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Characterization of agro-morphological variation in exotic fenugreek (*Trigonella foenum-graecum* L.) germplasm

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Abstract

This study investigates the relationship among morphological parameters and with seed yield for 167 fenugreek accessions including two checks (Methi and Methray) under field at (NARC) Islamabad Pakistan. Augmented field design was used. An inter row distance was kept at 45cm and intra row distance was 1m. The results revealed that there was positive and significant correlation of seed yield with leaf length, days to maturity, days to flower completion. These characters might be enhanced instantaneously and given earlier stress for indirect selection of high yielding fenugreek accessions. PCA analysis showed that the presence of genetic diversity among the valued germplasms. Four out of the 21 principal components with an eigenvalue of ≥ 1.0 accounted for 53.37% of the overall differences found among 167 genotypes of fenugreek. Contribution of the first three PCs were 20.07%, 12.29% and 11.29%, accessions indicating that these accessions may be used in breeding programs for obtaining promising lines. The presence of considerable genetic differences, and highly significant, positive interrelated parameters can be utilized in breeding programmes for development of new fenugreek cultivars.

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Introduction

A number of markers system exists and numerous other being rapidly evolving through time that can be used as implement to differentiate genetic diversity among populations. Different means of assessing genetic diversity includes those traits that are visually measurable, or based on gene product, or those relying on a DNA assay and are discussed briefly: Variations in phenotypes are due to the interaction of genes with their environment and offer the most convenient marker system to study genetic diversity. The most commonly used morphological parameters may include qualitative and quantitative traits (Arriel *et al.,* 2007).

The annual dicotyledonous plant *Trigonella foenum-graecum* L. is a member of the Fabaceae family and subfamily Papilionaceae. It is a self-pollinating crop, which bears white automatous flowers and produces golden yellow seeds visited by insects. It is now widely cultivated in northern Africa, central Europe, North America and Australia. Fenugreek is cultivated as a spice in the world where it is grown. The most important feature of fenugreek is the existence of two types of flowering shoots in the plant. The collective type produces axillary flowers and exhibits an indefinite growth habit. On the contrary, the other type known as "blind shoots" produces both axillary and terminal flowers. By and large, fenugreek flowers are cleitogamous while open type flowers are less common (Petropoulos, 2002). Fenugreek is cultivated in worldwide, and several of these regions have a long history of its use. Asia is on the top of the continents in case of fenugreek production. India is leading in fenugreek production and about 90% of the world fenugreek is grown there (Jongebloed, 2004). Asian countries like Iran, China, Israel, and Pakistan also have a significant production of fenugreek. Africa ranks second after Asia in terms of fenugreek production in both acreage as well as richness of the genetically diverse fenugreek germplasm (Petropoulos, 2002). Overall 18 different members of fenugreek (*Trigonella*) are presently known in literature. Some of the well-known members are, *T. corniculata*, T. polycerata, *T. cariensis*, *T. rigida*,, *T. suavissima, T. torulosa,T. spinosa, T.anguina T.caeruleaT.arabica T. radiata, T. platycarpos, T. hamosa, T. occulta, T. striata, T. cretica and T. arcuata* (Fazli and Hardman, 1968);Fenugreek has been mentioned as a medicinal plant in ancient herbal documents, religious scriptures and other anecdotes. It has been used as a traditional medicine referred as Indian Ayurvedic, Greek, Chinese and Arabian medicines (Sur *et al.,* 2001). A wide range of medicinal properties has been reported in fenugreek including (but not limited to) wound-healing, bust enhancement, stimulated lactation, aphrodisiac, anti-diabetic, anti-hyperthyroidism, anticancer, antioxidant, antipyretic, antimicrobial and many others (Krishnaswamy, 2008). Variation in phenotypic, biochemical as well as genotypic traits may act as a marker (De Vicente and Fulton, 2003, Jan *et al.,* 2016). These markers are very simple to score as they are based on distinct phenotypes such as flower color, plant height, seed characteristic and cost minimum of time and resources. The apparent difficulty of such markers is that in studies of genetic variability the expression of the phenotypes is highly influenced by environmental conditions. These traits often show diversity due to phenotypic plasticity (Brown, 1978). In the present study we evaluated exotic fenugreek population for agro-morphological characterization to report the extent of genetic diversity among germplasm.

Materials and methods

Field experiments were conducted during October 2014-2015 at the National Agriculture Research Center (NARC) Islamabad Pakistan. A total of 167 exotic genotypes of *T. foenum-graecum* were evaluated in the current study. These genotypes have originated from different countries and seeds of these genotypes are maintained at the gene banks of NARC (Table 1).Seeds were sown at the end of October 2014 under field area at NARC Islamabad (33° 33' N and 73° 06'E), during the year 2014-2015. The annual average rainfall in this area ranges from 500-900 mm with 70% in summer and 30% in winter that is a unique area for its bio-geographic feature following augmented design. An 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.

Plant materials

Field experiments were conducted during October 2014-2015 at the National Agriculture Research Center (NARC) Islamabad Pakistan. A total of 167 exotic genotypes of *T. foenum-graecum* were evaluated in the current study. These genotypes have originated from different countries and seeds of these genotypes are maintained at the gene banks of NARC (Table 1).

Correlation and multivariate study 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 evaluated for all tested germplasm.

Statistical analysis

The Pearson correlation and Principal Component Analysis (PCA) were carried out using Statistica 7 software at P = 0.05.

Results

Correlation study among diverse fenugreek genotypes

A total of 89 values were obtained in the correlation analysis of which 7 values were highly significant and positively correlated, and another 11 were positively correlated. Similarly, 30 were negatively correlated (Table 2). DFI revealed highly significant and positive correlation with days to flower completion (0.82), and primary branches per plant (0.2) positive correlated with days to maturity (0.16). Similarly, LW (0.15) positively correlated with PeL (0.15). DFI exhibited highly negative correlation with thousand seed weight (-0.34) and plant height (-0.41), pod length (-0.21), pod width (0.17) and leaf width (-0.15). DFC was also showed significantly positive correlation with petiole length (0.24), PB/P (0.24), DM (0.11) and S/P (0.10). Similarly, highly significant but negative correlation was observed for thousand seed weight (-0.32) with leaf width (-0.17). DM revealed highly positive and significant correlation with PH (0.27) LL (0.24), while it was highly significant but negatively correlated with 1000 seed weight (-0.06) and leaf width (-0.02).

Table 1. List of fenugreek accessions used in the current study.

S.NO	Accessions	Country	S. No	Accessions	Country
1	PI -138685	USA	85	PI-660995	USA
2	PI-138688	USA	86	PI-660998	USA
3	PI-141724	USA	87	PI-660999	USA
4	PI-141726	USA	88	PI-661002	USA
5	PI-143501	USA	89	PI-661003	USA
6	PI-164141	USA	90	PI-661006	USA
7	PI-164374	USA	91	PI-661010	USA
8	PI-170834	USA	92	PI-661011	USA
9	PI-173819	USA	93	A7100003	Australia
10	PI-179057	USA	94	A7100005	Australia
11	PI-182308	USA	95	A7100006	Australia
12	PI-183911	USA	96	Trig-7	Canada
13	PI-196899	USA	97	Trig-13	Canada
14	PI-203151	USA	98	Trig-17	Canada
15	PI-208465	USA	99	Trig-19	Canada
16	PI-211636	USA	100	Trig-21	Canada
17	PI-212123	USA	101	Trig-35	Canada
18	PI-218116	USA	102	Trig-39	Canada

19	PI-220554	USA	103	Trig-42	Canada
20	PI-220555	USA	104	Trig-52	Canada
21	PI-222124	USA	105	Trig-54	Canada
22	PI-243230	USA	106	Trig-55	Canada
23	PI-250106	USA	107	Trig-56	Canada
24	PI-251281	USA	108	Trig-57	Canada
25	PI-251610	USA	109	Trig-65	Canada
26	PI-257604	USA	110	Trig-75	Canada
27	PI-268434	USA	111	Trig-85	Canada
28	PI-286436	USA	112	Trig-92	Canada
29	PI-286532	USA	113	Trig-94	Canada
30	PI-286532	USA	114	Trig-106	Canada
31	PI-286533	USA	115	Trig-114	Canada
32	PI-296391	USA	116	Trig-115	Canada
33	PI-296394	USA	117	Trig-119	Canada
34	PI-302448	USA	118	Trig-124	Canada
35	PI-302448	USA	119	Trig-128	Canada
36	PI-302449	USA	120	Trig-129	Canada
37	PI-302449	USA	121	Trig-135	Canada
38	PI-302978	USA	122	CN-19115	Canada
39	PI-302979	USA	123	CN-19120	Canada
40	PI-313170	USA	124	CN-19124	Canada
41	PI-338679	USA	125	CN-19125	Canada
42	PI-381061	USA	126	CN-19126	Canada
43	PI-381062	USA	127	CN-19130	Canada
44	PI-383791	USA	128	CN-19135	Canada
45	PI-383791	USA	129	CN-19137	Canada
46	PI-426970	USA	130	CN-19138	Canada
47	PI-426971	USA	131	CN-19139	Canada
48	PI-466972	USA	132	CN-19150	Canada
49	PI-426974	USA	133	CN-19154	Australia
50	PI-469264	USA	134	19112	Australia
51	PI-478616	USA	135	21882	Australia
52	PI-543073	USA	136	21889	Australia
53	PI-543075	USA	137	20639	Australia
54	PI-543076	USA	138	21025	Australia
55	PI-543079	USA	139	21071	Australia
56	PI-543080	USA	140	21117	Australia
57	PI-543081	USA	141	21131	Australia
58	PI-543082	USA	142	21173	Australia
59	PI-543083	USA	143	21176	Australia
60	PI-543084	USA	144	21196	Australia
61	PI-557489	USA	145	21217	Australia
62	PI-568214	USA	146	21255	Australia
63	PI-572538	USA	147	21256	Australia
64	PI-572539	USA	148	21294	Australia
65	PI-577111	USA	149	21365	Australia
66	PI-577713	USA	150	21378	Australia
67	PI-613629	USA	151	21437	Australia
68	PI-613630	USA	152	21487	Australia
69	PI-613631	USA	153	21506	Australia
70	Po-613632	USA	154	21596	Australia
71	Pi-613633	USA	155	21686	Australia
72	Pi-617075	USA	156	21748	Australia
73	PI-617076	USA	157	21789	Australia

74	PI-617077	USA	158	21824	Australia
75	PI-617078	USA	159	21844	Australia
76	PI-617079	USA	160	21856	Australia
77	PI-617080	USA	161	21884	Australia
78	PI-628786	USA	162	21895	Australia
79	PI-628787	USA	163	21901	Australia
80	PI-628788	USA	164	22060	Australia
81	PI-628789	USA	165	22073	USA
82	PI-628790	USA	166	Ch.-Methray	Pakistan
83	PI-639185	USA	167	Ch. Methi	Pakistan
84	PI -660994	USA			

The LL of all accessions was significantly associated with SY/P (0.19) and PH (0.16), while it showed a negative but significant correlation with thousand seed weight (-0.20) and PW (-0.06). Similarly, positive correlation was observed between LW and 1000 seed weight (0.20), PH (0.18) and PB/P (0.17). PH showed highly significant and positive correlation with 1000 seed weight (r= 0.19) and PL (0.16) while it was very negatively correlated with PL (-0.09), SY/P (-0.08) and S/P (-0.05). B/P were found to be negatively correlated with the PL (-0.16), PW (-0.07), S/P (-0.13), PH (-0.07) and thousand seed weight (-0.08). While PL showed highly significant and positive correlation with S/P (0.28), PW (0.19) and 1000 seed weight (0.15). Seeds per pod were very negatively related with thousand seed weight (-0.06). PL was expressively greater and associated positively with stem thickness (r = 0.33) and with shattering percentage (r = 0.17), while it showed highly negative and correlation with 1000 seed weight (-0.03) (Table 2).

Table 2. Correlation analysis of different agro-morphological traits of *T. foenum-graceium* genotypes.

Traits	DFI	DFC	DM	LL	LW	Br/P	PH	PL	PL	PW	S/P	TSW	SY/P
DFI													
DFC	** 0.82												
DM	*0.16	0.11											
LL	0.10	0.09	**0.24										
LW	-0.15	-0.17	-0.02	0.02									
Br/P	** 0.24	** 0.24	0.05	0.11	*0.17								
PH	-0.41	-0.34 **	0.27	-*0.16	*0.18	-0.07							
PL	*0.15	**0.24	0.00	-0.03	0.12	0.07	-0.09						
PL	-0.21	-0.12	0.07	0.02	-0.06	-0.16	*0.16	0.05					
PW	-0.17	-0.15	0.02	-0.06	0.07	-0.07	0.11	0.08	*0.15				
S/P	0.12	0.10	0.11	0.02	-0.15	-0.13	-0.05	0.02	** 0.28	-0.03			
TSW	-0.34	-0.27	-0.06	-0.20	*0.20	-0.08	*0.19	-0.01	*0.19	0.10	-0.06		
SY/P	0.00	0.09	0.13	*0.19	-0.13	0.07	-0.08	0.02	0.03	0.03	0.09	-0.03	1.00

*: significant at 0.05, **: significant at 0.01

PCA analysis of diverse fenugreek germplasm

Generally PC analysis, often conducted to constructs a new set of orthogonal manage reduces and to find out the relative consequence of taxonomic diversity. In our valuations, the first PC contributed 20.07% of the overall variance of the agronomic data, the second 12.29%, the third 11.29%, the fourth 9.72%, (Table 3, Fig. 1).

PCA analysis revealed that leaf length, seed yield per plant, plant height was the most vital characters which accounted for a considerable level of phenotypic variation recorded in this fenugreek genotypes. It is suggested that work on these character would be very cooperative selection and taxonomy of elite fenugreek genotypes in Pakistan.

Table 3. Diversity among *Trigonella* accessions accounted for first four principal components.

Traits	PC1	PC2	PC3	PC4
Eigenvalue	2.61	1.60	1.47	1.26
Cumulative Eigenvalue	2.61	4.21	5.67	6.94
Percent variance	20.07	12.29	11.29	9.72
Cumulative Percent variance	20.07	32.37	43.65	53.37
		Eigen vectors		
DFI	0.89	0.01	-0.01	-0.12
DFC	0.85	0.04	0.01	-0.22
DM	0.12	0.65	-0.33	0.00
LL	0.17	0.54	-0.44	0.24
LW	-0.27	-0.22	-0.58	-0.37
Br/P	0.34	-0.13	-0.56	-0.16
PH	-0.55	0.39	-0.40	0.05
PL	0.22	-0.03	-0.06	-0.72
PL	-0.31	0.49	0.35	-0.37
PW	-0.29	0.11	0.01	-0.42
S/P	0.13	0.49	0.49	-0.16
TSW	-0.53	-0.10	0.01	-0.33
SY/P	0.15	0.40	-0.01	0.04

Discussion

Crop genetic resources maintained at PGRI, Islamabad including that of fenugreek germplasm represents vital resources of genetic diversity within the country. Conservation of these resources are imperative, however it poses a challenge to plant breeders as the number of accessions maintained at this resource are rising tremendously. Success stories in all crop breeding and improvement programs including that of fenugreek heavily rely on identification of useful genetic diversity within the accessions (Khan *et al.,* 2011; Ali *et al.,* 2016).

Knowledge of genetic variations among genotypes and relationships of economic characters would assist fenugreek breeders to develop appropriate breeding plans and to develop the most adaptive and productive cultivars. As much as the precise identification of vital resources is important, equally important is the elimination of duplicated or multireplicated accessions from gene banks. 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).

Here in our analysis, almost all of the 21 agro morphological traits have shown significant variation among the genotypes assessed. The diversity and interrelationship for the studied traits among the genotypes studied is described briefly below:

Some of the most important traits of varietal development such as PH showed positive and highly significantly correlation ($p \leq 0.01$) with 1000 seed weight (r=0.19**), pods length (r=0.16 **). Thus these traits must be taken into consideration in fenugreek breeding and cultivar improvement programs. Similar associations of PH with a number of agronomic ally important traits such as seeds per pods, 100 seed weight have been well documented (Basalma, 2008; Azadgoleh *et al.,* 2009). On the other hand, correlations between days to flower initiation and days to flower completion were positive and highly significant ($p \leq 0.01$).

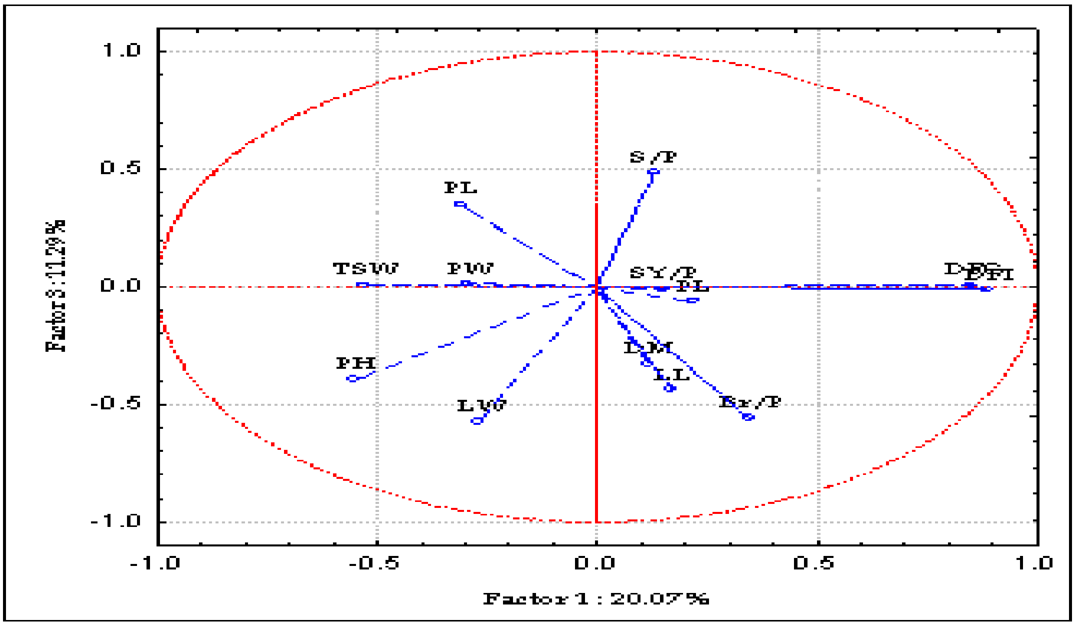

Fig. 1. Contribution of agronomic and morphological parameters in PC1 and PC3 during the present study.

Negative significant correlation of days to flowering completion with plant height had negative non-significant correlation with seed per pod is in contrast to the findings of Shinwari *et al.* (2013). Non-significant correlations have been found for seed pod-1 with 100 seed weight, primary branches plant-1, plant height, seed pod-1 and pod length (Emrani *et al.,* 2012).

Nonetheless, all genotypes are indicating to strong genotype x environment interaction and some of this diversity and correlation anomalies may be credited to the genetic and agro-ecological environments under which the accessions were assessed here. The principal components analysis revealed that the first three principal components with eigenvalue greater than one explained 53.37% of the total variation among accessions analyzed here. The PC was associated with DF, DFC, PB/P, PH, PL, LL and SY/P as positive contributors. The first principal component accounted for the highest variability in the data compared to other components. The second PC was positively related with the number of DM, LL, PL, S/P, PH, DFI and DFC. The results show that potential characters for breeding material selection and evaluation may be inferred from the PCA. Malik *et al.* (2014) reported that DF, DM and 100 seed weight were positively associated with 2nd principal component in chickpea. Muniraja *et al.* (2011) also reported the first principal component was associated with number of pods plant-1, seed yield, plant height and number of secondary branches plant-1 while the second PC correlated with days to flowering and days to maturity in chickpea.

Conclusion

The current study was extremely important and our results highlighted the existence of sufficient genetic diversity amount genotypes in terms of studied traits which is a pre-requisite for any crop improvement program. Seed yield was significantly correlated with leaf length, primary branches per plant, days to flower completion and days to maturity. Thus these traits in association with other must be accounted in genotype selection in *T. foenum-graecum* breeding and cultivar improvement programs.

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