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DIVERSITY ANALYSIS OF FABA BEAN (*VICIA FABA* L.) GERMPLASM OF BIHAR USING AGRO-MORPHOLOGICAL CHARACTERISTICS

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Abstract

Investigation was carried out to know the extent and pattern of the genetic variability present in faba bean (*Vicia faba* L.) germplasm collected from Bihar. In order to understand and establishing the relationship among 71 accessions, agronomical and morphological characterization and evaluation was carried out. A set of 20 traits (13 quantitative and seven qualitative) were undertaken to determine the variability in the faba bean. Diversity index of faba bean germplasm was worked out following of Shannon-Weaver Diversity Index (SDI). Time taken to complete 50% germination ranges between 9.6 and 12.5 days. In case of yield and yield attributing trait maximum diversity was noticed for seed/pod and harvest index also supported by coefficient of variance 24.4 and 28.9 per cent, respectively. Over all maximum amount of diversity was noticed in qualitative traits (seed shape, seed coat color and cotyledon colour) having >30% CV, reasonable amount was noticed in protein and starch contents. Location-wise Samastipur germplasm got maximum variability. Results on frequency distribution indicated that most of germplasm falls under medium category for maximum traits. Faba bean productivity can be scaled up further through effective selection of elite germplasm or lines as well as through cross breeding with its close relatives, which exists in cultivated, wild or weedy form.

Introduction

Faba bean (*Vicia faba* L.) being among the most ancient plants in cultivation and easiest to grow (Singh *et al*. 2013b). Faba bean is a native of North Africa and southwest Asia (Harlan 1974). It can be grown in soils having pH ranging anything between 5.5 and 9.0 (Singh et *al*. 2010). In India it is still treated as a minor legume/unutilized/underutilized crop (Singh *et al*. 2012a).

It is mainly *rabi* pulses/legume in plains of India, though it is successfully grown during *kharif* (rainy season) in hilly and mountainous regions (Singh and Bhatt 2012). This crop can be taken as sole crop and as intercropped/mixed crops with variety of combination even as border/ guard crop especially in Bihar and eastern Uttar Pradesh of India (Singh *et al*. 2012b).

It has tremendous genetic potential to develop resistance against biotic and abiotic stress. Breeding varieties with tannin-free seeds and more recently, with low *vicine-convicine* content, offers new perspectives for enriching nutritional aspects (Singh *et al*. 2014). There is a need to diversify the gene pool base of this crop by various means and sources and exploration for collection of germplasm is quickest and sometime easiest method of enriching genetic resources of any crop (Singh and Bhatt 2012). Collection for introduction through exploration, collection and exchange of germplasm is one of the major sources for broadening genetic base, though collection of germplasm is the quickest method (Singh *et al*. 2013b, Verma *et al*. 2015). Characterization and evaluation of germplasm (71 accessions) collected from Bihar was carried out with an objective to enhance the understanding on extent and pattern of diversity in faba bean germplasm and to establish relationships among them.

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Materials and Methods

Faba bean (*Vicia faba L.*) germplasm was collected from diverse eco-geographical regions of Bihar. The geographical areas surveyed for diversity collection are located in Samastipur, Muzaffarpur, Vaishali and Sitamadhi districts of Bihar State of India. Total 71 accessions were collected during the exploration trip (Singh and Bhatt 2012). The field experiment was conducted in an augmented block design (ABD) with released variety "Vikranta" as check, at main research farm of ICAR Research Complex for Eastern Region, Patna (Bihar), during *rabi* seasons of 2010-11 and 2011-12 for agro-morphological evaluation. This experimental design i.e. Augmented Block Designs was used due to its uniqueness as it facilitate comparison of several treatments (i.e. germplasm) with standard (i.e. check) under limited replication conditions. The existing treatments are referred as checks and are generally replicated multiple times. Vikranta was replicated five times whereas all collected germplasm were replicated twice. Individual plot size was 2.0 M × 1.0 M, which accommodated four lines of each accession in each plot; row to row distance was kept at 30 cm, whereas it was planted at 25 cm apart.

The soil of experimental site is sandy loam in texture, neutral in soil reaction (7.1 pH), having 0.67% organic carbon content, medium in fertility status (available N 244.5 kg, P 25.6 kg and exchangeable K 195 kg, sulphur 8.9 kg and zinc 1.0 kg). Agro-climatic conditions of Research Farm were conducive for faba bean cultivation. Recommended agronomic management practices were followed for growing the crops. Basal dose of nitrogen, phosphorus and potash was provided @ 25: 40: 30 kg/ha. Observations were made and data were recorded simultaneously, for different agro-morphological traits *viz.*, days to 50% germination, germination (%), plant height (cm), branch/plant, days to anthesis. At post-harvest stage data were also recorded on pod/plant, pod length (cm), seeds/pod, 100-seed weight (g), biomass yield (g/plant), seed yield (g/plant) and harvest index. At post harvest qualitative data were also recorded for the traits i.e. seed size (to know the boldness of seed (small, medium and bold); seed shape (oblong, semi spherical and semi-oval); seed coat colour (light green, pale green, light brown to copper colour); cotyledon colour (light yellow, pale yellow, brown colour). Leaf angle was recorded before the onset of flowering. Biochemical analysis for estimation of protein and starch content was also done for quality determination. All the data generated during the course of study was subjected to statistical analysis to have minimum, mean, maximum, coefficient of variance (CV), and frequency distribution. Coefficient of variance (CV) is useful in estimating diversity, high CV (%) indicate presence of more diverse germplasm as suggested (Bond 1966, Hayward and Breese 1993). Diversity index of faba bean germplasm was also worked out with help of Shannon-Weaver Diversity Index (SDI) for individual accessions, location wise as well as for total collection by using formula (Poole 1974) based upon frequency distribution.

Results and Discussions

Observation and data recorded during the course of investigation have been analyzed. Results obtained were summarized in suitable sub-head and presented in tabular form. Results of each district as well as whole germplasm has been analyzed and presented at one place to make it easy in understanding about diversity present in particular trait at one or different locations as a whole. Since data contain two different nature (traits *viz.*, quantitative and qualitative) results were presented in separate table.

Diversity in quantitative traits: Time taken (days) by faba bean plants to germinate is mainly governed by depth of seeding and size of seed (reserve food material). Maximum, minimum and mean time taken to complete 50% germination is presented in Table 1. The standard deviation (Sd) value (0.75) and CV stand for whole population was 7.6% (Table 1). Days taken to 50%

germination by faba bean germplasm suggest that good amount of variability existed on this particular trait, that can be potentially utilized for reducing overall crop duration right from initial phase of crop cycle. Germination percentage is crucial factor which can effectively reduce the cost of seed, especially in case of this crop in which 100-seed weight varies from less than 20 g to more than 100 g.

Table 1. Extent and pattern of diversity in quantitative traits in faba bean germplasm.

Traits	Min.	Max.	Mean	SD (%)	CV (%)
Days to 50% germination	9.6	12.5	11.1	0.75	6.7
Germination (%)	50.4	88.4	67.9	12.4	18.2
Pant height (cm)	63.4	94.3	78.4	6.1	7.8
Branches/plant	8.0	12.7	10.1	1.1	10.5
Days to anthesis	39.8	70.3	58.9	4.7	8.0
Days to maturity	95	118.3	102.5	4.4	4.3
Pods/ plant	29.8	65.9	43.2	7.6	17.5
Pod length (cm)	3.5	5.9	4.1	0.40	9.6
Seeds/pod	2.3	3.9	3.0	0.2	8.2
100-seed weight (g)	15.2	35.7	21.4	3.2	15.
Biomass (g/plant)	96.8	259.6	167.6	36.5	21.8
Seed yield (g/plant)	21.9	73.1	45.1	11	24.3
Harvest index	0.19	0.67	0.38	0.11	28.9

Total germination per cent is basically governed by seed quality and favorable agro-climatic conditions. Faba beans having high plasticity can accommodate in any situation to complete life cycle successfully. In general taller plants bear more pods and consequently produce more seeds per plant. This trait can be used efficiently for evolving new genotype for this crop (Bora *et al.* 1998, Hoey *et al*. 1996, Verma *et al*. 2015). Indeterminate growth habit is a major genetic feature of faba bean being a leguminous crop, which provides unique opportunity, by virtues of that plants start both the stages i.e. vegetative and reproductive simultaneously, after certain period of vegetative phase. At the onset of reproductive stage first observation was made for days taken to anthesis (flowering), the minimum and maximum and statistical mean given (Table 1). Early onset of flowering, provide extra time for economic produce (seed formation). Result revealed that day taken to mature has considerable diversity among the tested landraces/germplasm of Bihar. Present study revealed that showing existence of very good amount of diversity in this trait, which can be exploited for breeding cultivars of extra short duration types for utilization as green manure, animal feed. Days to maturity are one of the crucial traits of any crop/variety, very important for inclusion in cropping system under certain agro-climatic condition. Minimum, maximum and mean number of days taken to complete life-cycle is shown in Table 1.

This particular trait can be utilized for producing cultivars of extra short duration, for any climatic hazardous condition. Such varieties can also fit under multiple/intercropping system, improving total system productivity (Alan and Geren 2007, Bora *et al.*1998, Verma *et al*. 2015).

Minimum, maximum and overall average of number of pods per plant were recoded and presented in Table 1. Variability in this trait indicates that pod per plant is mainly influenced with genetic makeup and partially with the environmental conditions.

Among others yield determining traits *viz*., length of pod being one of yield deciding factors, contributing positively in seed yield. Other yield attributing traits shown in Table 1 indicates about the existence of considerable amount of diversity in this parameter being a functional output of polygene, having positive influence on faba bean seed yield. Seed size has considerable influence on grain per pod. Data revealed that seed per pod has good amount of range and diversity, presence of good amount of CV confirms existence of fair amount of diversity for this particular trait (Bora *et al*. 1998).

Biomass production is a indicator of ability of plant to adopt well under certain agro-climatic conditions. It is partially, true because sometime plant failed to produce seed and unable to complete its life cycle successfully. The collected germplasm has got good amount of variability in respect to biomass production. The range per plant biomass at harvest with mean value, CV and SD are given in Table 1. This result indicates that there is ample scope of improvement in this important traits having direct and positive bearing on seed yield. This trait indicates that the crop has good plasticity and having great potential to improve its performance in leap. These results are in the close conformity with the findings of Bora *et al.* 1998, Veasey *et al*. 1999. Very good amount of diversity was recorded with seed yield per plant (g). This variability was also supported by SD and CV (Table 1). Harvest index is an effective indicator of source to sink relation and translocation of photosynthesis. The range in HI was 0.19 to 0.37 with mean value of 0.28. Data presented in Table 1 show the range of HI with good amount of diversity with SD and CV. The results of present study are in conformity with earlier findings of Bora *et al.* 1998 and Veasey *et al*. 1999. It is worth mentioning here that variety Vikranta, used as check, exhibited diversity in medium range for all the quantitative parameters tested.

Diversity in qualitative traits: Diversity analysis of qualitative traits was also carryed out. In case of total seed protein content, it was within the range of 25.7 to 29.3 per cent with a mean value of 27.6% and SD and CV. Faba bean is also one of the good sources of energy, containing more than 50% carbohydrates. Perusal of data shows that there is good range in the carbohydrate percentage. Other qualitative parameters *viz.*, seed size (boldness), seed shape, cotyledon colour, seed coat colour and leaf angle was also studied. As per need to make them quantifiable they were converted in quantitative terms (Table 2). To know the boldness of seed, they were classified into three categories *viz*., small, medium and bold. Good amount of variability exited in this trait. Seed size, seed and shape were also classified into three categories, *viz.*, oblong, semi-spherical and semi-ovals to know the degree of diversity in this trait.

Table 2. Extent and pattern of diversity in qualitative traits in faba bean germplasm.

Traits	Min.	Max.	Mean	SD (%)	CV (%)
Total protein content	25.73	31.28	27.57	0.78	2.8
Carbohydrates (%)	55.92	63.6	59.90	1.70	2.8
Seed size (boldness)	1.00	3.00	1.58	0.41	25.8
Seed shape	1.00	3.00	2.01	0.66	32.8
Cotyledon colour	1.00	3.00	2.14	1.11	43.83
Seed coat colour	1.00	3.00	2.54	0.93	36.4
Leaf angle (phylotaxy) (º)	107.50	138.75	122.24	6.88	5.6

(i) Seed size (boldness of seed (1= Small, 2 = Medium and 3 = Bold). (ii) Seed shape (1 = Oblong and 2 = Semi spherical and 3 = Semi oval). (iii) Seed coat colour (1 = Light green. 2 = Pale green. 3 = Light brown to copper colour). (iv) Cotyledon colour (1 = Light yellow, 2 = Pale yellow, 3 = Brown colour) and leaf angle (phylotaxy).

Data presented in Table 2, indicate presence of considerable amount of diversity in this qualitative trait. Seed coat colour was also classified into light green, pale green and light brown to copper colour and converted into numerical value 1 to 3 and there is presence of good amount of variability and diversity in this trait. To know the presence of diversity in cotyledon colour, it was also classified into light yellow, pale yellow and brown colour and converted into 1 to 3, good amount of variability exit in this trait. Cotyledon colour also varied.

The study was further extended to know the exact angle of attachment of leaf with stem, being a sheet of photosynthetic activity. This trait is very unique particularly for modeling to evolve new plant type with improved photosynthetic activity, efficient source-sink relation and conversion of photosynthate in efficient manner. Considerable amount of diversity in this trait has been recorded (Table 2). The check variety Vikranta, recoded diversity in all the qualitative traits as a medium.

Shannon-Weaver diversity indices (SDI): Shannon Weaver Diversity Indices (SDI) used to estimate the heterogeneity/variability present in the population under studies. The SDI was worked out to know the existence of diversity on different quantitative parameters.

Shannon-Weaver diversity indices (SDI) in quantitative traits: The data on diversity indices are encouraging; taking consideration of its nature of pollination. The corresponding values of Shannon-Weaver Diversity Index (SDI) for all parameters are ranged from 0.05 to 0.26 (Table 3). In case of yield and yield attributing trait maximum diversity was noticed in case of seed yield and harvest index. Other yield attributes also exhibit considerable amount of diversity, with SDI ranges, *viz*., pod/plant, seeds/pod and biomass production. Growth and development parameters also exhibited good amount of diversity, good amount of diversity was also recorded in case of qualitative parameters like seed coat and cotyledon colour, seed size and shape ranging in between. Samastipur collection site poses more diversity as compared to other collection site. Diversity and variability was in general present in each districts and overall collected germplasm (Bora *et al*. 1998, Verma *et al*. 2015). Similarly in case of check variety Vikranta, recoded SDI for quantitative traits was with in the medium range.

Table 3. Shannon-Weaver diversity indices (SDI) for quantitative traits in faba bean germplasm.

Traits	Samastipur	Muzaffarpur	Vaishali	Sitamadhi	Total collection
Days to 50% germination	0.21	0.17	0.16	0.19	0.19
Germination (%)	0.23	0.15	0.18	0.21	0.21
Pant height (cm)	0.16	0.15	0.17	0.25	0.17
Branches/plant	0.20	0.21	0.14	0.25	0.13
Days to anthesis	0.17	0.17	0.20	0.15	0.17
Pods/plant	0.19	0.07	0.05	0.14	0.14
Pod length (cm)	0.18	0.14	0.07	0.17	0.19
Seeds/pod	0.19	0.17	0.05	0.25	0.16
100-seed weight(g)	0.23	0.21	0.18	0.19	0.24
Biomass (g/plant)	0.21	0.23	0.19	0.22	0.21
Seed yield (g/plant)	0.24	0.19	0.18	0.21	0.23
Harvest index	0.26	0.21	0.23	0.22	0.26

Shannon-Weaver diversity indices (SDI) in qualitative traits: SDI on agro-morphological traits promises the presence of excellent amount of diversity among all the quantitative and qualitative parameters (Table 4). This might be due to genetic base evolved through the passing of

time. Being often cross-pollinated genetic base is further developed due to its nature of multiplication. This study showed that by adopting only traditional method of selection of best among available provides very limited scope for further crop improvement rapidly. On the other hand it is also providing a unique opportunity for selecting parent for distant hybridization programme. Correspondingly in case of check variety Vikranta, recoded SDI for qualitative traits it was recorded within the range.

Table 4. Shannon-Weaver diversity indices (SDI) for qualitative traits in faba bean germplasm.

Traits	Samastipur	Muzaffarpur	Vaishali	Sitamadhi	Total collection
Total protein content	0.23	0.18	0.23	0.17	0.21
Starch (%)	0.18	0.22	0.17	0.24	0.22
Seed size	0.19	0.15	0.18	0.25	0.17
Seed shape	0.13	0.16	0.09	0.18	0.13
Seed coat colour	0.19	0.15	0.11	0.19	0.19
Cotyledon colour	0.20	0.17	0.16	0.19	0.21
Leaf angle (phylotaxy) **(º)**	0.18	0.11	0.18	0.17	0.14

Frequency distribution: Frequency distribution for all the germplasms was worked out to cluster different traits into three major categories. The basic idea behind this exercise is to know hetero- and homogeneity based on pre decided yardsticks.

Frequency distribution in quantitative traits: Majority of accessions came under medium germination percentage and only 7.1% come under (<50%) low germination percentage. In respect of days taken to complete 50% germination, total germplasm fall under medium category. In case of plant height majority of accession were categorized under medium and a small percentage of faba bean germplasm comes under tall categories and remaining group in the small (Table 5).

Majority of accession took medium duration for anthesis. Number of seeds per pod also had same fashion, i.e., under medium group. Good population falls in medium group, however fair amount of accessions comes under large as well as lower as per frequency distribution pattern. Majority of accessions for banching per plant, pod length, seed yield , biomass yield and harvest index had fallen under minimum to medium category (Table 5), which once again revealed that there is still scope for further improvement through selection (Veasey *et al*. 1999). Intraspecific hybridization with its close relatives is also another aspect of crop improvement.

Frequency distribution in qualitative traits: Results for frequency distribution in qualitative traits have been summarized in Table 6. In case of protein content, majority of collection falls under low category. In case of starch content majority of germplasm falls under medium starch group. Similar pattern was noticed in case of seed size (Table 6). In case of seed shape, seed coat colour and cotyledon colour majority of faba bean accession group as oblong, light green and light yellow respectively. Leaf orientation was recorded as leaf angle and majority was medium.

Faba bean germplasm collected and evaluated has exhibited considerable amount of variability in one or other traits. Diversity present in seed yield per plant confirms that there is a great scope for improving seed yield/plant and per unit area.

Table 5. Frequency distribution for different quantitative traits in faba bean.

Traits	Frequency category	Frequency distribution (%)				
		Samastipur	Muzaffarpur	Vaishali	Sitamadhi	Total collection
Days to 50% germination	Minimum(<10 days)	0	0	0	0	0
	Medium (10-15 days	24	25	21	1	71
	Maximum (>15 days)	0	0	0	0	0
Germination (%)	Low (<50%)	03	02	0	0	05
	Medium (50-75%)	12	17	16	01	46
	High (>75%)	09	06	05	0	20
Pant height (cm)	Small(<70 cm)	10	09	02	0	21
	Medium (70-90 cm)	13	16	17	01	47
	Tall (>90 cm)	01	0	02	0	03
Branch/ plant	Minimum (<8.5)	01	02	01	0	04
	Medium (8.5-11)	14	20	19	01	54
	Maximum (>11)	09	03	01	0	13
Days to anthesis	Early (<50 days)	04	03	06	01	14
	Medium (50-65 days)	18	20	15	0	53
	Late (>65 days)	02	02	0	0	04
Days to maturity	Early (<100 days)	09	11	07	0	27
	Medium (100-110 days)	10	12	09	01	32
	Late (>110 days)	05	02	05	0	12
Pods/plant	Low (<30)	0	0	01	01	02
	Medium (30-50)	17	20	18	0	55
	High (>50)	07	05	02	0	14
Pod length (cm)	Small (<4 cm)	13	08	09	0	30
	Medium (4-5 cm)	10	16	12	0	39
	Long (>5 cm)	01	01	0	01	02
Seeds/pod	Minimum (<2.7)	02	01	01	0	04
	Medium (2.7-3.1)	10	22	16	0	49
	Maximum (>3.1)	12	02	04	01	19
100-seed weight (g)	Minimum (<25)	21	22	18	0	61
	Medium (25-35)	02	03	03	01	09
	Bold (>35)	01	0	0	0	01
Biomass (g/plant)	Low (<100)	01	0	0	0	01
	Medium (100-175)	19	20	16	01	56
	High (>175)	04	05	05	0	14
Seed yield (g/plant)	Low (<30)	02	04	0	01	07
	Medium (30-50)	22	18	19	0	59
	High (>50)	0	03	02	0	05
Harvest index	Low (<0.20)	02	03	07	0	12
	Medium (0.2-0.5)	18	20	11	01	50
	High (>0.5)	04	02	03	0	09

Table 6. Frequency distribution for different quantitative traits in faba bean.

Traits	Frequency category	Frequency distribution (%)				
		Samastipur	Muzaffarpur	Vaishali	Sitamadhi	Total collection
Total protein content (%)	Low (<26)	11	42	13	0	36
	Medium (26-29)	08	10	08	01	27
	High (>29)	05	03	0	0	08
Starch (%)	Low (<0.20)	07	05	04	0	16
	Medium (0.2-0.50)	09	11	11	0	31
	High (>50)	08	09	06	01	24
Seed size	Small (<1.5)	10	08	07	0	25
	Medium (1.5-2.5)	09	14	12	01	36
	Bold (>2.5)	05	03	02	0	10
Seed shape	Oblong (<1.3)	11	14	12	01	38
	Semi spherical (1.3-2.3)	11	09	07	0	27
	Semi oval (>2.3)	02	02	02	0	06
Seed coat colour	Light green (<1.4)	10	12	11	0	33
	Pale green (1.4-2.4)	09	10	08	0	27
	Brown to copper (>2.4)	05	03	02	01	11
Cotyledon colour	Light yellow (<1.2)	09	08	08	0	33
	Pale yellow (1.2-2.1)	11	14	09	01	27
	Brown (>2.1)	04	03	04	0	11
Leaf angle (phylotaxy) (º)	Low (<100)	0	0	0	0	0
	Medium (100-200)	12	07	10	0	29
	High (>200)	12	18	11	01	41

(i) Seed size (boldness of seed (1= Small, 2 = Medium and 3 = Bold). (ii) Seed shape (1 = Oblong and 2 = Semi spherical and 3 = Semi oval). (iii) Seed coat colour (1 = Light green, 2 = Pale green, 3 = Light brown to Copper colour) and (iv) Cotyledon colour (1 = Light yellow, 2 = Pale yellow, 3 = Brown colour) and leaf angle (phylotaxy).

Characterization and evaluation provide unique tool to gauge potentiality present in the particular gene pool/germplasm. Faba bean is often indeterminate in growth habit; provides ideal scope for crop improvement. It was found that productivity of faba bean can further scaled through effective selection of elite germplasm for numerous traits at breeders' will. Overall crop duration can be reduced, right from time consumed for germination to series of phonological stages specially time took to anthesis and finally maturity. Similarly one can easily select germplasm tagged for high seed yield, protein yield etc.

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