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Effect of morphological trait variance on plant yield in different *Trigonella foenum-graecum* L. varieties

Annu Sindhu[1, 2], Suresh Kumar Tehlan[3], Ashok Chaudhury[1*]

[1]Plant Molecular Biology Laboratory, Department of Bio and Nano Technology, Bio and Nano Technology Centre, Guru Jambheshwar University of Science and Technology, Hisar-125 001, Haryana, India
[2]Present Address: 8/21-29, Trickey Avenue, Sydenham, Victoria, VIC-3037, Australia
[3]Department of Vegetable Sciences, CCS Haryana Agriculture University, Hisar-125004, Haryana, India

[*]Corresponding author: ashokchaudhury@hotmail.com

Abstract

Trigonella's rich biochemistry globally signifies it as a medicinal herb. India is the largest producer and exporter of *Trigonella* seeds. Its products are used on a large scale as raw material preparation of food and are also used in cosmetics and pharmaceutical industry especially for steroid hormones synthesis. Therefore, to meet global production requirements, superior trait varieties are in constant demand. In the present investigation, seeds of ninety *T. foenum-graecum* L. varieties from and around the most productive *Trigonella* belt in India were collected. The varieties procured from the States of Gujarat, Rajasthan and Haryana were employed to study nine quantitative and nine qualitative traits. These traits include plant height, pods per plant, seeds per pod, yield per plant, days to 50% flowering, days to maturity, days to field emergence, pod length, branches per plant, plant growth habit, leaf margin, leaf margin pigmentation, leaf tip, number of pods per axis, seed color, seed size, seed luster and plant category. The numerical data obtained was subjected to analysis of variance, genotypic and phenotypic variance, heritability, path coefficient analysis, genetic advance, coefficient of variance and analysis of variance, covariance, multivariate analysis and cluster analysis. Traits profoundly contributing towards yield were traced as growth habit, flowering date, branches, and pods per plant and seeds per pod. Path coefficient analysis revealed that seeds per pod had highest positive impact on yield per plant followed by pods per plant, branches per plant, plant height and days to 50% flowering. Therefore, it is concluded that high heritability estimates would be of great significance for breeding programs to attain superior varieties on the basis of phenotypic performance.

Keywords: Breeding; Diversity; Heritability; Variety; Selection; Trait Analysis.

Introduction

Genus *Trigonella* is one of the largest genera of the third largest family of flowering plants *Fabaceae* and sub-family *Papillionaceae* (Marzouk and Bakatoushi, 2011). It is dicotyledonous strongly aromatic herb locally known as Methi in Hindi. *Trignella foenum-graecum* is a leguminous crop with autogamous white flowers. Plants are robust, erect, strongly scented annual herbs with long compound leaves possessing trifoliate leaflets and large petiole having stipule at base. Favorable seed sowing season varies from mid-October to mid-November. *Trigonella* requires well drained, good soil of medium texture with tolerable pH range of 5.3-8.2. Fruit is a legume which is long, pointed and narrow containing 10-20 seeds in it. Root bear nodules and seeds are rhomboid, golden yellow colored with a unique hook like groove. India is well known as the "Spice Bowl of the World. India is the largest producer and exporter of *Trigonella* seeds, with extensive cultivation in tropical and subtropical regions of the country (Kumar et al., 2012). India proudly contributes 47 percent in quantity and 40 percent in value of the total spice production of the world (Krishnadas and Mundinamani, 2011). Rajasthan produces the lion's share followed by Gujarat, Uttarakhand, Uttar Pradesh, Madhya Pradesh, Maharashtra, Haryana and other States. According to the Indian National Standard Organization, out of 109 *Trigonella* species cultivated globally, 63 species are grown in India alone (Fazli and Hardman, 1971) and 99,554 ha land under cultivation during year 2011-2012 produced1, 42,949 metric ton seeds. Out of the total seed produced 21,800 ton seed was exported which generated 727.52 million INR as foreign exchange to Indian economy (Spices Board India, Ministry of Commerce and Industry, Government of India, New Delhi). Mainly exported value-added products include seeds, powder and oleoresins. *Trigonella foenum-graecum* L. is an imperative and versatile spice crop and one of the oldest medicinal herbs recognized in recorded history (Lust, 1987). The genus is native of South Eastern Europe and Western Asia and indigenous to the Eastern shores of Mediterranean Sea. The plant products are majorly consumed for forage, green manure, seed and vegetable (Rao and Sriramulu, 1977; Duke and Reed, 1981; John, 2003). Galactomannan and diosgenin are among the most imperative constituents of *Trigonella* extract accounting for its multidimensional therapeutic properties (Fazli and Hardman, 1968; Al-Habori, 1998; Mehrafarin et al., 2011; Giridhar et al., 2016). These act as raw precursor for the production of steroidal drugs and hormones which include testosterone, glucocorticoids and progesterone (Mehrafarin et al., 2011). Galactomannans are globally utilized as food and industrial thickeners (Whistler, 1993; Garti et al., 1997; McCormick et al., 2009). Leaves and seeds are consumed routinely for flavoring food items in culinary practices in Asian dishes (Basch et al., 2003; Kakani et al., 2011). Seeds were revealed to have worthy therapeutic potential against diabetes (Mowla et al., 2009; Baquer et al., 2011) and digestive disorders (Jain et al., 1987). *Trigonella* has been proved very effective against skin disorders such as inflammation, acne, burns and skin-mycosis. It not only suppresses the growth of pathogenic microorganisms (Pandey et al., 1993; Devi et al., 1997), but it also acts as an efficient nitrogen fixer (Nutman, 1976; Desperrier et al., 1986). What is more, it accounts for array of therapeutic and medicinal assets. It is proved to be anti-cancerous (Amina et al., 2005; Alshatwi et al., 2013), anti-ulcer and gastro-protective (Thirunavukkarasua and Anuradha, 2007; Helmy, 2011), antioxidant (Bukhari et al., 2008; Acharya et al., 2011), anti-diabetic and cholesterol lowering (Kumar et al., 2005; Vijayakumar and Bhat, 2008), anti-hyperthyroidism (Tahiliani and Kar, 2003a), effective against thyroxine-induced hyperglycaemia (Tahiliani and Kar, 2003b; Parmar and Kar, 2007), effective against hepatoxicity, apoptosis and nephrotoxicity (Kaviarasan and Anuradha, 2007; Sushma and Devasena, 2010). *Trigonella* also possess antinociceptive (Laroubi et al., 2009), antimicrobial (Dash et al., 2011; Nandagopal et al., 2012), anti-fertility, anti-androgenic (Kassema et al., 2006; Priya et al 2012), anti-inflammatory, antipyretic actions (Ahmadiani et al., 2001; Kawabata et al., 2011). Industrial applications use its seed for synthesizing dye and extracting alkaloids and steroids. Genetic diversity analysis in medicinal therapist *Trigonella foenum-graecum* L. belonging to North Indian States using DNA based molecular markers such as RAPD and SSR was recently reported from laboratory by Sindhu et al. (2017). The active constituents of *Trigonella* includes galactomanans (20%), hemicellulose (23.6%), cellulose (8.9%), lipids (7.9%), soluble dietary fibre gum (19%), lysine (2.4%), trigonellin (0.13%), choline (0.05%), gentianine, carpaine, yellow colouring materials, trigocoumarin, trigomethylcoumarin, piperidine, tannins, cyanogens glycosides, volatile oil (<0.02%), trigofoenosides (A-D, F and G) (methanolic extract), 7-glucuronides: apigenin, luteolin, chrysoeriol, 3-robinobioside; 3, 7- diglucoside: kaempferol, quercetin-3, 7-diglucoside, 4, 7-dihydroxyflavone, 3, 4, 7-trihydroxyflavone, formononetin, daidzein, 3 isomeric (2S, 3R, 4R, 2S, 3R, 4S-, 2S, 3S, 4R-)-4-hydroxyisoleucine ketones, 4-hydroxyisoleucyl-4-hydroxyisoleucine lactone, C14-dipeptide (Sanyal, 1984; Max, 1992). In addition to seeds, *Trigonella* leaves also own significant nutritional value with great physiological properties. Incorporation of *Trigonella* seeds in diet effectively reduces the probable UV-A and UV-B mediated radiation damage to skin cells and also helps reducing air pollution sickness. Lipids, cellulose starch, calcium, iron, β-carotene and ascorbic acid are the constituents having imperative applications as functional foods. Value-added products of the herb including seeds, powder and oleoresins are exported to Europe, North America, South Africa and other Asian countries. Agriculture and civilization have progressed simultaneously along with the history of development of new varieties. Sustained development of improved varieties is a cornerstone for increasing crop productivity. Investment in other agricultural practices would be of least significance unless the farmers have improved varieties. Keeping in mind the enormous potential and demand for this spice crop, systematic characterization effort has been made to catalogue the morphological diversity existing among ninety *Trigonella* varieties from North Indian States. Quantitative analysis revealed the chief traits associated with and affecting seed yield and thus highlighted commercially productive varieties. Genotypic selection on the basis of identified highly heritable characters could assist in the selection of most suitable varieties required by plant breeders and commercial producers. These varieties can be effectively utilized in future breeding programs to improve the quality and quantity of production or can be directly used for commercial purposes.

Results

Analysis of variance

The nine different traits analyzed showed significant differences ($P<0.01$) among the varieties. Mean sum of squares for all varieties revealed that substantial variation is present among the varieties (Table 1). Similarly, mean, range, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (broad sense) and expected genetic advance as percentage of mean for all characters (Table 2) revealed the presence of sufficient range of variation among varieties for each trait.

Coefficient of variation

Phenotypic coefficient of variation was found more than the genetic coefficient of variation (Table 2). Yield per plant exhibited highest PCV (18.04) and GCV (17.86) estimates followed by days to field emergence, pods per plant, seeds per pod, plant height and branches per plant. High values of PCV and GCV for these traits suggests the wide range of variability present among the set of varieties used for present investigation. Lowest PCV and GCV was observed for days to 50% flowering (4.91 and 4.68, respectively) followed by days to maturity (5.73 and 5.67).

Heritability estimates and coefficients of variation

Yield per plant was found to have the maximum genetic advance as % of mean (36.45%) while days to 50% flowering had lowest (9.18%) value (Table 2). High heritability (broad sense) values for the traits reflect that these traits could possibly respond effectively to phenotypic selection. High estimates of heritability as well as genetic advance were also observed for pods per plant, yield per plant, plant height and seeds per pods. High heritability was reported for days to 50% flowering and days to maturity (90.8% and 97.8%, respectively) even though a low genetic advance of 9.18% 11.54%, respectively were obtained for these traits as shown in Table 2. The traits with high heritability and genetic advance had moderate to high GCV and PCV estimates. Low GCV and PCV estimate with high heritability value was reported for days to 50% flowering and days to maturity (Table 3). The trait having high heritability and genetic advance put forth the ample scope of improvement of these traits through simple selection procedures.

Correlation coefficient

Association at phenotypic and genotypic level among different traits was analyzed using correlation coefficient analysis. Pod

Table 1. Analysis of variance for various traits in 90 Trigonella varieties.

Mean sum of squares										
Source of variatior	D.F.	Plant height	Pods pe plant	Seeds Per pod	Yield per plant	Days to 50% flowering	Days to matur	Days to field emergence	Pod length	Branches per plant
Replications	2	26	28.82	4.37	1.05	139.83	42.87	0.25	1.78	1.87
Varieties	89	356.41	596.72	2.04	17.12	28.06	142.42	2	4.66	0.88
Error	178	1.36	0.87	0.02	0.11	0.91	1.07	0.14	0.06	0.02

Where D.F. denotes Degree of freedom

Table 2. Mean, range, phenotypic and genotypic coefficient of variation, heritability and genetic advance for various traits in 90 Trigonella varieties.

Characters	Mean ± SE (m)	Range	GCV	PCV	Heritability (%)	Genetic advance (% mean)
Plant height	114.14 ± 0.67	84.8-138.6	9.538	9.59	98.9	19.52
Pods per plant	117.84 ± 0.54	84.4-160	11.968	11.99	99.6	24.58
Seeds per pod	7.97 ± 0.08	5.4-11.2	10.28	10.44	96.9	20.84
Yield per plant	13.33 ± 0.19	7.6-18.6	17.86*	18.04*	98.1	36.45**
Days to 50% flowering	64.31 ± 0.55	50-76	4.68*	4.91**	90.8	9.18*
Days to maturity	121.15 ± 0.60	110-140	5.67*	5.73*	97.8	11.54*
Days to field emergence	5.29 ± 0.22	4-9	14.88	16.52	81.1	27.59
Pod length	16.65 ± 0.15	13.4-20.6	7.44	7.59	96	15.01
Branches per plant	6.03 ± 0.08	5-8	8.87	9.16	93.8	17.70

* = Significant at P = 5%, ** = Significant at P = 1%.

Table 3. Phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficient among 90 Trigonella varieties.

	Plant height	Pods per plant	Seeds per pod	Yield per plant	Days to 50% flowerin	Days to maturity	Days to field emergence	Pod length	Branches per plant
Plant height		0.0203	0.0494	0.1607**	-0.1877	-0.1329	-0.0887	-0.0198	-0.1371
Pods per plant	0.0196		0.2832*	0.3233*	0.0029	-0.0653	0.0547	0.0831	0.4973
Seeds per pod	0.0503	0.1869*		0.0127	-0.1631	-0.1574	-0.2508**	0.6763*	0.2337
Yield per plant	0.1640*	0.2251*	0.0170		0.0723	0.0079	0.0465	0.0183	0.0958
Days to 50% flowering	-0.1982	0.0037	-0.1704	0.0692		0.0057	0.0331	0.0149	0.0891
Days to maturity	-0.1362	-0.0657	-0.1624	-0.0419	-0.0366		0.0776	-0.1276	-0.0141
Days to field emergence	-0.1000	0.0608	-0.2786	0.0987	0.1632	0.1434		0.0153	0.0685
Pod length	-0.0202	0.0860	0.7003*	-0.1378	0.0139	-0.2146	-0.1861		0.1174
Branches per plant	-0.1430	0.5146*	0.1432*	-0.0134	0.0718	0.1232	-0.0011	0.0018	

* = Significant at P = 5%, ** = Significant at P = 1%.

Table 4. Direct (diagonal) and indirect effect of component traits on grain yield per plant of Trigonella varieties.

	Plant height	Pods per plant	Seeds per pod	Days to 50% flowering	Days to maturity	Days to field emergence	Pod length	Branches per plant	Correlation with Yield per plant
Plant height	0.1948	0.0038	0.0098	-0.0386	-0.0265	-0.0195	-0.0039	-0.0279	0.1640
Pods per plant	0.0043	0.2182*	0.1408*	0.0008	-0.0143	0.0133	0.0188	0.1123	0.3251
Seeds per pod	-0.0018	-0.0066	0.6062*	0.0060	0.0057	0.0098	-0.0248	-0.0051	0.5170
Days to 50% flowering	-0.0217	0.0004	-0.0186	0.1094	-0.0046	0.0108	-0.0151	-0.0015	0.0723
Days to maturity	-0.0055	-0.0027	-0.0066	-0.0017	0.0405	0.0066	0.0006	0.0029	0.0078*
Days to field emergence	-0.0035	0.0021	-0.0097	0.0034	0.0057	0.0356	-0.0075	0.0043	0.0465
Pod length	-0.0010	0.0043	0.0352	-0.0069	0.0007	-0.0108	0.0505	-0.0001	0.0183**
Branches per plant	-0.0015	0.0955	0.0015	-0.0001	0.0008	0.0013	0.0000	0.2117**	0.2425

* = Significant at P = 5%, ** = Significant at P = 1%

Table 5. Mean values of different clusters for 9 traits in Trigonella varieties.

	Plant height	Pods per plant	Seeds per pod	Yield per plant	Days to 50% flowering	Days to matur	Days to field emergence	Pod length	Branches per plant
Cluster I	120.88	124.07	7.50	14.17	64.26	125.92	5.000	15.755	6.558
Cluster II	112.15	123.83	7.61	13.89	64.95	116.39	5.806	16.107	6.113
Cluster III	98.50	153.75	7.80	13.38	65.25	125.17	7.333	16.183	7.433
Cluster IV	121.18	121. 6.77	7.26	13.36	64.71	117.44	5.061	15.524	5.564
Cluster V	103.49	104.98	7.21	15.01	68.43	127.31	5.929	16.300	5.671
Cluster VI	99.21	109.76	8.02	9.60	65.87	130.70	5.167	17.200	6.053
Cluster VII	118.40	129.46	8.93	14.86	64.02	122.92	4.956	17.776	6.142
Cluster VIII	113.97	108.54	8.66	11.28	64.49	117.14	4.844	17.539	5.764
Cluster IX	114.64	112.69	8.04	12.46	55.64	122.611	5.250	17.033	6.033
GM	114.14	117.84	7.97	13.33	64.313	121.152	5.285	16.649	6.033

Table 6. Intra (diagonal) and inter-cluster average D^2 (bold) and D values among 90 Trigonella varieties.

Clusters	Number of varieties	Variety code	D^2
I	11	H1, H2, H4, G7, G8, G10, G20, G28, R7, R9, R12	10.54
II	18	H6, H9, H19, H21, H22, G3, G5, G6, G11, G12, G13, G15, G16, G17, G23, G25, R16, R30	8.05
III	2	H10, R23	33.45
IV	11	H20, H24, H27, H28, H29, G1, G2, G19, G21, G22, R5	6.14
V	7	H15, R1, R2, R3, R4, R6, R14	13.30
VI	5	R11, R13, R17, R22, R24	8.28
VII	15	H3, H8, H11, H13, H14, H23, H25, H26, H30, G9, G24, G26, G30, R28, R29	9.06
VIII	15	H5, H16, H17, G29, H18, G4, G18, G27, R8, R15, R18, R19, R20, R21, R27	9.27
IX	6	H6, H12, G14, R10, R25, R26	20.86

Table 7. Clustering of 90 varieties of Trigonella for D^2 Statistic analysis.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX
Cluster I	10.54	12.96	33.06	15.06	21.18	20.15	15.72	20.02	25.67
	3.24	3.60	5.75	3.88	4.60	4.48	3.96	4.47	5.06
Cluster II		8.05	29.43	11.90	16.89	18.79	15.12	15.29	24.94
		2.83	5.42	3.45	4.11	4.33	3.88	3.91	4.99
Cluster III			33.45	49.09	41.95	38.88	37.23	48.24	51.65
			5.78	7.00	6.47	6.23	6.10	6.94	7.18
Cluster IV				6.14	17.51	20.84	20.12	14.86	25.53
				2.81	4.18	4.56	4.48	3.85	5.05
Cluster V					13.30	18.20	25.22	23.13	37.33
					3.64	4.26	5.02	4.81	6.11
Cluster VI						8.28	20.59	15.16	28.07
						2.87	4.53	3.89	5.29
Cluster VII							9.06	14.53	24.66
							3.01	3.81	4.96
Cluster VIII								9.27	23.57
								3.04	4.85
Cluster IX									20.86
									4.56

Table 8. Diverse and superior varieties selected from clusters.

Cluster	Variety code	Character
I	H2	Pods per plant, seed per pod, yield per plant, pod length
I	G20	Pod per plant, pod length, branches per plant
I	G28	Pod per plant, seed per pod, branches per plant
I	R7	Pod per plant, yield per plant
VII	H3	Seed per pod, yield per plant, pod length
VII	H11	Pod per plant, seed per pod, yield per plant, branches per plant
VII	H25	Pod per plant, seed per pod, yield per plant, pod length, branches per plant
VII	G9	Pod per plant, seed per pod, yield per plant, pod length
VII	G30	Pod per plant, seed per pod, branches per plant
VII	R28	Pod per plant, seed per pod, branches per plant

length showed positive and significant association with seeds per pod with the highest GCC of 0.7003 and PCC of 0.6763(Table 3). Number of branches per plant was found to be positively and significantly correlated with number of pods per plant (GCC=0.5146, PCC=0.4973) and with number of seeds per pod (GCC=0.1432, PCC=0.2337). Yield per plant was in turn positively correlated with number of pods per plant (GCC=0.2251, PCC=0.3233). Pods per plant were positively associated with number of seeds per pod (GCC=0.1869, PCC=0.2832) and plant height was found to be positively associated with yield per plant (GCC=0.1640, PCC=0.1607). On the other hand days to field emergence had negative and significant correlation with number of seeds per pod (-0.2508) (Table 3). It is evident from above results that number of pods per plant exhibited positive and significant correlation with yield per plant and branches per plant. In the same way, the traits of branches per plant, pod length and pods per plant were found highly correlated with number of seeds per pod.

Path coefficient analysis: Correlation studies alone were not sufficient to make the picture of association analysis crystal clear. Therefore, assessment of real contribution of an individual trait towards grain yield per plant becomes essential. Path coefficient analysis provides a clear and more realistic picture of a complex situation that exists at correlation level. It measures the direct as well as indirect effects of one variable on the dependent variable through the other traits. The path coefficient analysis was carried out on genotypic correlations and presented in Table 4.

Direct effects: A critical perusal of path coefficient analysis revealed that seeds per pod had highest positive impact (0.6062) on yield per plant followed by pods per plant (0.2182), branches per plant (0.2117), plant height (0.1948) and days to 50% flowering (0.1094) (Table 4).

Indirect effects: It was observed that pods per plant (0.1123) had contributed to yield per plant mainly through branches per plant which in turn contributed to yield per plant largely by direct effect (0.2117). Pods per plant had indirect positive effect on yield per plant via seeds per pod (0.1408) as well as direct effect also (Table 4). Due to lowest coefficient values of days to maturity (0.0078) and pod length (0.0183) showed that these traits have minimal affect on yield per plant. The overall picture indicated that major significant positive correlations were due to the number of seeds present in each pod.

Cluster mean

Mean values of different clusters for 9 quantitative traits have been presented in Table 5. The genetic differences between the clusters were reflected by cluster mean. The cluster differed from each other for/in one or more traits. Varieties in Cluster III took maximum days for days to field emergence (7.3), had maximum number of pods per plant (153.70) and branches per plant (7.43). Cluster IV varieties had tallest plants (121.18 cm). Maximum yield (15.01) and days to maturity (130.7) was observed for cluster V varieties. Varieties in cluster II were early maturing (116.39). Late maturing varieties (130.70) were grouped in cluster VI. Varieties with longest pod length (17.77 cm) and maximum seeds per pod (8.93) were grouped to form cluster VII. In addition, this cluster also had second highest pods per plant and seed yield. Cluster VIII and cluster IX varieties were early emerging and early flowering as well (Supplementary Figure 2).

Inter and intra-cluster average D^2 values: Intra-cluster distances of 33.75 among the varieties of cluster III indicated the presence of considerable diversity present in the cluster. Varieties of cluster IV were found out to be more similar to each other as reflected by least intra-cluster distances (Table 6). The intra-cluster distances reflect the amount of diversity existing within a cluster and thus, always less than the inter-cluster distances. Inter-cluster distance was least between cluster I and II, whereas highest between cluster III and IX varieties. The next more diverse clusters were cluster III and IV followed by cluster VIII and III with inter-cluster distances of 49.09 and 48.24, respectively.

Cluster analysis

Based on relative magnitude of D^2 values, 90 varieties were grouped into 9 clusters. The distribution pattern of varieties in different clusters was presented is Table 7. Cluster II was found out to be the largest one, comprised of 18 varieties. 15 varieties each were present in cluster VII and VIII, followed by 11 varieties each in cluster I and cluster IV. Cluster III, V and IX possess two, seven and six varieties, respectively (Table 7). As per the clustering pattern obtained no clear association was traced among the varieties with respect to their eco-geographical region of distribution. All clusters consisted of varieties from different eco-geographical regions. It was found that varieties within the cluster were more closely related in terms of traits under consideration than those present in significantly distant clusters. Table 8 enlists superior and diverse varieties selected from these clusters based on their performance.

Cluster I consisted of 11 varieties collected from three eco-geographical regions. Varieties performed above average for all the traits except days to field emergence and pod length. Cluster II consisted of 18 varieties possessing plant height, seeds per pod, and days to maturity and pod length below that of average values. Cluster III consisted of two varieties, one each from Haryana and Rajasthan. Below average mean values for plant height reflected the prostrate nature of varieties with all other superior yield related traits. Cluster IV consisted of 11 varieties from all eco-geographical regions undertaken in present investigation and showed fewer days to maturity, days to field emergence, pod length, branches per plant and long plants with intermediate yield. Cluster V consisted of 7 varieties majority of which were from Rajasthan scoring highest mean values for yield per plant, seeds per pod, below mean values of plant height and maximum days to maturity and days to 50% flowering. Cluster VI grouped 5 varieties from Rajasthan. Plants were short heighted having long pods with more seeds per pod but less number of pods per plant resulting in lowest yield per plant.

Cluster VII consisted of 15 varieties, belonging to all three regions possessing second highest mean values for pods per plant and longest pods among all the clusters thus, resulting in second highest seed yield per plant. Branches per plant were also above average. Cluster VIII consisted of 15 varieties bearing moderate height with less number of pods per plant, high number of seeds per pod, pod length, moderate days to 50% flowering and branches per plant. Cluster IX consisted of 6 varieties, two from Haryana, one from Gujarat and three from Rajasthan region. The varieties were categorized by moderate plant height, branches per plant, days to days to field emergence, more number of seeds per pod but less number of pods per plant and low seed yield. Magnitude of heterosis largely depends upon the genetic diversity among parental lines. In the present study varieties of cluster IX showed maximum divergence with respect to cluster III. If the varieties possessing maximum diversity are involved in hybridization program, it is expected that more heterotic F1's and most promising segregants in the segregating generations will be produced.

Evaluation of qualitative traits

Varieties differ in their growth habit as thirty five varieties were found with erect posture. Forty four varieties categorized as semi-erect category and the rest eleven showed prostrate growth habit (Supplementary Table 2 and Supplementary Figure 3). Leaves of seventy seven varieties were deeply serrated while on thirteen varieties they were less serrated. On the basis of presence of pigmentation on leaf margin seventy six varieties were classified as margin pigmentation present and fourteen categorized as pigmentation absent. Among ninety varieties, forty four varieties had round leaf tip while rest forty six varieties had pointed leaf tip. Based on number of pods per axis, sixty eight varieties had single pod and the other twenty two varieties had double pods.

Majority of varieties (53) had yellow seed color, followed by brown (30 varieties) and few varieties had light dark brown seed color (7 varieties). Twenty five varieties were categorized as small seeded while sixty five were large seeded. Based on the seed coat appearance forty six varieties had dull seeds and forty four varieties had shiny seed luster (Supplementary Table 2 and Supplementary Figure 6 and 7). On the basis of number of leaves present on the plants the varieties were classified into more (>300) and less (100-300) leafy types. Among ninety varieties, ten varieties were leafy type while eighty varieties were categorized into seedy type.

Discussion

Ninety *Trigonella* varieties used in present investigation showed sufficient range of variation at genotypic and phenotypic levels for the traits studied and thus were found suitable for further analysis. Among yield components, highest range of variation were observed for pods per plant followed by plant height, days to maturity and days to 50% flowering in agreement with Gangopadhyay et al. (2009) and Dashora et al. (2011). Wide range of variation existing among these traits had their respective affect on seed yield of different *Trigonella* varieties and should be considered during bringing out further improvements in seed yield of these varieties. The overall estimate of phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all traits studied in respect to yield parameters (Table 2). Phenotypic coefficient of variation includes environmental components and hence, indicates the additive effect of environment on the expression of trait. The higher estimates of PCV and GCV indicated the presence of high variability for seed yield per plant followed by days to field emergence, pods per plant, seeds per pod, plant height, pods per plant and branches per plant. These estimates pointed towards the major contribution of genetic factors on the variability present among the varieties which is in agreement with the findings of Rakesh and Korla (2003), Raje et al. (2003), Banerjee and Kole (2004), Datta et al. (2005), Dashora et al. (2011), and Naik (2012). Low estimates of PCV and GCV was observed for days to maturity and days to 50% flowering supported by the findings of Fikreselassie et al. (2012). They reported high GCV for pods per plant, number of seeds per plant and pod length. Seed yield per plant showed the highest PCV value in comparison to GCV indicating less environmental influence on this trait, which was also confirmed by high heritability values. Small differences in GCV and PCV for seed yield, pod length and days to 50% flowering was also reported by Dashora et al. (2011).

The get the most reliable outlook of the degree of advance to be expected from the selection, genetic coefficient of variation should be considered with the heritability estimates (Burton, 1952). Heritability estimates in the present study were high for all the traits (Table 2). Environmental influence is relatively less on such traits as a result these can be further improved through direct selection procedures. High heritability estimates were also observed by Shukla and Sharma (1978) for seed weight by Feysal (2006) for seed yield, seeds per pod and plant height. These findings therefore strengthen the result outcome of present investigation. Traits with low GCV such as days to 50% flowering and days to maturity showed high heritability estimates indicating that heritable portion of variability in these traits is higher as observed by Dashora et al. (2011) as well.

However, Johnson et al. (1955) reported that heritability estimates along with genetic gain proved to be more significant for the selection of best individual for breeding purpose other than the heritability values alone. High heritability together with high genetic advance was indicative of additive gene effects and high heritability associated with low genetic advance was an indication of dominance and epistatic effects as comprehended by Panse (1957). Therefore, it is concluded that high heritability estimates would be of significant importance for breeding programs to attain superior varieties on the basis of phenotypic performance of quantitative traits.

The present study also reported high heritability estimates accompanied with high genetic advance as % of mean for seed yield per plant, pods per plant, plant height and seeds per pod, providing ample scope for improvement of these traits through simple selection procedures. Similar results for heritability and genetic advance have been reported for pods per plant by Rakesh and Korla (2003) and Raje et al. (2003), for seed yield and pods per plant by Dashora et al. (2011).

Correlation between traits is probably a result of linkage or pleiotropy. Correlation due to linkage can be modified through recombination, but correlation due to pleiotropy may not be easy to overcome. In later cases, the genetic improvement in yield trait is not possible without bringing improvement in yield component traits. The inclusion of various component traits in a selection scheme is obviously not practicable and under these situations, knowledge with respect to the association to various traits with seed yield would be of immense help in formulating an effective and efficient selection and screening strategy.

The correlation coefficient analysis (Table 3) between yield and its component traits indicated that number of pods per plant exhibited strong positive and significant correlation with yield per plant and branches per plant, also branches per plant, pod length and pods per plant were highly correlated with number of seeds per pod. Similarly, the association of seed yield with one or more than one trait have been reported in past by Saha and Kole (2001), Banerjee and Kole (2004), Gangopadhyay et al. (2009), Prajapati et al. (2010), Dashora et al. (2011), Soori and Nejad (2012) and Kole and Saha (2013) for pod length and seeds per pod with seed yield. Positive and significant correlation was observed between plant height and yield per plant, as supported by the findings of Sharma et al. (1990) and Dash and Kole (2000). However, Banerjee and Kole (2004) and Saha and Kole (2001) reported non-significant correlation among these traits. The existence of strong positive correlation among yield and its component traits assist in identification of suitable varieties that could be used for direct selection of high yielding genotypes.

Negative or non-significant correlation among days to maturity, days to 50% flowering and days to field emergence was reported in current investigation and by Dash and Kole (2000), Dashora et al. (2011) and Kole and Saha (2013). On the flip side it was found to be positively significant by Saha and Kole (2001). Therefore, these traits should be considered while making selection for yield improvement in *Trigonella*. Branches per plant had positive and significant correlation to number of pods per plant. This indicates that rise in number of branches per plant will lead to increase in number of pods and seeds per plant and ultimately yield per plant. The results showed that genotypic correlation of traits was higher than corresponding phenotypic ones, thereby suggesting strong inherent association among these traits. Majority of the traits were significantly and positively correlated to yield per plant indicating the scope of selection for improving seed yield of *Trigonella*.

Path coefficient analyses the direct and indirect effects of independent traits on the dependent traits by partitioning the correlation coefficient. It is apparent that many of these traits are correlated because of a mutual association, which may be positive or negative. More variables when considered in correlation tables make indirect association complex. At this point, path coefficient analysis provides an effective means of separating direct and indirect causes of association and permits critical evaluation of specific forces to produce a given correlation and measure the relative importance of each causal factor.

The below one values of direct effects indicated that inflation due to multicolinearity was minimal (Gravois and Helms, 1992). Partitioning of genotypic correlations between yield per plant and its component traits showed direct effects were in general of higher magnitude than that of indirect effects for most of traits. Number of seeds per pod registered the highest direct and positive effects followed by pods per plant, branches per plant, plant height and days to 50% flowering which reflected their relative contribution to seed yield per plant in *Trigonella.* Pods per plant showed indirect positive effect on yield per plant via seeds per pod as well as direct effect also. Pods per plant had contributed indirectly to yield per plant mainly via branches per plant. Similar results were reported by Chandra et al. (2000), Mahey et al. (2003), Ayanoglu et al. (2004), Banerjee and Kole (2004), Datta et al. (2005), Singh and Kaur (2007), Gangopadhyaya et al. (2009), Dashora et al. (2011), Naik (2012) and Soori and Nejad (2012). The results of correlation and path analysis showed the factors affecting and associated with seed yield in *T. foenum-graecum*. It is envisaged that the data presented here would assist plant breeders while selecting plants on the basis of these qualitative traits, to improve seed yield in *Trigonella.* The present study improved the understanding of many interrelated processes involving the genetic control of variation in seed yield. The results thus observed would help in providing some guidelines in selection procedures and predicting possible merits for genetic recombination and formulating model plant type for selection in segregating generations.

Based on the divergence observed, the 90 *Trigonella* varieties were grouped to form nine clusters (Table 7). All clusters except cluster VI had varieties originating from different eco-geographical regions. Varieties belonging to same eco-geographical region were included in different clusters so that geographic diversity does not necessarily correlate with genetic diversity existing among the species which was further confirmed by molecular marker analysis. Similarly, based on morphological traits Soori and Nejad (2012) divided six Iranian ecotypes in two groups on the basis of number of seeds per plant and 100 seed weight. These reports concluded that geographic diversity alone is less significant for tracing genetic divergence existing among a species. Fikreselassie et al. (2012) observed that 144 Ethiopian fenugreek accessions formed six different clusters and accessions within a cluster were from different eco-geographical regions. The clustering of varieties from different eco-geographical locations into one cluster could be attributed to frequent exchange of breeding material from one place to another and its further selection in different geographic regions could result in genetic drift. The hybridization among diverse parents is likely to produce heterotic hybrids and desirable transgressive segregants in further generations. However, caution may be exercised while selecting very divergent varieties because such crosses may not yield proportionate heterotic response. Therefore, a hybridization program may be initiated involving the varieties belonging to diverse clusters with high mean for almost all component traits.

In present investigation varieties present in cluster IX were found out to be most diverse with respect to varieties present in cluster III followed by varieties in cluster III and IV, cluster VIII and III (Table 8). Varieties present in cluster IV possess least variability among themselves. Varieties of cluster V, cluster VII and cluster I possess superior traits related to seed yield per plant, therefore hybridization between the varieties is advocated in order to achieve high yielding segregants. The diverse and superior varieties selected from these clusters could be best exploited in future breeding programs for improving yield related traits.

Based upon qualitative constellation, 11 varieties showed prostrate growth habit, majority of which were from Rajasthan. Leaf margins were observed deeply serrated in 85.5% of the varieties studied. Less serrated leaves were observed mainly in varieties collected from Gujarat. Leaf margin was found pigmented in 76 out of 90 varieties. Rounded and pointed leaf tip shape was observed with equal frequencies. 75.5% of the varieties studied had single pod (Supplementary Figure 5). According to seed color yellow, dark brown and brown seeds were observed. Yellow seeds were most predominant followed by brown seeds while dark brown seeds were observed in few varieties. Seed size was reported large among two third of the total varieties (Supplementary Table 2 and Supplementary Figure 6). Dull and shiny seed luster type was noted with equal frequencies. 88.8% of plants were seedy type bearing less number of leaves. Similar results have been reported on seed shape, seed color, seed weight, seedling pigmentation and other traits on fenugreek lines by Chauhan (2003).

Materials and methods

Germplasm collection

In total ninety *Trigonella foenum-graecum* L. varieties belong to three different states i.e. Gujarat, Rajasthan and Haryana as shown in Supplementary Table 1and Supplementary Figure1 were employed in this investigation. Seeds of the varieties were procured from Regional Spice Research Station, Rajasthan Agriculture University, Jobner, Rajasthan; SD Agricultural University, Jagudan, Gujarat and CCS Haryana Agriculture University, Hisar, Haryana. Plants were raised in University Fields at Plant Breeding Research Area, CCS HAU, Hisar, Haryana. Subsequently, different traits were analyzed and evaluated at Plant Molecular Biology Laboratory, Department of Bio and Nano Technology, Bio and Nano Technology Centre, Guru Jambheshwar University of Science and Technology, Hisar, Haryana.

Environmental condition

Hisar (29°10’ N, 74°46’ E, ele. 215.2 m) has a semi-arid climate with cold winter and hot dry summer. During the study, variation in temperature ranges from 7.3 °C to 35.8°C and relative humidity varies between 62.5 to 97.8%. Sunshine was observed for a minimum of 3.8 hour to maximum of 9.7 hour per day while 2.5 to 35.2 mm of rainfall was experienced during the whole experiment season.

Field experiment management

Varieties were sown in experimental field area by dibbling method (Chandrasekaran et al. 2010) in randomized complete block design (RCBD) with three replications and evaluated during two consecutive growing seasons first in late October 2011 to April 2012 and then in mid November

2012 to April 2013. The experimental units consists of two rows each 2 m long having 10 cm plant to plant spacing and inter row distance of 14 cm and intra row distance of 30 cm in each replication. To accommodate ninety accessions in a block approximately 68m x 2m sized plot was utilized. Irrigation applied to the experimental area to provide water supply was equivalent to average crop growing season irrigation for the region. The agricultural care and management practices were followed for raising a good field crop including weed control and fertilization are based on scientific advices and recommendations.

Data collection

The range of morphological and phonological characters was observed and data was recorded. The mean data of these plants was used for statistical analysis. Emergence of seedlings in the field was recorded by visual observations three days after sowing. At maturity data for plant height from the base of the plant to the terminal tip was measured (in cm) and branch number was assessed. Total number of pods per plant was counted. The number of days was calculated from the date of sowing to the date of opening of at least one flower in about 50% plants of the varieties. Seeds from all the pods used for recording pod length from selected plants, were counted and average number of seeds per pod was computed. Total grain weight of selected plants was weighed and averaged. Number of days to maturity was calculated from the date of sowing to the date on which pods of 50% plants for a variety dried off. Pod length was measured and average length was calculated. The numbers of pods per plant on primary branches were counted at the time of harvesting.

The growth habit or phenology of the plant was observed visually and categorized as erect, semi-erect and prostrate. Based on serration intensity of the leaf margin, leaves were categorized into deeply serrated and less serrated. The leaf margins were observed visually for the presence or absence of pigmentation. The varieties were categorized into pointed and rounded leaf tip based on leaf tip shape. The number of pods per axis at the time of harvest varied from one to two. Seed color of different varieties was perceived as yellow, dark brown and brown. The seed size was assessed visually and differentiated into small and large. Depending upon luster, the seeds were classified into dull and shiny types. Based on leaf richness plants were categorized as leafy and seedy type.

Data analysis

Numerical values for the all traits from two seasons were recorded. The mean value for each replication was subjected to statistical analysis. The analysis of variance (ANOVA) was performed to figure out the significance variation existing among different traits under study in present set of varieties using OPSTAT software (http://14.139.232.166/opstat/default.asp). Computation of genetic parameters such as phenotypic and genotypic coefficient of variation, broad sense heritability, expected genetic advance at 5 per cent selection intensity, correlation coefficient and path coefficient following the standard statistical methods utilizing the Agriculture Module of Indostat software (http://www.indostat.org/agriculture.html). The data for different characters studied were analyzed following randomized complete block design model. Path coefficient analysis revealed the direct and indirect effects of trait on seed yield. From analysis of variance and covariance, the error variance and covariance values were subjected to multivariate analysis. The original correlated variables were first transformed to uncorrelated one's and then D^2 values were worked out through Mahalanobis's D^2 statics (1936). Constellation of varieties into clusters was done following Tocher's method (Rao, 1952) based on relative magnitude of D^2 values.

Conclusion

This paper illustrates the determination of key traits and their mutual association with respect to seed yield trait in *Trigonella* varieties through combining phenotypic and genotypic correlations, heritability and genetic advance, thus, providing plant breeders ample scope for desired traits selection in future crop improvement programs and commercial production of *T. foenum-graecum*. Also, it a concluded that parental selection should be based on systematic study of genetic diversity as geographical diversity could not be directly correlated to the genetic diversity present among the population. Qualitative assessment of data assisted identification of superior varieties. The superior and diverse traits identified from the variability studies carried out on field data of most productive North Indian *Trigonella* could be utilized as the basis of future breeding programs in other parts of the world with similar environmental conditions.

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