

## Genetic Diversity and Nutritional Components Evaluation of Bangladeshi Germplasms of Kidney Bean (*Phaseolus vulgaris* L.)

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### ABSTRACT

Considering the crucial focus on plant developments as high yielding, protein, and disease-resistant varieties, in this study, the genetic diversity and nutritional traits of available kidney bean germplasms found in Bangladesh have been evaluated based on seventeen quantitative and six nutritional traits. Analysis of genotypic, phenotypic variance and covariance showed that higher environmental influence was found in 1000-seed weight and seed yield/plant than other characters. High heritability was observed in dry weight (94.59%), leaf area (96.83%), days to 5 leaves stage (88.80%), number of leaves (87.38%) and number of pods per plant (87.32%), while high genetic advance was found in leaf area (59.46), 1000-seed weight (52.80), seed yield/plant (39.89). The genotypes were grouped in four clusters by diversity ( $D^2$ ) analysis where clusters I and III consisted of seven and one genotypes, respectively. Furthermore, the highest inter and intracluster distance was found between Cluster I and Cluster III (23.742) and cluster IV (0.900) respectively. Principle component analysis revealed that days to 5-leaves stage, days to 1<sup>st</sup> flowering and days to 50% flowering were major characters contributing towards genetic diversity (74.8%) in Kidney bean. Analysis of nutritional traits showed that the germplasms of the Sylhet region contained more carbohydrate (60.24-64.03%), fiber (2.08-2.46%) and ash (2.31-2.95%), whereas the germplasms of Bandarbanhad had more protein content (23.05-23.11%) than the released varieties used as control. In addition, the genotypes of the Sylhet region e.g. G6 (2.68t/ha), G5 (2.56 t/ha) and G4 (2.49 t/ha) showed maximum seed yield/ha. The results suggest that the germplasms of Sylhet and Bandarban could serve as valuable genetic resources to breed high yielding and super quality Kidney bean varieties.

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### Introduction

Beans are intricately woven into the fabric of human diet history. The health benefits and savory taste of Kidney beans have made it sound familiar all over the world. Kidney beans are also known as French bean, haricot bean, common bean, snap bean or navy bean, are

valued for their protein-rich (23%) seeds and high mineral contents (Ali and Kushwaha, 1987). The bean is becoming more popular due to its dual-purpose as pulse and also consumed as immature tender fruits. Presently, the largest commercial producers of Kidney bean are India, China, Indonesia, Brazil and the United States (FAOSTAT, 2017). In Bangladesh, the total



production was recorded as 110116 t in 2014, where the yield of the fresh bean was recorded 6.043 t/ha and the seed production was 729 tons (FAOSTAT, 2017). According to National Nutrient Database of USDA, one standard cup of canned Kidney beans (about 150 grams) contains 0.55 g of fat, 5.66 g of carbohydrate, 2.6 g of fiber, 1.94 g of sugar, 1.42 g of protein, 17 mg of calcium, 1.2 mg of iron, 18 mg magnesium, 30 mg of phosphorus and 130 mg potassium (USDA, 2017). The presence of various immune system-boosting antioxidants e.g. flavonoids and carotenoids in the beans are well known. This power-packed legume has been shown to help manage and regulate diabetes symptoms in many patients and reduce the risk of heart diseases due to their high levels of flavonoids (Baloch and Zubair, 2010).

Kidney bean is relatively less cultivated in Bangladesh due to lack of high yielding variety and less popularity in consumers' level and in vegetable markets in Bangladesh compared to the developed countries. As a matter of fact, Kidney beans are often overlooked as a source of incredible nutrient components. However, the bean is very popular among the ethnic people living in the hilly regions of Bangladesh (Nazrul and Shahed, 2016). The ethnic people of the hilly districts of Bangladesh cultivate the bean and consume it as a source of protein and high minerals. Although some varieties of Kidney bean have been released from Bangladesh Research Institute (BARI), lack of high yielding varieties and high disease infestation e.g. the leaf curl and mosaic virus of the crop are considered as the main impediment for wide cultivation of Kidney bean in Bangladesh (Noor *et al.*, 2014). Thus, the development of kidney bean variety with high yield, high protein content and disease resistant are the main breeding focus for in Bangladesh.

In order to crop improvement, consideration of germplasm and genetic resources is of great importance. Collection, characterization, and conservation of germplasm are the vital activities in a breeding program (Upadhyaya *et al.*, 2008) The success of an efficient plant breeding program is dependent upon the existence of genetic variability and diversity because the efficiency of selection largely depends upon the magnitude and nature of genetic variability

present in the germplasm. Genetically diverse parents are likely to produce high heterotic effects and desirable sergents (Niveditha *et al.*, 2017). Mahalanobis's  $D^2$  analysis is a useful tool for identifying the best parents and their combinations for generating variability with respect to various traits (Mahalanobi, 1936). In addition, yield and its contributing characters are polygenically controlled and an environmental factor influences these traits. Hence, it is essential to partition the overall variability into its heritable and non-heritable components with the help of genetic parameters viz. genetic coefficient of variation, heritability and genetic advance (Tuppad *et al.*, 2017). Heritability is the only component that is transmitted to the next generation. The heritability value alone does not have much significance as it fails to account for the magnitude of absolute variability. It is, therefore, necessary to utilize heritability along with genetic advance, while advocating for selection (Tuppad *et al.*, 2017). In the present work, we collected the available germplasms of Kidney bean found in different growing regions of Bangladesh, then determined the genetic diversity of the collected germplasm with respect to morphological and nutritional component traits to make the best use of these genetic treasures for further improvement of yield and nutritional traits.

## Materials and Methods

### Plant materials

The available germplasms of Kidney bean in Bangladesh were collected from its growing regions directly from farmers of the hilly regions of Bangladesh and Bangladesh Agricultural Research Institute (BARI). The local entries comprised of local germplasms collected from different parts of Sylhet and Bandarban hilly districts of Bangladesh. Here, a total of eighteen germplasms of Kidney bean were collected and evaluated for morphological and nutritional traits which are listed below.

### Design and layout of the experiment

The experiment was designed in a randomized complete block design with three replications at the research farm of Sher-e-Bangla Agricultural University, Dhak-1207. The plot size was 20×10

m<sup>2</sup>. A distance of 30 cm from row to row and 15 cm from plant to plant was maintained. The genotypes were randomly distributed to each row within each line. All recommended package of intercultural practices described by Nazrul and Shahed (2016) was followed to raise a good crop.

**Table 1.** List of Kidney bean germplasm collected and characterized

Name	Kidney bean germplasm	Site of collection
G1	BARI Jharseem-1	BARI
G2	BARI Jharsheem-3	BARI
G3	Local	Sylhet
G4	Local	Sylhet
G5	Local	Sylhet
G6	Local	Sylhet
G7	Local	Sylhet
G8	Local	Sylhet
G9	BARI Jharsheem-2	BARI
G10	Advanced Line	BARI
G11	Advanced Line	BARI
GB 12	Advanced Line	BARI
GB13	Local	Bandarban
GBN14	Local	Bandarban
GBN15	Local	Bandarban
GBN16	Local	Bandarban
GBN17	Local	Bandarban
GBN18	Local	Bandarban

BARI – Bangladesh Agricultural Research Institute

### Morphological parameters recorded

The data was recorded from ten randomly selected plants per genotype per replication for seventeen morphological characters including, days to 5-leaves stages (LS), days to 1st flowering (FF), days to 50% flowering (50%F), days to maturity (M), days to 1st pod setting (FPS), plant height (cm) (PH), number of leaves (NL), number of pod/plant (NPP), leaf area (cm<sup>2</sup>) (LA), petiole length (cm) (PL), pod length (cm) (PdL), pod diameter (cm) (PdD), dry weight of pod (DWP), number of seeds/pod (NSP), 1000 seed weight (SW), seed yield/plant (g) (SY), seed yield (t/ha) (Y).

### Nutritional components analyses

For nutritional component traits, protein, fiber, fat, ash and carbohydrate and moisture content of dry bean seeds were estimated from the

grinding powder of five g of dry seeds of different Kidney bean germplasm. Quantitative determination of moisture, protein, fiber, fat, ash and carbohydrate of dry seeds (50.0 g) were done following the protocols described by the association of official analytical chemist (AOAC, 1995).

$$\text{Protein (\%)} = 5.71 \times \{[(\text{ml HCL for Sample} - \text{mL HCL for Blank}) \times N_{\text{HCl}} \times 0.014] \times 100\} \div \text{Weight of sample (mg)}$$

$$\text{Crude fiber (\%)} = 100 \times \text{Weight of the crude fiber} \div \text{Weight of sample}$$

$$\text{Fat (\%)} = 100 \times (\text{Final weight of the test tube} - \text{Initial weight of the test tube}) \div \text{Weight of sample}$$

$$\text{Ash (\%)} = 100 \times \{ \text{weight of ash (g)} \div \text{Weight of the sample (g)} \}$$

The moisture content of the bean flour sample was determined by drying at 105°C overnight in an electric oven. Carbohydrate (%) was determined using the formula: 100 - (moisture + ash + fat + protein + fiber).

### Statistical analysis

Analysis of variance (ANOVA) was performed and error variance was tested for homogeneity (Cochran and Cox, 1957). The mean values for all characters were calculated. The data were analyzed for estimation of the genotypic and phenotypic coefficient of variation (GCV and PCV) following by Burton (1953). Heritability (h<sup>2</sup>) in the broad sense (in percent) was computed by the formula given by Lush (1949) and Hanson *et al.*, (1956). The genetic advance was calculated according to the methods of Johnson *et al.*, (1955). Genetic advance (GA) was estimated following the formula suggested by Johnson *et al.*, (1955). Multivariate analysis was done by computer using GENSTAT 5.13 software through four techniques viz., Principal Component Analysis (PCA), Principal Coordinate Analysis (PCO), Cluster Analysis (CA) and Canonical Vector Analysis (CVA). The genetic diversity among the genotypes was assessed by Mahalanobi (1936) general distance (D<sup>2</sup>) statistic and its auxiliary analyses.

### Results and Discussions

#### Morphological and yield-related traits

The germplasms of kidney bean were characterized through the evaluation of evaluating 17 seventeen morphological and yield-related traits. The results were pertained to mean values of three replicated data, range, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in a broad sense ( $h^2$ ) and expected genetic advance as percent of the mean (GA) (Table 2 and 3). The morphological variations in color, shape, and size of dry seeds of different kidney bean germplasms presented in figure 1. The phenotypic appearance of dry seeds of different kidney bean germplasm was varied in color, shape, and size (Figure 1). The germplasm collected from Sylhet region appeared as various attractive color e.g. black (G3), chocolate color at the hilum (G4), brown (G5), reddish black (G6), deep brown spotted on light brown seed body (G7) and were broad shaped seed (Figure 1). Moreover, these genotypes produced more yield (2.21-2.68 t/ha) (Table 2) in comparison with the released varieties (G1 and G2) from BARI (Bangladesh Agricultural

Research Institute). Unlike these genotypes, the Sylhet local germplasm G8 which was black in color with light brown spot and produced comparatively lower yield (1.97 t/ha). The germplasms collected from Bandarban e.g. G13, G14, G15, G16, G17 and G18 were brown to deep brown in seed color. Interestingly, these genotypes were found lean and weak at the vegetative growth stage, hence produced low yield (0.80 -1.87 t/ha). The bean seed colors of advanced lines collected from BARI were purple (G10), very light brown (G11) and brown spotted red (G12). The recorded yield of advanced lines was 1.75 t/ha in G10, 1.80 t/ha in G12 and 2.00 t/ha in G11. The released variety of kidney bean G1 (BARI Jharsheem- 1) was white-colored small-sized seed exhibited yield 1.47 t/ha (Table 2). Seeds of G2 (BARI Jharsheem-3) were robust and cultivated generally for dry seed consumption. While G9 (BARI Jharsheem-2) was characterized as late variety (89 days) (Table 2) and was generally cultivated for fresh green beans for its slender pods.



**Fig. 1.** Variation in seed color, shape, and size in kidney bean germplasm.

**Table 2.** Mean (of three replications) performance of various growth parameters and yield components of kidney beans.

Genotypes	LS	FF	50%F	M	FPS	PH	NL	NPP	LA	PL	PdL	PdD	DWP	NSP	1000SW	SY(g/plant)	Y(ton/ha)
G1	13.67	34.00	36.00	79.00	38.67	36.59	14.00	21.33	158.84	8.37	11.00	8.13	1.91	4.12	277.54	66.17	1.47
G2	7.33	33.00	36.00	80.00	39.33	33.33	12.67	14.44	161.95	10.77	10.67	7.81	1.84	4.03	370.11	73.37	1.63
G3	7.33	30.00	33.67	79.33	37.67	24.17	15.33	25.67	123.72	6.70	10.30	7.33	2.17	4.60	278.00	101.07	2.25
G4	7.33	32.33	36.00	80.00	39.00	29.67	16.47	27.93	121.09	9.30	11.09	7.03	2.33	4.70	290.24	112.03	2.49
G5	7.67	30.00	34.33	80.00	37.33	30.00	18.33	27.67	169.59	9.00	11.18	8.35	2.50	5.04	283.36	115.07	2.56
G6	7.67	30.00	34.33	78.00	39.33	29.72	21.93	30.68	169.67	12.67	12.38	7.48	2.75	5.50	318.21	120.43	2.68
G7	7.67	33.67	35.33	79.67	39.33	28.50	14.73	15.49	83.80	6.93	10.47	8.12	1.92	3.44	348.97	99.47	2.21
G8	8.00	30.00	33.33	82.33	38.67	29.73	14.00	14.13	147.69	9.80	10.33	8.04	1.79	3.76	335.77	88.80	1.97
G9	12.67	38.67	42.00	89.33	47.00	36.38	13.83	17.67	152.89	12.48	8.00	8.36	1.92	4.03	291.70	72.60	1.61
G10	8.67	35.67	38.67	86.00	42.00	24.43	13.00	14.33	127.85	8.07	8.67	7.76	1.91	4.64	299.86	78.60	1.75
G11	7.00	30.00	33.33	82.33	36.67	30.30	17.00	22.00	106.04	8.90	9.99	7.76	2.05	4.53	338.86	90.15	2.00
G12	13.33	34.33	36.67	78.00	41.33	20.53	14.67	19.17	114.67	7.33	9.99	8.33	1.62	4.19	443.33	81.15	1.80
G13	9.00	30.33	34.33	80.33	38.67	24.64	13.40	16.00	104.08	7.37	9.51	8.40	1.69	3.98	337.05	64.05	1.42
G14	9.33	34.67	37.00	78.67	39.67	26.89	11.93	20.67	106.00	7.37	9.60	7.41	1.43	4.10	244.61	84.00	1.87
G15	7.67	30.00	35.00	82.67	39.00	33.33	16.37	18.67	117.33	7.17	9.63	7.21	1.42	4.17	213.64	58.50	1.30
G16	7.67	34.00	37.33	82.33	39.67	31.79	14.67	19.67	108.10	8.47	10.09	8.92	1.27	4.23	245.77	51.30	1.14
G17	12.00	31.67	33.67	80.67	38.33	29.00	9.17	12.50	94.33	5.87	6.67	7.85	1.11	3.37	277.45	38.55	0.86
G18	11.67	31.00	33.00	85.33	38.33	27.21	7.93	10.67	91.33	5.43	6.53	8.37	1.00	3.23	225.32	35.85	0.80
Min	7.00	30.00	33.00	78.00	36.67	20.53	7.93	10.67	83.80	5.43	6.53	7.03	1.00	3.23	213.64	35.85	0.80
Max	13.67	38.67	42.00	89.33	47.00	36.59	21.93	30.68	169.67	12.67	12.38	8.92	2.75	5.50	443.33	120.43	2.68
Mean	9.20	32.41	35.56	81.33	39.44	29.23	14.41	19.37	125.50	8.44	9.78	7.92	1.81	4.20	301.10	79.51	1.77
SE	0.642	0.932	0.928	2.190	1.130	2.350	0.933	1.700	4.330	0.337	0.724	0.549	0.087	0.174	49.210	11.450	0.255
LSD0.05	2.431	3.526	3.511	8.271	4.278	8.884	3.529	6.442	16.396	1.276	2.739	2.739	0.330	0.657	18.218	4.325	0.964

LS-Days to 5-leaves stages; FF-Days to 1st flowering; 50%-F-days to 50% flowering; M: days to maturity, FPS: days to 1st pod setting, PH: plant height (cm), NL: number of leaves, NPP: number of pod/plant, LA-Leaf area (cm<sup>2</sup>), PL: petiole length (cm), PdL: pod length (cm), PdD: pod diameter (cm), DWP: dry weight of pod, NSP: number of seeds/pod, SW: 1000 seed weight, SY: seed yield/plant (g), Y: seed yield (t/ha).

**Table 3.** Estimation of genetic parameters in seventeen characters of eighteen genotypes of kidney beans.

Traits	MSS	CV(%)	$\sigma^2_g$	$\sigma^2_e$	$\sigma^2_P$	GCV	ECV	PCV	$h^2_b$	GA	GA (% mean)
Days to 5 leaves stage	15.34**	8.53	4.91	0.62	5.52	24.00	8.52	25.46	88.80	4.30	46.58
Days to 1st Flowering	29.53**	3.47	9.41	1.30	10.71	9.33	3.47	9.96	87.84	5.92	18.02
Days to 50% flowering	24.83**	3.16	7.85	1.29	9.14	7.79	3.16	8.40	85.87	5.35	14.86
Days to Maturity	39.57**	3.27	10.80	7.17	17.97	4.02	3.27	5.18	60.12	5.25	6.42
Days to 1st Pod Setting	22.05**	3.48	6.71	1.92	8.63	6.51	3.48	7.38	77.78	4.71	11.82
Plant Height (cm)	94.66**	9.55	28.80	8.27	37.06	17.82	9.55	20.22	77.69	9.74	32.36
No of Leaves	28.38**	7.94	9.03	1.30	10.33	20.88	7.94	22.34	87.38	5.79	40.20
No of Pod/Plant	94.11**	10.66	29.92	4.35	34.27	27.97	10.66	29.93	87.32	10.53	53.83
Leaf Area (cm <sup>2</sup> )	2609.06**	4.14	860.30	28.16	888.46	22.88	4.14	23.25	96.83	59.46	46.39
Petiole Length (cm)	16.26**	4.73	5.36	0.17	5.53	26.53	4.73	26.94	96.92	4.70	53.79
Pod Length (cm)	6.46**	9.05	1.89	0.79	2.68	14.05	9.05	16.71	70.65	2.38	24.32
Pod Diameter (cm)	0.72	8.47	0.09	0.45	0.54	3.74	8.46	9.25	16.37	0.25	3.12
Dry Weight of Pod	0.61	5.88	0.20	0.01	0.21	24.53	5.87	25.22	94.59	0.89	49.14
No of Seeds/Pod	0.94	5.06	0.30	0.05	0.34	13.00	5.06	13.95	86.86	1.05	24.96
1000 Seed Weight	9355.92**	20.21	1907.79	3632.54	5540.33	14.65	20.21	24.96	34.43	52.80	17.70
Seed Yield (g/Plant)	1748.91**	17.75	517.43	196.63	714.06	28.79	17.75	33.83	72.46	39.89	50.49
Yield (t/ha)	0.86**	17.77	0.26	0.10	0.35	28.72	17.72	33.75	72.42	0.89	50.34

**Note:** \*\* indicates significant at 1% level, Gen MSS: Genetic mean sum of square, CV: Coefficient of variation,  $\sigma^2_g$ : Genotypic variance,  $\sigma^2_P$ : Phenotypic variance,  $\sigma^2_e$ : Environmental variance, GCV: Genotypic coefficient of variance, PCV: Phenotypic coefficient of variance,  $h^2_b$ : Heritability in broad sense, GA: Genetic advance, GA (% of mean): Genetic advance as percent of mean.

The analysis of variance showed that the mean sum of square was highly significant ( $>0.01$ ) in days to 5 leaves stage, days to 1st flowering, days to 50% flowering, days to maturity, days to 1st pod setting, plant height (cm), number of leaves, number of pod/plant, leaf area (cm<sup>2</sup>), petiole length (cm), pod length (cm), 1000 seed weight, seed yield (g/plant) and yield (t/ha) (Table 3). The germplasms showed different flowering time and days to maturity. The days to 1<sup>st</sup> flowering was ranged from 30.00 to 38.67 DAS (days after sowing) with a mean value of 32.41 (Table 2). The earliest flowering was recorded as 30 DAS in genotypes Sylhet varieties (G3, G5, G6, G8), G11 and Bandarban varieties G13 and G15. Neupane *et al.*, (2008) reported that the number of days required for flowering in kidney bean was influenced by the genotype varying from 40 to 84 days depending on the genotype. For days to maturity, the average of 81.33 days with a range of 78.00 to 89.33 was recorded. The G6 and G12 required the least number of days to maturity (78.00 days) followed by G14 (78.67 days), whereas the maximum number of days to maturity was observed in the genotype G9 (89.33 days) followed by G10 (86.00 days) (Table 2). In the present study, G11 indicated the earliest pod setting (36.67days) followed by genotypes G5 (37.33 days). The shortest time required for first pod maturity in BARI Jharsheem-1 (81.5 days) and the longest time from BB-3 (96.0 days) reported by Hussain (2005). The maximum number of pods per plant was noticed in genotype Sylhet variety G6 (30.68) followed by advanced line G11 (22.00). The genotype G18 recorded the minimum number of pods per plant (12.50) followed by genotype G17 (10.67) (Table 2). Prakash and Ram (2014) reported the variation in the number of pods per plant which varied from 10.46 to 30.22 in kidney bean. The highest dry weight of pod was recorded in G6 (2.75 g) followed by G5 (2.50 g) and G4 (2.33 g). Furthermore, the maximum number of seeds per pod was recorded in genotype G6 (5.50) followed by genotype G5 (5.04). The genotype G12 was exhibited the maximum 1000 seed weight (443.33g) followed by genotype G2 (370.11g). Interestingly, Bandarban variety G12 exhibited the maximum 1000 seed weight, whereas other Bandarban varieties showed very

poor performance in seed weight. However, significant variations in 1000 seed weight were also observed by Noor *et al.*, (2014). The highest seed weight was recorded 368.50 g and the lowest 116.38 g by Noor *et al.*, (2014). Seed yield per plant ranged from 35.85g to 120.43g, with a mean value of 79.51g. The maximum seed yield per plant was recorded in the local varieties of the Sylhet region G6 (120.43g) followed by G5 (115.07g) and G4 (112.03g) than BARI released varieties (G1 and G2). Advanced line G11 also produced an optimum yield in comparison with the varieties collected from Bandarban. The maximum seed yield per hectare was recorded in genotype G6 (2.68 t/ha) followed by genotype G5 (2.56 t/ha) and genotype G4 (2.49 t/ha) (Table 2). It would be suggested that the genotypes showed a high level of traits might have the high genetic potential to use them in breeding programs in order to improve seed yield in kidney bean.

#### **Analysis of genetic parameters, heritability, and genetic advance**

The success in any crop improvement program depends on the ability of the breeder to determine and assemble the genetic variability and highly heritable characters after the elimination of the environmental component of phenotypic variation (Mather, 1949). Therefore, it is necessary to have prior information on both PCV and GCV, so that the estimation of heritability that helps the breeder to predict the expected GA possibly by selection for a character can be computed. In this study, analysis of phenotypic and genotypic coefficients of variation and heritability was estimated for seventeen traits of the collected germplasm. The phenotypic coefficient of variance was higher than the genotypic coefficient of variance in all characters (Table 3). This indicated that the apparent variation was caused not only for genotypes but also for the environmental effects on those traits. The trait of pods per plant showed the high phenotypic coefficient of variation (29.93%) and genotypic coefficient of variation (27.97%). Pod length exhibited moderate GCV (14.05%) and PCV (16.71 %) (Table 3) values which were consonance with Singh *et al.*, (1994). But Raffi and Nath (2004) reported the highest genotypic

and phenotypic variations for pod length. The number of seeds per pod also showed moderate GCV (13.00%) and PCV (13.95%) which was opposite of the results of Angadi *et al.*, (2011) who assessed low genotypic and phenotypic coefficient of variation was for this trait. In the case of the dry weight of pod, high values were estimated in GCV (24.53) and PCV (25.22), heritability (94.59%) and genetic advance as percent of the mean (49.14%). The low GCV (3.74%) of pod diameter suggested the influence of the environment on the expression of this character (Table 3). Therefore, selection based only on the phenotypic expression of this character would be ineffective for the improvement of this crop. Moderate GCV and PCV were observed as 13.00 and 13.95 respectively (Table 3) in the number of seeds/plants. However, moderate-high PCV (24.96 %) and moderate GCV (14.65%) were recorded in 1000 seed weight but PCV and GCV were high for 100 seed weight according to Patil *et al.*, (1993). Similarly, a high phenotypic coefficient variation for 1000 seeds weight (43.22%) was found by Prakash *et al.*, (2015). A high estimation of PCV (33.75%) and GCV (28.72%) for seed yield (t/ha) in the current study was supported by Angadi *et al.*, (2011). High heritability with a combination of high genetic advance as percent of mean allows the plant breeders to speculate the presence of additive gene effects on this trait. High heritability coupled with high genetic advance as percent of mean was observed in following characters, days to 5 leaves stage (88.80, 46.58), plant height (cm) (77.69, 32.36), number of leaves (87.38, 40.20), number of pod/plant (87.32, 53.83), leaf area (cm<sup>2</sup>) (96.83, 46.39), dry weight of pod (94.59, 49.14), pod length (cm) (70.65%, 24.32%), number of seeds/pod (86.86, 24.96), seed yield (g/plant) (39.89, 50.49) and yield (t/ha) (72.42%, 50.34%) (Table 2). A similar result was also found by Raffi and Nath (2004), Patil *et al.*, (1993) and Singh *et al.*, (1994) for seed yield (g/ plant). On the contrary, low heritability for seed yield per plant was observed in segregating the F<sub>3</sub> generation of cowpea by Padi and Ehlers (2008). High values for heritability and genetic gain as a percent of the mean for seed yield (t/ha) reveals that this trait might be controlled by additive gene effects

and selection would be effective. Omoigui *et al.*, (2006) calculated heritability in selected varieties of cowpea and noted that yield had high heritability. High heritability coupled with moderate genetic advance may be due to moderate values for phenotypic standard deviation as the heritability is high for these characters and selection differential is always constant (Nadarajan and Gunasekaran, 2012). In the present study, 1000 seed weight showed moderate heritability (34.43%) and genetic advance as percent of the mean (17.70%) (Table 3) whereas, Kamaluddin (2011) and Prakash *et al.*, (2015) found high heritability and high genetic advance for 100 seed weight.

### Genetic diversity

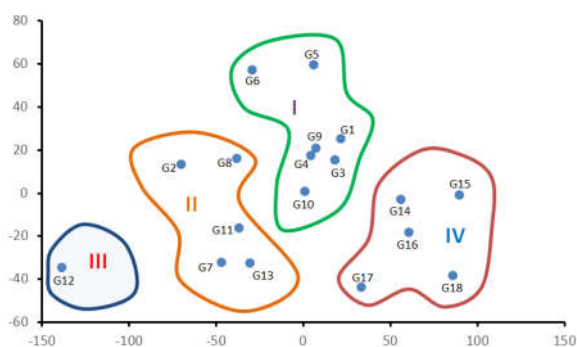
The principal component analysis was carried out with eighteen genotypes of a kidney bean. The first three Eigen values for three principal coordination axes of genotypes accounted for 74.8% variation (Table 4). Out of seventeen characters studied, principal component I contributed maximum towards the total diversity with the value of 42.9%, followed by component II (22%), component III (9.9%), and component IV (7%) (Table 4). Govanakoppa *et al.*, (2002) reported a high contribution of pods per plant, 100 seed weight, plant height and reproductive branches towards divergence in kidney bean.

**Table 4.** Eigen values and yield percent contribution of each character toward genetic divergence.

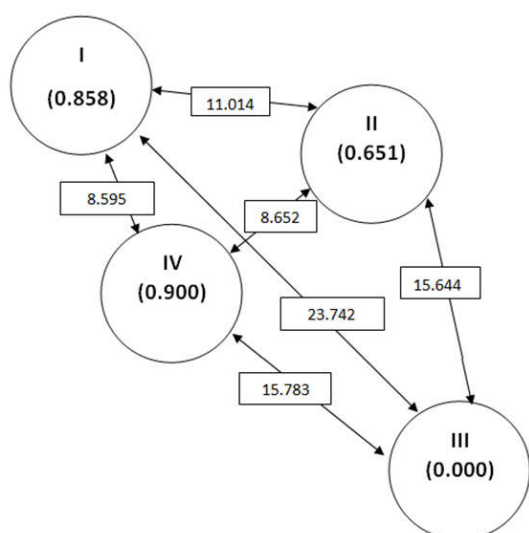
Principal Component Axis	Eigen values	Percent variation	Cumulative % of Percent variation
I	7.29	42.9	42.9
II	3.73	22.0	64.9
III	1.69	9.9	74.8
IV	1.19	7.0	81.8
V	0.84	4.9	86.7
VI	0.66	3.9	90.6
VII	0.59	3.5	94.1
VIII	0.35	2.1	96.2
IX	0.28	1.7	97.8
X	0.13	0.8	98.6
XI	0.12	0.7	99.3
XII	0.04	0.3	99.6
XIII	0.04	0.2	99.8
XIV	0.03	0.2	100.0
XV	0.01	0.0	100.0
XVI	0.00	0.0	100.0
XVII	0.00	0.0	100.0



The genetic diversity in the collected kidney germplasm was assessed by using Mahalanobis's  $D^2$  statistic. Grouping the genotypes into clusters using Tocher's method resulted in the formation of four clusters, of which cluster I had the highest seven genotypes followed by cluster II and IV had five genotypes and cluster III was solitary (Figure 2). The inter-cluster distances ranged from 8.595 (between clusters I and IV) to 23.742 (between clusters I and III). The highest intracluster distance (0.900) was in cluster IV (Figure 3). The results also showed that inter-cluster distance was always higher than intracluster distance (Figure 3).



**Fig. 2.** Clusters of the different genotypes of kidney beans.



**Fig. 3.** Nearest and farthest clusters in kidney bean genotypes.

A similar result was found by Nandi *et al.*, (1996) Sureja and Sharma (2001), Singh and Mishra (2008) and Savitha (2008).

Theoretically, a crossing of genotypes belonging to the same cluster is not expected to yield superior hybrids or segregants. A general notion exists that the larger in the divergence between the genotypes, the higher will be the heterosis (Falconer, 1981). Therefore, the result suggested that an attempt would be taken to crosses between genotypes belonging to the clusters I and III for getting high heterotic combinations.

The cluster mean analysis results showed that the genotypes under the cluster I (G1, G3, G4, G5, G6, G9, and G10) showed high mean values for the characters viz., plant height (30.14), number of leaves (16.13), number of pods per plant (23.61), leaf area (146.24), petiole length (9.51), pod length (10.37), dry weight of pod (2.21), number of seeds per plant (4.66), seed yield per plant (95.14) and yield per hectare (2.12) (Table 5). The single genotype G12 under cluster III showed a high mean value for pod diameter (8.33) and 1000 seed weight (443.33). While the genotypes included in cluster IV showed the lowest values for almost the characters above.

The analysis of the contribution of characters towards the divergence of the genotypes present in table 6. Vector I obtained from PCA expressed that days to 5 leaves stage (0.477), days to 50% flowering (1.046), plant height (0.151), pod length (0.016), number of seeds per pod (0.781), 1000 seed weight (0.097), seed yield per plant (0.101), yield (4.152) were major characters that contribute to the genetic divergence. It was the reflection of the first axis of differentiation. In vector II days to 1st flowering (1.822), days to maturity (0.126), days to first pod setting (0.674), number of leaves (0.885), number of pod per plant (0.764), leaf area (0.110) showed their important role toward genetic divergence.

The value of Vector I and Vector II revealed that both Vectors (I and II) had positive values for days to maturity and the number of leaves per plant indicating the highest contribution of these traits towards the divergence among 18 genotypes of kidney bean. Negative values in both vectors for a dry weight of pod had a lower contribution towards the divergence. In the

present study, eighteen genotypes of kidney bean were grouped into four clusters.

**Table 5.** Cluster mean values of different characters of kidney bean genotypes.

Parameters	Clusters			
	I	II	III	IV
Days to 5 leaves stage	9.29	7.8	13.33	9.67
Days to 1 <sup>st</sup> Flowering	32.95	31.4	34.33	32.27
Days to 50% flowering	36.43	34.46	36.67	35.2
Days to Maturity	81.67	80.93	78	81.93
Days to 1st Pod Setting	40.14	38.53	41.33	39
Plant Height (cm)	30.14	29.3	20.53	29.64
No of Leaves	16.13	14.36	14.67	12.01
No of Pod/Plant	23.61	16.41	19.17	16.44
Leaf Area (cm <sup>2</sup> )	146.24	120.71	114.67	103.42
Petiole Length (cm)	9.51	8.75	7.33	6.86
Pod Length (cm)	10.37	10.19	9.99	8.5
Pod Diameter (cm)	7.78	8.03	8.33	7.95
Dry Weight of Pod	2.21	1.86	1.62	1.25
No of Seeds/Pod	4.66	3.95	4.19	3.82
1000 Seed Weight	291.27	346.15	443.33	241.36
Seed Yield (g/Plant)	95.14	83.17	81.15	53.64
Yield	2.12	1.85	1.8	1.19

**Table 6.** Relative contributions of different characters to the total divergence of kidney bean genotypes.

Parameters	Vector-1	Vector-2
Days to 5 leaves stage	0.477	-0.290
Days to 1st Flowering	-1.196	1.822
Days to 50% flowering	1.046	-1.863
Days to Maturity	0.095	0.126
Days to 1st Pod Setting	-0.511	0.674
Plant Height (cm)	0.151	-0.342
No of Leaves	0.749	0.885
No of Pod/Plant	-0.043	0.764
Leaf Area (cm <sup>2</sup> )	-0.015	0.110
Petiole Length (cm)	0.067	-0.801
Pod Length (cm)	0.016	-0.997
Pod Diameter (cm)	0.149	-1.304
Dry Weight of Pod	-24.087	-10.215
No of Seeds/Pod	0.781	-1.225

The magnitude of D2 values confirmed that there was a considerable amount of diversity in the experimental material evaluated. Days to 5 leaves stage, days to 1<sup>st</sup> flowering, days to 50% flowering, days to maturity were the maximum contributors for divergence in the present study which should be given utmost importance for the selection of the genotypes for crossing program. The relative contribution of different plant characters to the total genetic divergence estimated by D<sup>2</sup> analysis indicated that seed yield per hectare ranked first days to 5-leaves stage which contributed maximum towards the total diversity with the value of 42.9%, followed

by days to 1st flowering 22%, days to 50% flowering 9.9% (Table 4), suggesting that these are potent factors in differentiating the germplasm of bean. Apart from the high divergence, the performance of the genotypes (cluster I- G1, G3, G4, G5, G6, G9, G10 and cluster III- G12) and the characters with the maximum contribution towards divergence should also be given emphasize for improvement of a kidney bean. Here, it is worthy to note that the superiority of a particular genotype in respect of a given character gets diluted by other genotypes that are related and grouped in the same cluster which is inferior or intermediary for that character in question. Hence, apart from selecting lines from clusters that have a high inter-cluster distance for hybridization, one can also think of selecting parents based on the extent of divergence with respect to the trait of interest.

Our data showed that there was no perfect relationship between genetic diversity and geographical diversity. This may be attributed to the genotypes of the present study were indigenous, landraces, local types and released varieties collection from BARI. The obtained results showed that the genotypes have overlapped in different clusters with some distinctness. The absence of correlation between genetic diversity and geographic diversity supported by the previous work of Dikshit *et al.*, (1999), whereas Zeven *et al.*, (1999) and Barelli *et al.*, (2005) reported the correlation between genetic diversity and geographic diversity. The random pattern of genotype distribution into various clusters from different eco-geographic regions suggested that geographic influence such as exchange of breeding material, genetic drift, natural and artificial selections might be responsible for diversity as reported earlier by Murthy and Arunachalam, 1966.

### Nutritional components

Most of the research on kidney beans have been related to the varietal selection. Therefore, a study of the nutritional quality of kidney beans would be of great interest. The contents of different nutritional components *e.g.* protein, carbohydrate, fat, fiber, ash and moisture of the collected germplasms were estimated (Table 7).

**Table 7.** Analysis of nutrient components content in kidney bean genotypes.

Genotypes	Protein (%)		Moisture (%)		Fat (%)		Fiber (%)		Ash (%)		Carbohydrate (%)	
G1	21.62	d	10.18	i	3.11	a	1.5	ef	2.08	cde	61.52	abcd
G2	22.41	c	11.91	de	3	ab	1.39	f	1.79	gh	59.5	cdef
G3	21.93	d	11.52	f	3.1	a	2.08	c	2.94	a	58.43	ef
G4	17.76	h	10.23	hi	2.5	de	1.17	g	2.31	b	64.03	a
G5	21.97	d	11.99	cd	2.7	bcd	1.39	f	1.71	h	60.24	cdef
G6	21.84	d	11.2	g	2.6	cd	1.49	ef	1.98	ef	60.89	bcde
G7	19.93	f	12.26	b	2.2	e	1.8	d	1.87	fgh	61.94	abc
G8	20.14	f	10.41	h	2.5	de	2.46	a	2.95	a	61.54	abcd
G9	18.2	g	11.77	e	3.1	a	1.78	d	1.76	gh	63.39	ab
G10	23.76	a	11.83	de	2.5	de	1.77	d	2.18	bcd	57.96	f
G11	17.78	h	12.68	a	2.5	de	1.75	d	1.78	gh	63.51	a
G12	20.01	f	11.9	de	2.6	cd	1.53	e	1.92	efg	62.04	abc
G13	23.11	b	12.16	bc	2.7	bcd	1.47	ef	1.93	efg	58.63	ef
G14	20.54	e	11.72	ef	2.6	cd	2.25	b	2.21	bc	60.68	cde
G15	23.05	b	11.71	ef	3.1	a	1.85	d	2.2	bc	58.09	f
G16	23.09	b	11.85	de	2.9	abc	1.44	ef	2.01	def	58.71	ef
G17	22.44	c	11.88	de	3.1	a	1.43	ef	1.84	fgh	59.31	def
G18	21.92	d	11.84	de	3.2	a	1.42	ef	1.94	efg	59.69	cdef
LSD (0.05)	0.389		0.211		0.362		0.112		0.181		2.57	

The analysis of variance showed that the nutrient components were significantly varied among the genotypes (Table 8). In the present investigation, significant differences ( $P < 0.01$ ) of protein

(10.50), carbohydrate (11.107), fat (0.267), fiber (0.336), ash (0.386) and moisture (1.41) was found in the collected eighteen genotypes of kidney bean (Table 8).

**Table 8.** ANOVA table for nutrient components of the kidney bean.

Source of variance	DF	Protein	Moisture	Fat	Fiber	Ash	Carbohydrate
Treatments	17	10.50**	1.41**	0.267**	0.336**	0.386**	11.107**
Error	34	0.0161	0.0048	0.014	0.0013	0.0035	0.7068

\*\*Significant at 1% level

Genotype, of G10, possessed significantly high protein (23.76%) content that was statistically different from other genotypes (Table 7). This component was also found high in G13 (23.11%), G16 (23.09%) and G15 (23.05%). The similar content was reported by Ofuya and Akhidue, (2005) in another study with a range of 20.9-26.9 g/100g. Carbohydrate content in the genotypes of kidney bean ranged from 57.96% (G10) – 64.03% (G4). A similar percentage of carbohydrate was also found by Hsieh *et al.*, (1992) and Salunkhe *et al.*, (1989). The highest content of carbohydrate was recorded in G4 (64.03) which was also statistically similar with G11 (63.51%), G9 (63.39%), G12 (62.04%) G7 (61.94%), G1 (61.52) and G8 (61.54%) (Table 7). The highest fat content was observed in G18 (3.2%), while the lowest fat content was found in G7 (2.2%). However, Chaudhary and Sharma (2013) and Hsieh *et al.*, (1992) found 1.5% fat in kidney bean. The fat content of the kidney bean variety is less in amount, making them a foodstuff with positive nutritional implications.

The highest fat-containing genotype was G18 (3.2%) which was statistically similar to genotypes of G3, G9, G15, G17 (3.1%) and G2 (3%).

Kidney beans are a very good source of cholesterol-lowering fiber which prevents heart disease, cancer, diabetes, insulin resistance and rising blood sugar (Menotti *et al.*, 1999). Interestingly, fiber content was calculated in a range of 1.17%- 2.46% which was different from the study of Chaudhary and Sharma (2013) who found high fiber content (4.0±0.34 g/100g). The highest fiber content was found in genotype G8 (2.46%) and the lowest in G4 (1.17%).

Kidney bean's contribution to heart health lies not just in their fiber but in the significant amount of ash (minerals) from kidney beans (Bazzano *et al.*, 2003). Minerals lessen the resistance of veins and arteries and improve the flow of blood, oxygen, and nutrients throughout the body (McIntosh and Miller 2001). While the highest content of ash (2.95%) was found in G8 which could also be categorized with G3

(2.94%) in same class (Table 6). Ash was found at a low level (1.79%- 2.95%) (Table 7) comparing with the estimation of Chaudhary and Sharma (2013) who found the ash content in the level of  $3.6 \pm 0.36$ g/100g and Hsieh *et al.*, (1992) who achieved the ash content as 4.5%. The present investigation estimated the moisture content 10.18- 12.68% which were possessed by genotype G1 and G11, respectively. The values were not statistically similar to the moisture content of other genotypes. Similar moisture content (10.8%) was found in mature kidney bean by Hsieh *et al.*, (1992). Khatoon and Prakash (2004) also found moisture content in the range of 10.1-12.7g/100g. In the present investigation, genotype G1 showed the highest fat (3.11%) and lowest moisture (10.18%) content. Genotype G4 showed the highest carbohydrate (64.03%) content and the lowest protein (17.76%) and fiber (1.17%) content. G8 showed the highest-fiber (2.46%) and ash (2.95%) content while G10 showed the highest protein (23.76%) and the lowest carbohydrate (57.96%) content (Table 7). In the present study, protein content was found higher in local genotypes collected from Bandarban (G13- 23.11%, G15- 23.05%, G16-23.09%, G17- 22.44%, G18- 21.92%) and advanced line (G10- 23.76%) than control check variety (G1- 21.62% and G2- 22.41%) (Table 7). In case of carbohydrate content, local varieties of Sylhet (G4- 64.03%, G5- 60.24%, G6- 60.89%, G7- 61.94%, G8- 61.54%) and advanced line (G11- 63.51%, G12- 62.04%) were higher than Bandarban local varieties. The maximum amount of fiber was found in local varieties of Sylhet (G3- 2.08%, G8- 2.46%) and advanced line (G14- 2.25%) among all genotypes. Higher amount of ash was found in local varieties of Sylhet (G3- 2.94%, G4- 2.31%, G8- 2.95%), and advanced line (G10- 2.18%) than control variety (G1- 2.08%, G2- 1.79%). Two of the Bandarban varieties (G14- 2.21%, G15- 2.2%, G16- 2.01%) (Table 6) also possessed high ash content. Most of the local varieties of both Sylhet and Bandarban along with all three advanced lines had shown lower fat content than control varieties.

### Conclusions

The study of genetic variability showed that the traits e.g. plant height (cm), pod length (cm), number of seeds/pod, number of pod/plant, leaf area (cm<sup>2</sup>), seed yield (g/plant) could be considered as selection criteria as these traits have higher GCV, heritability and genetic advance for crop improvement. The highest inter-cluster distance was found between Cluster I and Cluster III indicating that hybridization among these genotypes could generate novel recombinants in transgressive segregates population. For nutritional traits, local genotypes of Bandarban (G13, G15, G16, G17, G18) and advanced line (G10) were found comparatively higher in protein content. However, local varieties of Sylhet (G4, G5, G6, G7, G8) and advanced line (G11 and G12) had higher carbohydrate contents. In addition, the highest amount of fiber and ash were found in Sylhet local genotypes (G3 and G8). In the case of yield performance, the genotypes of the Sylhet region *viz.* G6, G5, and G4 showed the maximum seed yield per plant and seed yield per ha. It might be concluded that G1 (BARI Jharseem -1), G3, G4, G5, G6, G9 (BARI Jharseem-2), G10 (advanced line) and G12 (advanced line) had potential for improvement based on the genetic merit of yield and contributing factors.

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### Conflicts of interest

The authors have declared that no competing interests exist.

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