genotypes and produced a maximum of 16 bands in the range from 10 to 180 kDa. Only clear unambiguous and reproducible bands were used for analysis. Of these bands, 6 (37.5) were highly polymorphic and present in half of the population while the remaining bands were present in almost all accessions. Maximum number of bands was reported in Turkish PI171872 and Indian PI175321 genotypes. Accessions i.e. PI141725, PI164325, PI250627 and PI251640 produced only 4 bands. For convenience, the gel was divided into three regions as depicted in figure 5 where region I has 6 bands in the range of 100~180kDa including 2 highly polymorphic bands (1 and 6). The middle region of the gel covered 6 bands in the range of 30~100kDa and 4 among them were found highly polymorphic. The third region comprised of 4 less polymorphic bands of low molecular weight (>30 kDa). Cluster analysis was performed to generate banding pattern-based dendrogram revealing maximum of 25% dissimilarity (see figure 6). At dissimilarity level 0.738 all the genotypes were distributed among four major clusters where first cluster was further subdivided in to two clusters i.e. A and B. Similar banding pattern among populations was observed in conformity with Geographical distribution. This was evident from grouping model as accessions from similar habitat or spatial proximity were closer in dendrogram. The cluster1A had 43 accessions mainly from India (10), Ethiopia (8), Afghanistan (7), Iran (6) and Turkey (6). Cluster 1B comprised of 18 accessions mostly of Indian and Pakistani origin. The third cluster was diverse with four Iranian and 2 Ethiopian accessions while one each contributed from Angola, Egypt, Italy Spain and Yemen. Cluster 4 included Indian and Iranian accession but cluster 5 comprised solely of 8 Iranian accessions. Some accessions from different countries i.e. Ethiopia, Turkey and Syria settled in small clusters while outliers like PI250627 (Egypt), PI-251640 (Ethiopia), PI-164325 (Tamil Nadu India), PI-208465 (Nepal) and PI-250235 (Pakistan) stood as independent of grouping due to unique banding pattern.

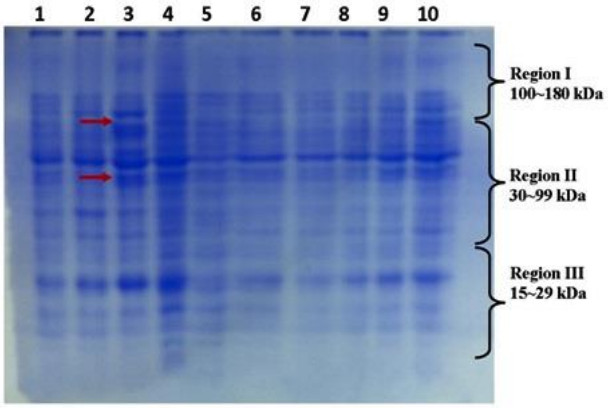


Fig. 5. From left to right: Total seed protein polymorphism revealed by SDS-PAGE in *Trigonella foenum-graceum* L. accessions i.e. PI220687, PI220839, PI-222124, PI-222274, PI-222841, PI-223022, PI-226572, PI-226479, PI-227392 and PI-229626.

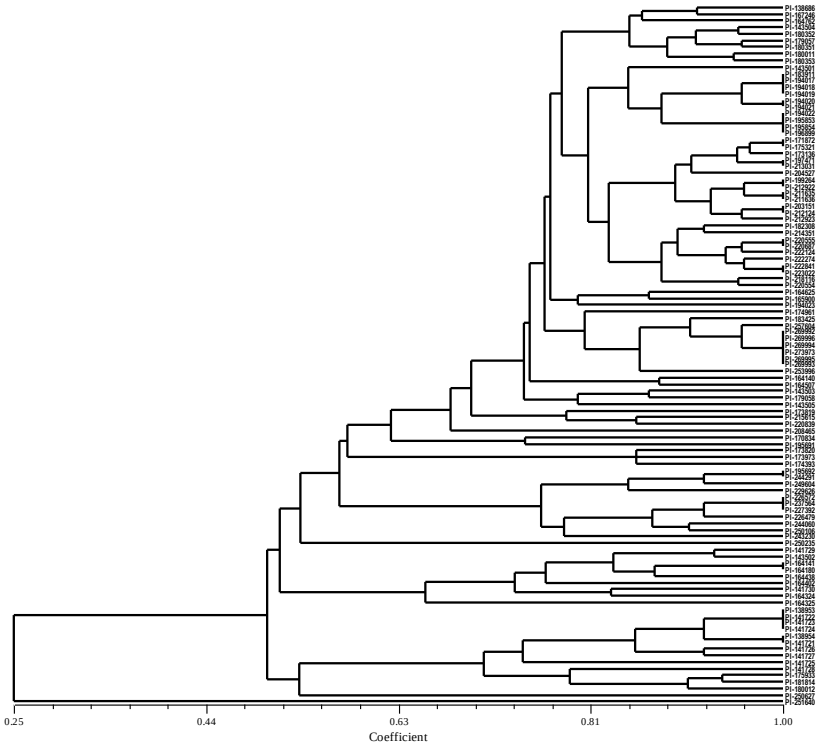


Fig. 6. Total seed protein polymorphism based distribution of accessions in UPGMA generated dendrogram

## DISCUSSION

Genetic diversity estimation has been a focus of plant curators as it is the preliminary step of crop improvement in changing global scenarios of diminishing food security, malnutrition, global warming and climate change. The threshold for marginal crops and medicinal plants like fenugreek has been higher due to low attention as more resources are allocated for staple and commercial crops (ACHARYA *et al*., 2006). The various biochemical and molecular methods play a key role in the identification of diverse crop genotypes (NIKOLIC *et al*., 2016; HLADNI *et al*., 2016; CHANDRAWATI *et al*., 2016; PERIC *et al*., 2014). In the present study a geographically diverse fenugreek population was evaluated for a range of agro-morphological and phonological diversity along with total seed protein variation. Studies have been conducted to determine genetic diversity level in European (BANYAI, 1973; PROVOROV *et al*., 1996), Indian (PANT *et al*., 1983) and American (BERTI *et al.*, 1993) populations. We observed a considerable amount of genetic diversity for days to 50% flowering, yield/plant, plant height, days to flower initiation, days to flower completion, plant habit and seed yield. MCCORMICK *et al.* (2009) studied phenotypic divergence in relatively larger set of fenugreek genetic resources from different countries and in conformity with our results, they reported significant variation for traits like growth habit, flowering time, seed color, seed size and seed yield. We noted positive effect of plant height, number of branches plant$^{-1}$, pod length and width on overall seed yield. Contribution of these traits towards seed yield was also observed by SADEGHZADEH-AHARI *et al*. (2010). Higher variance for characters e.g. plant height, seed yield/plant, flowering initiation and completion stressed the need for using these traits in breeding programs for crop improvement. Recently, FIKRESELASSIE (2012) while comparing performance of Ethiopian fenugreek germplasm with commercial cultivar "Challa", also suggested to utilize variability of various agronomic traits in fenugreek breeding programs. Classification of accessions in multivariate analyses proved to be driven not only by phenotypic and phonological variation but also geographical distribution and country of origin. Morphological similarity was exhibited by accessions of neighboring countries e.g. India-Pakistan-Iran, Iran-Turkey, Turkey-Greece-Italy, Syria-Jordan, etc. which resulted in close depiction of PCA scatter plot and dendrogram. In recent past, similar origin-specific or latitude-specific grouping pattern have been observed in regional germplasm collections of fenugreek (MCCORMICK *et al*., 2009). Iran as a gateway to the Mediterranean region, which along with India is one of the proposed origin of *Trigonella*, has been cited to have phenotypically diverse fenugreek population (MCCORMICK *et al*., 2009; SADEGHZADEH-AHARI *et al*., 2010). Along with Iran, Pakistani and Indian accession also were found highly diverse for flowering duration which was confirmed by MCCORMICK *et al*. (2009). Total of six different types of sub-populations were identified on the basis of PCA scatter plot. Interestingly, Iran origin accessions figured in all 6 of these sub-population followed by Indian accessions in 5 sub-populations. This revealed broad genetic base of these genotypes viz-a`-viz traits e.g. days to flowering initiation, yield plant$^{-1}$ plant height, plant habit and vigor. Of 11 prostrate low vigor Afghanistan origin accessions, seven clustered in a single clad showing close resemblance for flowering initiation, completion and flower color. In addition to morphological characterization, we resolved total seed proteins from T*rigonella* germplasm on SDS-PAGE gels, which is considered as a reliable technique to study peptide profiling that is independent of environmental fluctuations. Unique peptide banding patterns among populations and related genotypes make proportionally specific genetic makeup as peptides are direct product of genes (HALIEM and AL-HUQAIL, 2013). This technique has been widely used in legumes for total seed

protein variation (GHAFOOR *et al*., 2002; NIKOLIC *et al.,* 2012). Moreover, total seed protein profiling in T*rigonella foenum-graceum* L. germplasm has been rarely utilized to infer population structure or genetic diversity level. We observed a maximum of 16 bands in accessions which was in congruence with the findings of NIKNAM *et al.* (2004) while comparing seed proteins of different Iranian fenugreek species. Variability among peptide molecular weight was high in the middle range between 30~100kDa unlike lower molecular weight bands which were relatively monomorphic. Similar results were earlier reported by ERUM *et al*. (2011) as they evaluated Pakistani *Kasurimethi Trigonella* germplasm to establish geographical indicator (GI) right for the country. A recent study (HALIEM and AL-HUQAIL, 2013) also highlighted higher level of polymorphism in fenugreek protein bands. Still larger grouping in cluster analysis among accessions of seemingly diverse ecological regions suggests intact genetic relatedness which could be the result of hybridization or common ancestor. Like phenotypic resemblance, generally similar banding pattern was exhibited by accessions from same country or countries with geographical proximity hence placed to gather in dendrogram. Indian, Iranian and Turkish germplasm revealed versatile banding pattern from as low as 6 bands per accession to 14 bands. Therefore, protein polymorphism can be utilized for distinguishing region specific ecotypes in a larger population set. This peptide polymorphism in some ways is induced by variable cellular response mechanism towards different eco-geographical conditions (JAN *et al.,* 2016; IBRAHIM *et al.,* 2017; KHURSHID and RABBANI, 2012; MONDINI *et al*., 2009). The technique also helps in studying genetic relatedness, variety identification, population structure analysis and evolutionary mechanism.

## CONCLUSIONS

Agro-morphological and biochemical characterization for total seed protein has revealed higher extent of genetic divergence in fenugreek regional germplasm. Accessions from Iran, India, Turkey and Ethiopia possess increased variability for flowering initiation, duration, plant height vigor and seed yield. Selection of superior genotypes and their subsequent use in breeding programs can be utilized for further crop improvement. Also, total seed protein banding pattern of large populations distinguished genotypes from diverse ecologies into different sub-groups. This divergence implicates evolution which may have been resulted by inter-crossing, gene flow or induced molecular mechanism in a response to different agro-ecological conditions. However to understand complex mechanism of inter-regional divergence, we suggest more focused advanced techniques and molecular analysis of genetic structure in global fenugreek germplasm.

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# PROCENA PROSTORNE GENETIČKE STRUKTURE U INTER-REGIONALNIM POPULACIJAMA *Trigonella foenum-graceum L.* VRSTA POMOĆU FENOTIPSKE VARIJABILNOSTI I PROTEINSKOG KOMPLEKSA SEMENA

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Izvod

*Trigonella foenum-graceum* L. je važna leguminoza koja se uglavnom uzgaja zbog farmaceutskih i nutritivnih vrednosti u regionu Mediterana, zapadne Azije, Indije i Afrike. Ispitivali smo 110 genotipovaiz različitih agro-ekoloških regiona Južne Azije, Mediterana, Srednjeg Istokat, Evrope i Afrike za fenotipsku različitost i variranje proteina semena. Značajno agro-morfološko variranje je otkriveno sa germplazma viz-a`-viz svojstvo t.j dani do početka cvetanja, dani do završetka cvetanja, prinos zrna$^{-1}$, okruženje biljke, vigor, boja cveta i visina biljke. Na osnovu PCA i Euclidian distance dobijen dendrogram rasporedio je sve genotipove u 6 i 9 različitih grupa za morfo-agronomske razlike. Četiri PCs sa Eigen vrednošću E>1 predstavlja 65% varijabilnosti germplazme. Geografska distribucija je bila evidentna sa plotom kako germplazma figuriše u 6 različitih subpopulacija. Iranska germplazma je bila najraznovrsnija, prisutna u svim subpopulacijama, sledi Indijska, Turska, Etiopijanska, Pakistanska i Egipatska koje su rangirane u 5,4,4,3 i 3 sub-populacije. Electroforetski obrazac proteina semena takođe je pokazao polimorfizam u obsegu od 30~100 kDa. Maximum od 16 traka je dobijen u Turskoj PI171872 i Indijanskim PI175321 genotipovima. UPGMA zasnovana klaster analiza rasporedila je sve genotipove u 5 gropa gde genotipovi bliski geografski su grupisani zajedno.

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