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Estimating phenotypic diversity in diverse Fenugreek (*Trigonella foenum-graceum* L.) population through morphometric analysis

Abdul Qadir¹, Niaz Ali¹, Sohail Ahmad Jan^{*2,3}, Malik Ashiq Rabbani², Aaqib Shaheen⁵, Hameed Shabab Alam¹, Naseeb Ullah¹, Aamir Numan¹, Mohib Abdullah², Haris Khurshid⁴

¹Department of Botany, Hazara University, Mansehra, Pakistan

²Plant Genetic Resources Institute (PGRI), National Agricultural Research Centre (NARC), Islamabad, Pakistan

³Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan

⁴Oilseeds Research Program, NARC, Islamabad, Pakistan

⁵National Institute for Genomics and Advanced Biotechnology, NARC, Islamabad, Pakistan

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Abstract

In order to estimate genetic variation in 167 genotypes of Fenugreek by morphological markers, an experiment was performed during 2014-2015 cropping years. The present study is useful to screened the elite genotypes among large Fenugreek population. Augmented field design with inter-row distance of 45cm and intra-row distance of 1m was kept. Range, mean, SD and CV results indicated significant diversity among the 13 important agronomic quantitative characters particularly the plant height, days to flower initiation and days to flower completion. The variances for the above characters were 175.7, 91.7, 77.7 and 24.4 respectively. The elite lines like PI543083, Trig 65, PI628790, 2736, 2720, Trig 94, Trig-42, A71000060, Methray, Methi etc. showed better agro-morphological performances as compared to other genotypes. The accessions PI-613629, PI-338679, PI-286532, PI243230 and PI-164141 gave maximum seed yield/plant and these genotypes are useful for improvement of this important plant species. Cluster analysis based on morphological traits divided all these studied accessions into four major groups. Direct relationship between yield and morphological parameters was recorded in Fenugreek germplasm. The current study may be helpful in the identification and development elite lines which can be utilized in breeding programmes for improvement of Fenugreek varieties with superior yield potential and broader genetic base.

*Corresponding Author: Sohail Ahmad Jan ✉ sjan.parc@gmail.com

Introduction

Fenugreek is an important medicinal annual herb, member of Papilionaceae family (Balodi and Rao, 1991). Among Trigonella species, Fenugreek is of tremendous commercial importance. The plant is native to Mediterranean areas of the world and grown mostly in India, Ethiopia, Morocco and Egypt (Davoud *et al.*, 2010). The seeds are used in sub-continental cooking as an element of different curry powder, in the paste and pickle preparation (Srinivasan, 2006). Although, Fenugreek seems to be barely cross-pollinated, still as its stigma becomes receptive before the anthers reach maturity (Petropoulos, 2002). This property of Fenugreek flowers can be exploited for cross-pollination at the beginning of the second stage of flower development, when the anthers are not opened but the stigma is accessible to pollination. Most of the conventional Fenugreek breeding and cultivar improvement programs running around the world are relying on this strategy to introduce desirable traits into elite germplasm (Basu *et al.*, 2014). Some of the important biotic (pest) stress, such as fungal, bacterial, viral and insect facilitated diseases have been associated with extensive dropping of fodder and seed yield in Fenugreek (Petropoulos, 2002).

The evaluation of genetic diversity is essential for breeding programs intended at crop enhancement. Analysis of agronomic and yield related traits and their impacts on seed yield or associated traits could provide insights for indirect selection of high yielding genotypes (Yimram *et al.*, 2009; Roy *et al.*, 2013). The morpho-biochemical and molecular methods are used to screen novel and improve genotypes for trait of interest (Qadir *et al.*, 2017; Jan *et al.*, 2016; Khan *et al.*, 2016). Among the agromorphological traits qualitative traits are less affected by environmental factors, unlike quantitative (Melese, 2006). Qualitative morphological parameters such as growth habit, early growth vigor, leaf colour, seed color and seed shape had high variability ranges depending on types of Fenugreek cultivars grown. The current study aimed to identify stable and superior Fenugreek accessions for important quantitative traits.

Materials and methods

Experimental materials

Morphological experiments were conducted at the field area of Genetic Resources Institute (PGRI), National Agricultural Research Center (NARC), Islamabad, Pakistan. A total of one hundred and sixty seven exotic Fenugreek diverse accessions were screened for their morphological evaluation. The fully matured seeds of these accessions were acquired from Gene bank of NARC, Islamabad, Pakistan.

Experimental method

Augmented design was used with inter row distance of 45cm and intra row distance of 1m was followed for this study. Standard agronomic practices were performed from sowing till ripening. Five randomly selected plants from each accession were used for data scoring.

The crop was not sprayed for any pesticide and also no fertilizers were used for its treatment. Recorded agronomic traits were lead following by standard Fenugreek descriptor. A total of 13 quantitative traits i.e. days to flowering initiation, days to flower completion, leaf length, leaf width, petiole length, primary branches per plant, pod length, pod width, days to maturity, plant height, number of seeds per pod, 1000 seed weight and seed yield per plant were studied.

Data analysis

Basic statistics (descriptive statistics) of the agronomic traits was carried out using Statistica 7 and Microsoft Excel (Microsoft Corporation, USA). Cluster analysis was performed on the data collected for the quantitative characters. The Euclidean dissimilarity coefficient matrices were used to calculate the relationship between the entries with a cluster analysis through NTSYS-pc, version 2.1 (Applied Biostatistics Inc., USA).

Result and discussion

In present experiment, 13 agro-morphological quantitative traits have shown significant variation among the genotypes assessed (Table 1).

Table 1. Descriptive statistics of economically important traits of Fenugreek germplasm.

Traits	Mean	Minimum	Maximum	SD	CV(%)	Variance
Days to flower initiation	89.79	68	132	9.57	10.67	91.75
Days to flower completion	106.19	85	141	8.81	8.304	77.75
Days to maturity	175.65	154	182	4.94	2.813	24.40
Leaf length	2.81	1.3	3.5	0.38	13.49	0.14
Leaf width	1.53	1.15	2.27	0.14	9.684	0.02
Primary branches/plant	8.09	3	16	2.33	28.78	5.43
Plant height	56.48	23.33	94.5	13.26	23.47	175.7
Pod length	9.99	7	13.25	1.10	11.06	1.22
Petiole length	6.50	1.5	9.06	1.16	17.94	1.36
Pod width	2.74	2	3.8	0.44	16.1	0.19
Seeds per pod	11.14	5	19.33	2.57	23.14	6.6
1000 seed weight	15.62	8.22	21.52	2.67	17.14	7.1
Seed yield per plant	9.22	4.95	2.55	3.73	40.45	13.9

The diversity and interrelationship for the studied traits among the genotypes studied is described briefly below:

Days to flowering initiation (DFI)

Number of days to flower initiation show significant variability among genotypes and ranged between 68 to 132 days. Maximum value of the DFI was 132 days was observed for genotype PI220554, while minimum value of 68 days was recorded for genotype PI46926 (Table 1, Fig. 1A). Mean value of 89.8, standard deviation (SD) of 9.6 and coefficient of variation (CV) was 10.7 for DFI. Our results were in agreement with Emrani *et al.* (2012) who noted high level of variability among all tested genotypes. Early flowering trait is of significant importance to both plant breeding as well as for practical utility on farmer's fields. Flowering stage is extremely sensitive and may be affected by extreme temperatures due to contrary effect on feasibility of pollen and pollination, which could result in poor fertilization and low seed set (Tefamichael *et al.*, 2014).

Days to flowering completion (DFC)

Days to flowering completion showed considerable diversity among the accessions and ranged from 85-141 days with a mean value of 105.2, SD of 8.8 and CV value was 8.3. Genotype PI22055 took maximum days for flowering completion while genotype

PI628790 completed the flowering in minimum time (Table 1). On the basis of class interval all 167 accessions were divided into five classes. Maximum 124 genotypes were present in group 2. While minimum 7 genotypes were present in group 4 while other have intermediate genotypes (Fig. 1B). Sadat *et al.* (2010) recorded similar results. They noted high variation for days to flowering completion. DFC have been mentioned in Atta *et al.* (2008) and Tefamichael *et al.* (2014), found significant and wide range of variation for days to flowering in Fenugreek accessions. However, not in all crops DFC varies significantly, for example in haricot beans there is hardly any variation in DFC (Zelalem, 2014).

Days to maturity (DM)

The record data for DM ranged between 154 to 183 days. The maximum DM was recorded for accession 1500 whereas minimum DM was recorded for 2720 (Table 1) with mean value was 175.7, SD was 4.9 and CV was 2.8 (Table 1). On the basis of class interval all 167 accessions were divided into five classes. Maximum 135 genotypes were present in group 4 while minimum 5 genotypes were present in group 2. The other genotypes have intermediate type (Fig. 1C). Similar results have been reported by Atta *et al.* (2008) and Tefamichael *et al.* (2014) in chickpea genotypes. The identified early maturing genotypes with reasonable yield related traits could be utilized

for development of early maturing Fenugreek cultivars and hence contribute to the promotion of Fenugreek in the region particularly during the rainy seasons.

Leaf length (LL)

Leaf length exhibited significant diversity among the diverse accessions of Fenugreek studied and varied from 1.3-3.5cm.

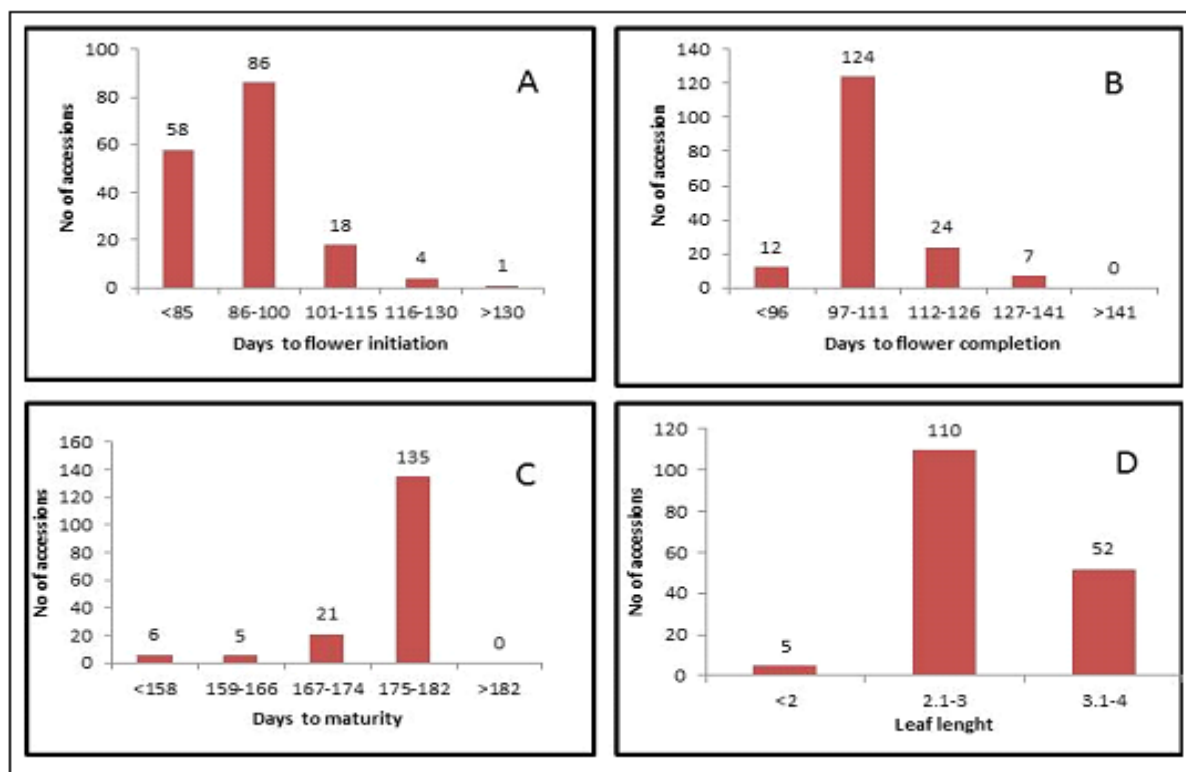


Fig. 1. Diversity in *T. foenum-graceium* Days to flower initiation (A), Days to flower completion (B), Days to maturity (C), and Leaf length (D).

The mean value for leaf length was 0.38cm and CV was 13.4 respectively. Maximum LL of 3.5cm was produced by the accession PI543083 while, Trig 119 had recorded minimum leaf length of 1.5cm (Table 1, Fig. 1D).

On the basis of class interval all 167 accessions were divided into three classes. Maximum 110 genotypes were present in group 2 While minimum 5 genotypes were present in group 1 while other have intermediate genotypes (Fig. 1D). Feven (2002) reported high variability in leaf length of Chickpea.

Leaf width (LW)

LW revealed significant variation among the genotypes studied and the LW value ranged from 1.2-2.3cm, with mean LW value of 2.74mm. Further, the SD recorded for LW was 0.149mm and CV was 9.684mm. Maximum LW value of 2.3cm was

observed for the control accession Check Methi, while accession Trig 65 recorded minimum LW of 1.2cm (Table 1). On the basis of class interval all 167 accessions were divided into four classes. Maximum 149 genotypes were present in group 2. While minimum 1 genotypes were present in group 1 while other have intermediate genotypes (Fig. 2A). Saleem *et al.* (2017) and Jan *et al.* (2017) also observed maximum variability in leaf width traits for *B. juncea* and *B. rapa* genotypes.

Petiole length (PL)

Petiole length exhibited significant diversity among the diverse accessions analyzed and varied between 1.5-9.1, with a mean value of 6.5, SD and CV values were 1.2 and 17.9 respectively (Table1). On the basis of class interval all 167 accessions were divided into three classes. Maximum 119 genotypes were present in group 2 while minimum 9 genotypes were present

in group 1 while other has intermediate genotypes (Fig.3A). Our results are similar with Saleem *et al.*

(2017) who found high variability in the petiole length of *Brassica juncea*.

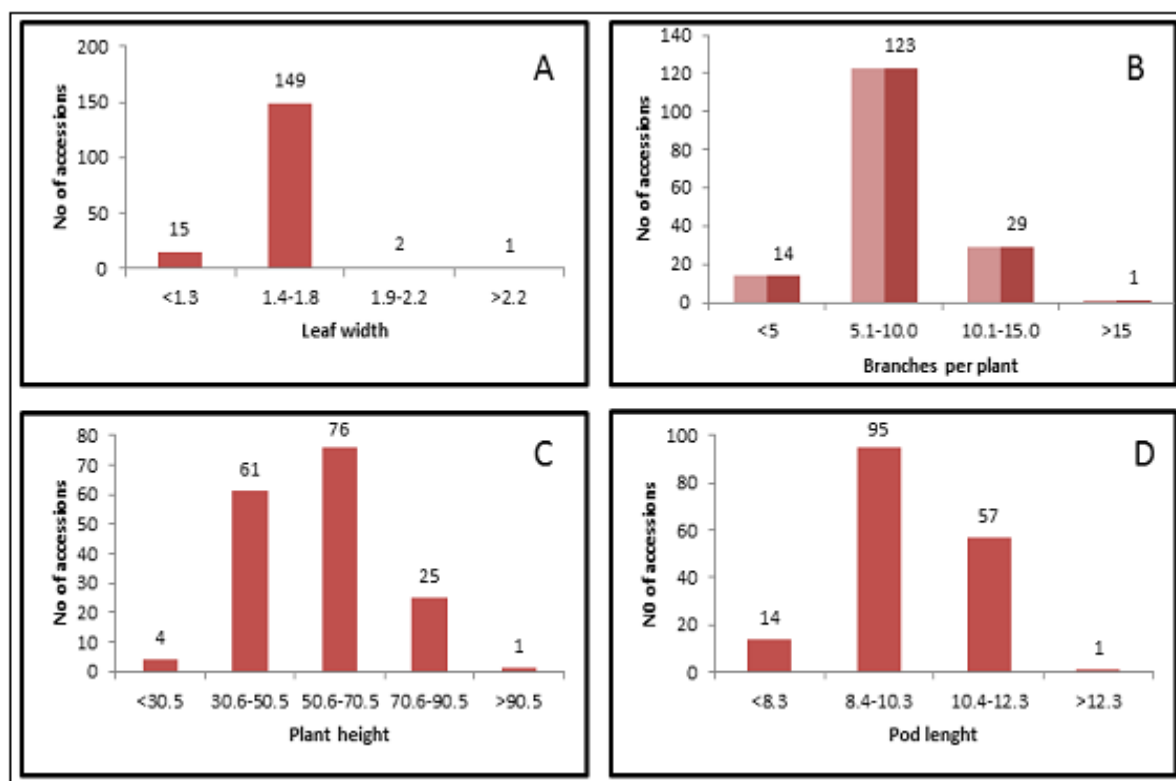


Fig. 2. Diversity in *T. foenum-graceium* leaf width (A), Branches per plant (B), plant height(C), and pod length (D).

Plant height (PH)

Plant height showed significant diversity among diverse accessions and ranged from 7-13.3. Accession PI628790 were the shortest plant height value while the control Check Methi show the tallest plant height value). Mean value was 10 cm SD was 1.1 and CV was 11.1 (Table 1). On the basis of class interval all 167 accessions were divided into five classes. Maximum 76 genotypes were present in group 3 while minimum 1 genotypes were present in group five while other have intermediate genotypes (Fig. 2C). Similar results were reported by (Emrani *et al.*, 2012; Shehzad and Farhatullah, 2012). Plant height is one of the most desirable traits in a Fenugreek varietal development. The estimated accessions revealed vast diversity with respect to plant height (Table 1). Existence of variation in PH is not a rare phenomenon in plant accessions of the same species, and similar results have been reported in a number of important crops species by Tesfamichael *et al.* (2014) in chickpea

germplasm and Yimram *et al.* (2009) in mung bean germplasm. Still, some crops accessions though of wider geographical origin (e.g. lentil) have been reported to bear little variation (Roy *et al.*, 2013). The present variations for PH from the studied genotypes can be exploited in Fenugreek breeding programs, and areas with arid climates and high winds may exploit the short stature genotypes. In the current analysis, the vast observed variations in PH may be probably attributed to the genetic differences and their response to environmental conditions of NARC, Islamabad. Earliness character is one of the major breeding objectives of Fenugreek as most farmers usually seek for early maturing cultivars in order to allow the crop to mature within the developing season and give reasonable yield. Upadhyaya *et al.* (2007) stated that early-maturity helps Fenugreek crop to avoid terminal heat and drought and increases its variation particularly in the sub-tropics.

Primary branches plant⁻¹ (PB/P)

PB/P showed considerable variability among genotypes and ranged from 3.0-16. The minimum number of primary branches per plant was recorded for the accession 2736 and whereas maximum number of primary branches for accession 223 (Table 1). Mean value was 8.09 branches where the SD and

CV values were 2.3 and 28.8 respectively (Table 1). On the basis of class interval all 167 accessions were divided into four classes. Maximum 125 genotypes were present in group 2 While minimum 1 genotypes were present in group four while other have intermediate genotypes (Fig. 2B). Results are in contrast to the findings of Khan *et al.* (2008).

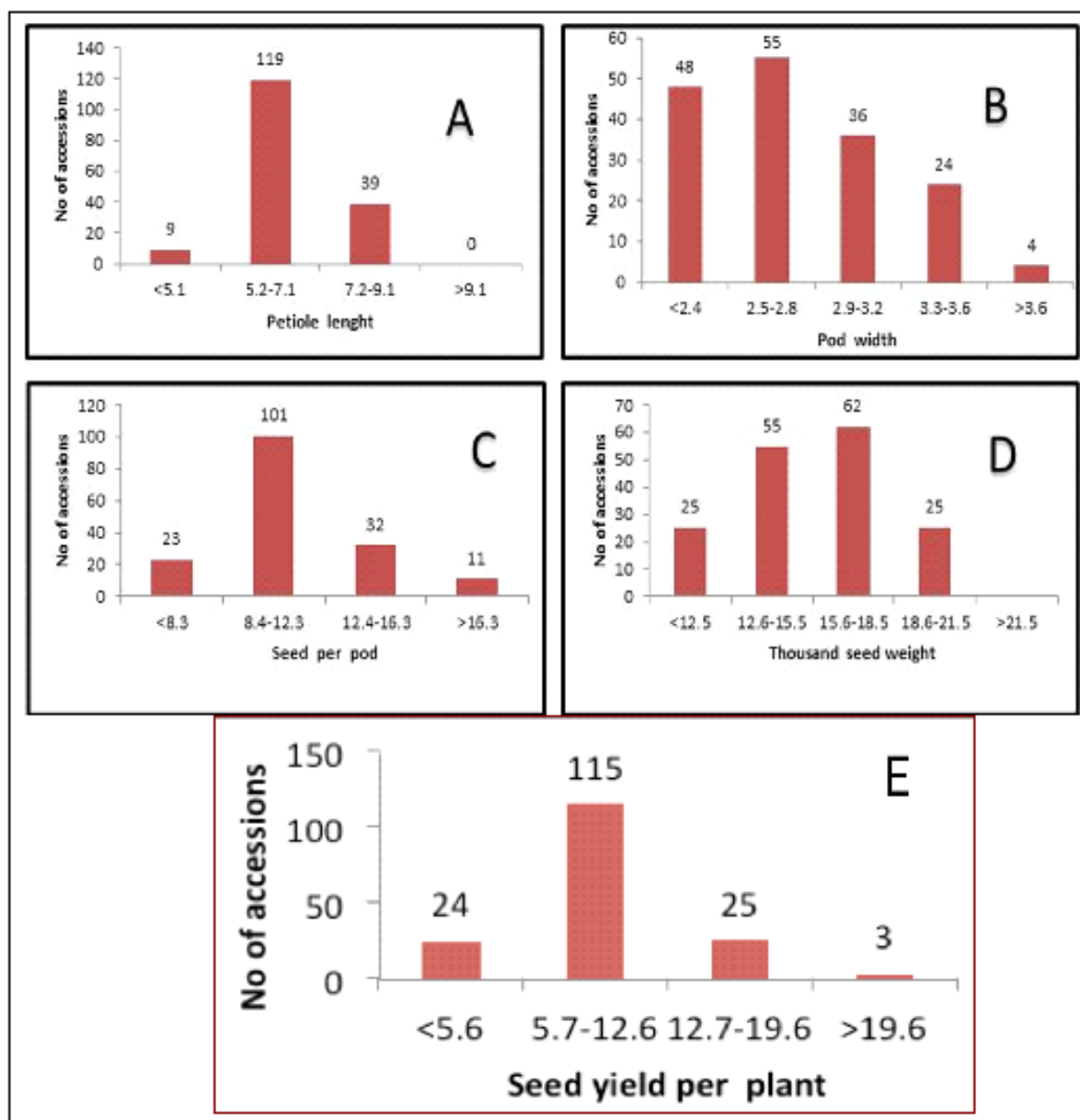


Fig. 3. Diversity in *T. foenum-graceium* petiole length (A), pod width (B), seed per pod (C), and thousand seed weight (D), seed yield per plant (E).

Seeds pod⁻¹ (S/P)

S/P showed considerable variability among accessions and exhibited a range of 5-19.3 seeds per pod. Maximum S/P were produced by the accession Trig 94 and minimum S/P were recorded for the

Accession 21596 (Table 1, Fig. 3C). On the basis of class interval all 167 accessions were divided into four classes. Maximum 101 genotypes were present in group 2 While minimum 11 genotypes were present in group four.

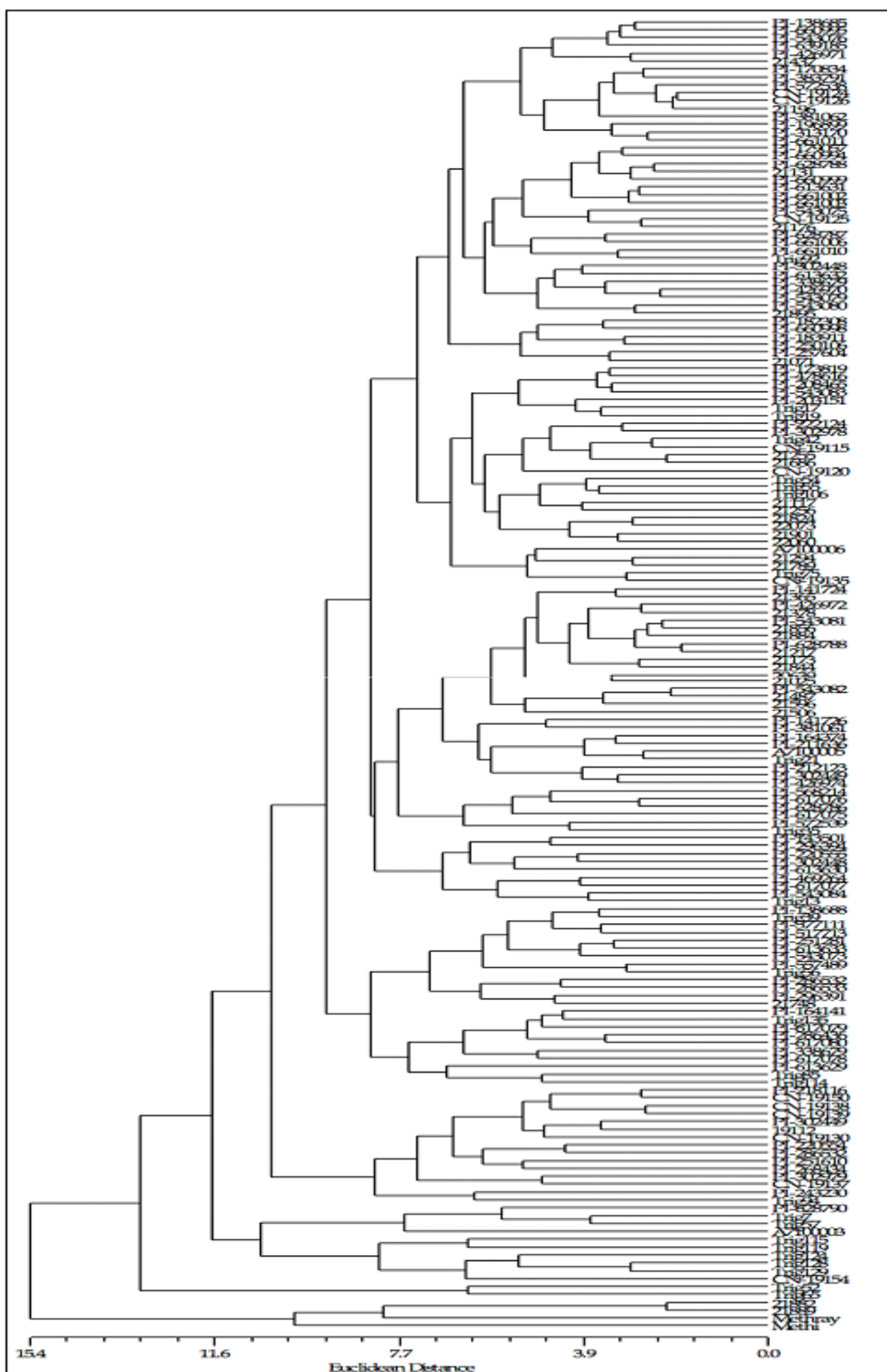


Fig. 4. Cluster analysis of the 167 Fenugreek accessions.

The other have intermediate genotypes (Fig. 2D). Emrani *et al.* (2012) reported the similar results whereas Sadat *et al.* (2010) reported opposite to the current study.

Pod length (PL)

PL showed considerable diversity among the different accession and varied from 1.5-9.1, with a mean value of 6.5, SD and CV values were 1.2 and 17.9 respectively (Table 1). On the basis of class interval all 167 accessions were divided into four classes. Maximum 95 genotypes were present in group 2 while minimum one genotypes were present in group four. The other have intermediate genotypes (Fig. 2D). Shehzad and Farhatullah (2012) also reported high heritability in F2:3 Brassica populations.

Pod width (PW)

Pod width revealed significant diversity among different genotypes and the recorded data varied from 2.0-3.8mm. Broadest pods were recorded for accession Trig-42 and thinnest pod width for accession 164141 mean value 2.7mm and SD of 0.4 and CV value recorded was 16.1 (Table 1). On the basis of class interval all 167 accessions were divided into four classes. Maximum 56 genotypes were present in group 2 while minimum 4 genotypes were present in group four. The other have intermediate genotypes (Fig. 3B). These finding are in line with Jan *et al.* (2017) and Saleem *et al.* (2017) who recorded maximum plant width Barssica genotypes.

1000-seed weight (TSW)

The record data for TSW values varied significantly and ranged from 8.2-21.5g with mean value was 15.62g, SD was 2.67 and CV was 17.14. Highest 1000 seed weight value was observed for accession A71000060, while the control Check Methray had lowest 1000 seed weight (Table 1, Fig. 3D). On the basis of class interval all 167 accessions were divided into four classes. Maximum 62 genotypes were present in group 3 While minimum 25 genotypes were present in group four. The other have intermediate genotypes. These results were in agreement with those of Singh *et al.* (2013) who described significant diversities for 1000 seed weight

in Fenugreek genotype. Further, seed shape and texture also influences the final seed weight. Thus the recorded variations for 1000 seed weight could be attributed to small seed size and undeveloped seeds. In short, the results of this study revealed considerable variation present within the Fenugreek accessions and this variation could be associated with the broad geographical collection of Fenugreek.

Seed yield per plant (SY/P)

SY/P exhibited considerable variability among the accessions and varied from 5-22.5g with mean value of 9.2, SD was 3.73 and CV was 40.45. Accession PI-613629 observed maximum seed yield per plant and genotype PI-21487 recorded in minimum SY/P. The results from this study revealed substantial variations among accessions for seed yield (Table 1). On the basis of class interval all 167 accessions were divided into four classes. Maximum 115 genotypes were present in group 2 while minimum 3 genotypes were present in group four (Fig. 3E). The existence of vast genetic diversity for seed yield showed the potential of the evaluated Fenugreek germplasm to develop high yielding accessions for definite and broad variation. Similarly previous studies have shown substantial genetic diversity for seed yield and this has been the most critical factor in genotypes selection of Fenugreek (Padmavathi *et al.*, 2013; Singh *et al.*, 2013). The most promising genotypes, for higher yield across different environments included PI-613629, PI-338679, PI-286532, PI243230 and PI-164141. These lines may be used directly as parents in obtaining potential Fenugreek varieties or can be crossed within before using them in breeding programs). This suggests that grain yield has been more influenced by environmental factors.

Cluster analysis

As shown that, there was considerable variation between accessions for morphological parameters. In this experimental study, accessions were classified into four different groups using cluster analysis based on quantitative parameters. Detailed estimate of clusters revealed that clusters were assorted within themselves and between each other based on main characters relations.

The cluster analysis executed with 13 quantitative characters divided 167 lines and 2 checks into 4 different cluster groups (Fig. 4). First cluster was the largest contained genotypes, had maximum days of flowering completion and minimum plant height. In this cluster, leaf width was 3.5cm and the length was 10.9cm, these being the smallest leaf values amongst the all germplasm. Leaf width was shorter in this cluster in contrast to other clusters, while the seed yield per plant was small in number and the highest 1000-seed weight was recorded in this cluster of Fenugreek accessions. The second cluster had characterized by high 1000-seed weight and lowest seed yield per plant and days to flowering completion). The third cluster represented genotypes had greater leaf length and leaf width as compare to other clusters. The fourth and final cluster including genotypes having longer leaf length petiole length and 1000 seed weight. Grouping of diverse genotype was not related with the geographical distribution instead Fenugreek accessions were mostly gathered due to their morphological diversity. Our results are in agreement with investigations of Gupta *et al.* (1998). This may be the association of diverse Fenugreek genotypes from one area to another. According to Baydar and Gurel (1999), a few environmental factors could also induce the gene flow among populations from various geographical sources.

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