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Genetic diversity in speckled bean (*Phaseolus vulgaris* L.) genotypes in Ethiopia

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In the present investigation, the genetic variability of 64 speckled type common bean genotypes were evaluated at Haramaya University during 2015 cropping season using 8 × 8 simple lattice design with three replications. The analysis of variance indicated highly significant (P<0.01) differences among genotypes for all the nine characters studied. High genotypic coefficient of variation (GCV) was observed for grain yield, while high phenotypic coefficients of variation (PCV) were recorded for number of seeds plant^{-1}, grain yield ha^{-1} and common bacterial blight resistance. For all the traits, estimates of PCV were higher than GCV indicating the presence of environmental influence. High heritability values and genetic advances were recorded in grain yield. Cluster analysis using Mahalanobis distance delineated the genotypes into six main groups. Cluster I contain the largest number of genotypes (43.75%) followed by clusters II (26.56%) and III (10.94%) while clusters IV, V and VI contain four genotypes each. The D^2 analysis indicated that there was a significant difference among the clusters. The maximum inter cluster distance was observed between cluster IV and V. Principal component analysis (PCA) was performed to assess the variation and correlation among genotypes for the traits and grouped them based on their performance. The combination of the first four principal components explained more than 85.74% of the genotypic variations. Therefore, exploiting the genetic diversity among clusters would broaden the genetic base of speckled bean breeding populations.

Key words: Clustering, genetic distance, genetic variability, *Phaseolus vulgaris*, speckled bean.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.*)* is an annual leguminous plant with a diploid chromosome (2*n* = 22) and is largely self-pollinated. It is used for human consumption and is the most widely consumed grain legume mainly in South America and Africa as well as worldwide (Mkanda et al., 2007; De La Fuente et al., 2012). It is grown on an area greater than 29 million hectares in the world (FAOSTAT, 2015) and over four million hectares in Africa which provides dietary protein to over 300 million people in rural and poor urban

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Table 1. List of speckled type common bean (*Phaseolus vulgaris* L.) genotypes used for the study.

S/N	Genotype	S/N	Genotype	S/N	Genotype	S/N	Genotype
1	Brown speckled	17	DAB-350	33	DAB-380	49	DAB-431
2	Cranscop	18	DAB-354	34	DAB-381	50	DAB-434
3	DAB-175	19	DAB-356	35	DAB-382	51	DAB-437
4	DAB-179	20	DAB-358	36	DAB-384	52	DAB-444
5	DAB-197	21	DAB-359	37	DAB-408	53	DAB-445
6	DAB-327	22	DAB-360	38	DAB-410	54	DAB-447
7	DAB-332	23	DAB-363	39	DAB-413	55	DAB-448
8	DAB-333	24	DAB-365	40	DAB-416	56	DAB-454
9	DAB-334	25	DAB-368	41	DAB-417	57	DAB-455
10	DAB-335	26	DAB-369	42	DAB-418	58	DAB-457
11	DAB-338	27	DAB-370	43	DAB-420	59	DAB-459
12	DAB-340	28	DAB-372	44	DAB-422	60	DAB-461
13	DAB-343	29	DAB-374	45	DAB-425	61	DAB-463
14	DAB-345	30	DAB-375	46	DAB-428	62	DAB-470
15	DAB-347	31	DAB-376	47	DAB-429	63	DAB-471
16	DAB-348	32	DAB-378	48	DAB-430	64	DAB-473

communities. In Eastern and Southern Africa, the annual common bean consumption is growing at 2.2 to 2.6% every year (Katungi et al., 2009). Ethiopia is ranked 4th in dry bean production in Africa with 323,326 ha with an average production of 513,725 tonnes (FAOSTAT, 2017).

In Ethiopia, common bean producers have varied preferences in different geographical regions. Small white and red are the dominant market class in the Central Rift Valley and Southern region, respectively while in Eastern Ethiopia, the preferred types are white, red and speckled type. The red, white and speckled beans have their own market niches both by the national and export market. However, there is a limitation of varieties for the speckled type to meet the market demand. The few available speckled type varieties are late maturing, low yielding and susceptible to diseases; hence, they are not preferred by the farmers. Therefore, there is an urgent need to develop varieties for these growing preferences.

The success of any breeding program depends on the amount of genetic variation present in a given crop. Knowledge of nature and degree of divergence in genotypes, the extent of transmissibility of the given trait guides a breeder to predict the behavior of the succeeding generations and helps to predict the responses to selection (Larik et al., 1989). Several studies have been conducted to assess the diversity of common bean genotypes (Yayis et al., 2011; Lima et al., 2012; Assefa et al., 2014; Kumar et al., 2014; Zelalem, 2014; Correa et al., 2015). However, limited work has been done in Ethiopia particularly in eastern Ethiopian for speckled bean type. Therefore, the present study was conducted to generate information on the extent of

genetic diversity among speckled type common bean genotypes, the heritability of important agronomic traits, and the genetic gain that can be made through implementing selection breeding.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted at Haramaya University research station in the main campus (9°26'N latitude; 42.0°E longitude; 1980 m above sea level altitude) during 2015 cropping season. The research station is situated in the semi-arid tropical belt of Eastern Ethiopia and is characterized by a sub-humid type of climate with an annual rainfall of 790 mm, annual mean temperature of 17°C with mean minimum and maximum temperature of 3.8 and 25°C, respectively and the research station soil type is alluvial.

Experimental and design

Sixty-two speckled type common bean genotypes along with two released varieties were used in the present study (Table 1). The genotypes were initially introduced from International Center for Tropical Agriculture (CIAT). The experiment was laid out in 8 × 8 simple lattice designs with three replications. The size of each plot was 4 m long with six rows. The spacing between rows and between plants within a row was 0.40 and 0.10 m, respectively. The trial was planted without fertilizer on July 25, 2015 and all other agronomic practices were applied uniformly to all plots as per the recommendations.

Data were collected for days to 50% flowering, days to maturity, plant height (cm), number of pods plant^{-1}, number of seeds pod^{-1}, 100 seed weight (g), and grain yield ha^{-1} (kg) as per the International Board for Plant Genetic Resources (IBPGR, 1982) descriptor. In addition, genotypes were evaluated for common bacterial blight severity using 1 to 9 disease scale (CIAT, 1988), where 1 = no visible disease symptoms and 9 = more than 25% of

Table 2. Mean performance of speckled type common bean genotypes and released varieties.

Character	Genotypes			Varieties			No. genotypes over	
	Minimum	Maximum	Mean±SD	Minimum	Maximum	Mean	Best performing variety	Mean of check
Days to flowering	49.0	66.0	52.71±0.29	51	60.0	56.0	24	56
Days to maturity	99.0	116.0	102.84±0.29	101	110.0	106	20	106
Plant height (cm)	34.6	48.6	41.66±0.28	39.5	46.1	42.83	4	42.8
No. of pods plant^{-1}	7.0	17.0	12.00±0.19	9.0	17.0	13.10	0	13.0
No. seeds pod^{-1}	3.0	6.0	4.61±0.04	4.0	6.0	4.97	1	5
No. of seeds plant^{-1}	27.0	80.0	55.32±1.03	51.0	74.0	62.31	2	62.0
100 seed weight (g)	44.1	72.17	54.01±0.36	51.6	53.4	52.50	31	52.5
Grain yield(kgha^{-1})	1052.1	4456.4	3318.40±62.87	3425.8	3770.5	3598.15	23	3598.15
CBB	2.67	6.67	3.79±0.08	3	4	3.67	13	3.67

CBB: Common bean bacteria blight resistance score; SD: standard deviation.

leaf surface area with large coalescing and generally necrotic lesions resulting in defoliation.

Data analysis

Data were subjected to analysis of variance (ANOVA) according to Gomez and Gomez (1984). The mean square values were used to estimate the genotypic and phenotypic variances according to Sharma (1998). Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were estimated according to the method suggested by Deshmukh et al. (1986). Broad sense heritability (H^2) was calculated as the ratio of genotypic variance to the phenotypic variance (Falconer and Mackay, 1996). The expected genetic advance (GA) was estimated in accordance with the formula described in Allard (1999) as follows:

$$GA = K^* \sigma_p \cdot H^2$$

where H^2 = heritability in broad sense, K = the selection intensity at 5% (K at 5% = 2.06), and σ_p = phenotypic standard deviation on mean basis.

Genetic advance as percent of mean (GAM) was also computed using the formula given as follows to compare the extent of predicted genetic advance of different traits under selection:

$$GAM = \frac{GA}{\bar{X}} \times 100$$

where $\bar{X}$ = grand mean of the trait; A = genetic advance under selection X.

The genetic advance as percent of the mean was categorized as low (0-10), moderate (10-20) and high (≥ 20) following Johnson et al. (1995).

Cluster analysis

Clustering of genotypes into different groups was carried out by average linkage method and the appropriate numbers of clusters were determined from the values of Pseudo F, cubic clustering criteria and Pseudo T^2 statistics using the procedures of SAS computer software version 9.1 so as to group sets of genotypes into homogeneous clusters (SAS, 2008).

Genetic divergence analysis

A measure of group distance based on multiple characters was given by generalized Mahalanobis D^2 statistics (Mahalanobis, 1936) for nine characters and was analyzed using the procedure Proc discrim of SAS version 9.1 facilities (SAS, 2008).

Principal component analysis (PCA)

Principal components analysis was performed using correlation matrix by employing SAS software version 9.1 (SAS, 2008) to evaluate the relationships among characters that are correlated among each other by converting into uncorrelated characters called principal components. The contribution of each character to PCA is determined by Eigen vector that is greater than half divided by the square root of the standard deviation of the Eigen value of the respective PCA as suggested by Johnson and Wichern (1988).

RESULTS

Analysis of variance and mean performance of genotypes

The analysis of variance for all the characters revealed the presence of highly significant differences among the genotypes under study (Table 3). The results revealed that the genotypes exhibited wide range of mean values for each character (Table 2). Days to 50% flowering ranged from 49 to 67 days while days to maturity ranged from 99 to 116 days. Hundred seed weight ranged from 44 to 72 g. Similarly, grain yield ranged from 1052 to 4456 kg ha^{-1} with an average yield of 3318 kg ha^{-1}.

Estimation of genetic variability

The variability components, *viz*., PCV and GCV, heritability, and genetic advance were estimated for all the nine characters. Genotypic coefficient of variation

Table 3. Mean squares from analysis of variance for nine characters of speckled type common bean genotypes.

Trait/Parameter	Replications (2)	Blocks (21)	Genotypes(63)	Error (105)	CV (%)	PCV (%)	GCV (%)	H^2(%)	GA (%)
Days to flowering	0.9	2.55	38.96**	3.64	3.62	7.45	6.51	76	11.72
Days to maturity	0.48	2.97	38.82**	4.15	1.98	3.85	3.31	74	5.84
Plant height(cm)	68.62	27.71	18.43**	5.32	5.54	7.47	5.02	45	6.94
No. of pods plant^{-1}	132.7	3.06	11.15**	2.78	13.9	19.67	13.91	50	20.28
No. seeds pod^{-1}	0.14	0.36	0.55**	0.22	10.28	12.52	7.14	33	8.4
No. of seeds plant^{-1}	3178.29	110.79	271.89**	105.06	18.53	22.91	13.48	35	16.34
100 seed weight (g)	16.74	5.56	56.05**	6.03	4.55	8.82	7.56	73	13.35
Grain yield (kg/ha)	106603.3	501010	1663561.9**	174836	12.6	24.69	21.23	74	37.61
Common bacterial blight severity score	1.33	0.67	1.73**	0.621	20.78	26.25	16.04	37	20.18

**p ≤ 0.01; PCV (%): phenotypic coefficient of variation; GCV (%): genotypic coefficient of variation; H^2(%): broad sense heritability; GA (%): genetic advance as percentage of mean; CV: coefficient of variation. Numbers in parenthesis indicated the degree of freedom.

ranged from 3.31 (days to maturity) to 21.23% (grain yield) while phenotypic coefficient of variation ranged from 3.85 (days to maturity) to 26.25% (CBB severity score). Heritability (broad sense) and genetic advance as percent of mean ranged from 33 to 76% and 5.84 to 37.61%, respectively. Based on the delineation of Deshmukh et al. (1986), the PCV and GCV were low for days to 50% flowering, days to maturity, plant height and 100 seed weight, while PCV was high for number of seeds plant^{-1} and seed yield ha^{-1}. Broad sense heritability (H^2) estimates were found more than 30% for all the characters studied and considered as medium to high (Falconer and Mackay, 1996). Moreover, the highest heritability values (>70%) were observed in days to flowering, days to maturity, 100 seed weight and grain yield (Table 3).

Cluster analysis

Based on D^2 divergence, the 64 common bean genotypes were grouped into six clusters (Table 4). The clustering pattern showed that cluster I was composed of the highest number of genotypes (28), followed by clusters II (17), III (7) and clusters IV, V and VI (four genotypes each). The two released speckled bean varieties, Brown speckled and Cranscop were grouped in clusters I and II, respectively. Cluster trait performance was evaluated and presented in Table 5. Cluster I was characterized as resistant (3.48) to common bacterial blight, relatively early for days to flowering and maturity (51.8 and 102.0 days, respectively), relatively high number of pods plant^{-1} (12.2), number of seeds pod^{-1} (4.7), 100 seed weight (55.25 g) and grain yield (3845.35 kg ha^{-1}). Cluster II was characterized by the highest number of seeds pod^{-1} (4.74) and relatively higher number of seeds plant^{-1} (57.33). Cluster III contained genotypes that needed more duration for flowering (53.9 days) and maturity (104.1 days). Cluster IV was characterized by late flowering (57.8 days) and maturity (107.8 days).

Cluster V was characterized as early in flowering and maturity (50.9 and 101.4 days, respectively), very high plant height (45.54 cm), highest grain yield (4317.8 kg ha^{-1}) and number of seeds plant^{-1} (60.82). Cluster VI was characterized by a short plant stature (38.36 cm) and highest common bacterial blight severity score.

Genetic divergence

Significant differences among the genotypes for all the characters would justify further calculation of D^2 (Sharma, 1998). The pair-wise generalized square distance (D^2) between the six clusters is presented (Table 6). There was a highly significant difference between all cluster pairs. The inter-cluster D^2 values ranged from 16.09 to 399.4. In the present study, the most divergent clusters (Table 6) were between clusters IV and V (D^2=399.4), followed by clusters I and IV (D^2= 276.01) and V and VI (D^2=228.8).

The minimum inter-cluster distance recorded between clusters I and II (D^2= 16.09) indicated close relationship among genotypes included in these clusters.

Table 4. Distribution of 64 common bean genotypes into six clusters based on D^2 analysis.

Cluster	No. of genotypes	Genotypes included in the clusters				
I	28	DAB-434	DAB-463	DAB-384	DAB-417	DAB-360
		DAB-437	DAB-359	DAB-380	DAB-370	DAB-473
		DAB-374	DAB-455	DAB-459	DAB-175	DAB-347
		DAB-420	DAB-470	DAB-381	DAB-376	DAB-428
		DAB-179	DAB-372	DAB-413	DAB-461	DAB-354
		DAB-368	DAB-333	Brown speckled		
II	17	DAB-358	DAB-471	DAB-197	DAB-429	DAB-431
		DAB-444	DAB-363	DAB-425	DAB-416	DAB-457
		DAB-356	DAB-375	DAB-335	Cranscop	DAB-327
		DAB-448	DAB-369	-	-	-
III	7	DAB-348	DAB-408	DAB-334	DAB-445	-
		DAB-430	DAB-454	DAB-422	-	-
IV	4	DAB-338	DAB-378	DAB-340	DAB-345	-
V	4	DAB-365	DAB-418	DAB-382	DAB-410	-
VI	4	DAB-343	DAB-447	DAB-332	DAB-350	-

Table 5. Mean values of clusters for nine characters of speckled type common bean genotypes.

Character	Clusters						Grand mean
	I	II	III	IV	V	VI	
Days to flowering	51.77	52.71	53.90	57.84	50.92	53.84	53.50
Days to maturity	101.96	102.67	104.14	107.75	101.42	104.00	103.66
Plant height (cm)	41.98	41.35	41.30	40.74	45.54	38.36	41.54
No. of pods per plant	12.19	12.12	11.98	9.75	13.39	11.12	11.76
No. of seeds per pod	4.71	4.74	4.30	4.24	4.58	4.32	4.48
No. of seeds per plant	57.30	57.33	51.58	41.09	60.82	48.22	52.72
Hundred seed weight (g)	55.25	52.84	52.22	51.78	55.81	53.83	53.62
Grain yield (kg ha^{-1})	3845.35	3263.72	2610.54	1333.90	4317.83	2086.00	2909.56
Common bacterial blight severity score	3.48	3.71	4.28	4.00	3.50	5.59	4.09

Principal component analysis

Principal component analysis, using 64 speckled bean genotypes for nine characters revealed that four principal components PC1, PC2, PC3 and PC4 with Eigen values of 2.92, 2.40, 1.40 and 1.00, respectively, accounted for 85.74% of the total variation (Table 7). The first two principal components, PC1 and PC2 with values of 32.42 and 26.67%, respectively, contributed significantly to the total variation indicating the vital role of the first two principal components.

In the first principal component, days to 50% flowering and days to 90% physiological maturity had high positive loadings, while plant height had high loading in the second and third principal components. Number of seeds

per pod has the highest loading in principal component four (Table 7).

DISCUSSION

Existence of genetic variation in a population is a decisive factor in common bean breeding program to improve the crop for the desired characters (Ceolin et al., 2007; Lima et al., 2012; Correa et al., 2015). In the present study, the analysis of variance showed the existence of adequate genetic variations among genotypes for all characters, which can be exploited through selection. Lima et al. (2012) and Correa et al. (2015) reported similar results of the current study that common bean genotypes showed

Table 6. Mahalanobis distance between common bean genotypes grouped in six clusters.

Cluster	I	II	III	IV	V	VI
I	0					
II	16.09*	0				
III	70.42***	22.45**	0			
IV	276.01***	164.1****	77.41***	0		
V	17.84*	56.65***	136.5***	399.4***	0	
VI	140.78***	66.86***	18.4*	38.72***	228.8***	0

$\chi 2$= 15.507, 20.090, and 26.13 at $p \leq 5$, 1 and 0.1%, respectively.

Table 7. Eigen vectors and Eigen values of the first four principal components (PCs) for nine characters of speckled type common bean genotypes.

Character	Eigen vectors			
	PC1	PC2	PC3	PC4
Days to 50% flowering	-0.49	0.31	0.05	0.06
Days to 90% physiological maturity	-0.48	0.32	0.05	0.05
Plant height (cm)	-0.10	0.42	0.48	-0.10
No. of pods per plant	0.24	0.41	-0.42	-0.40
No. of seeds per pod	0.28	0.07	0.08	0.83
No. of seeds per plant	0.36	0.41	-0.36	0.05
Hundred seed weight (g)	0.18	-0.36	0.37	-0.34
Grain yield (kg ha^{-1})	0.43	0.21	0.35	-0.12
Common bacterial blight resistance	-0.17	-0.32	-0.45	0.01
Eigen value	2.92	2.40	1.40	1.00
Total variance explained (%)	32.42	26.67	15.52	11.13
Cumulative variance (%)	32.42	59.09	74.61	85.74

PC: Principal component.

considerable variations for days to flowering and maturity, plant height, number of pods plant^{-1}, number of seeds pod^{-1}, mass of 100 seed weight and grain yield. The research results indicated the higher chance of obtaining better genotypes from the newly introduced breeding materials than the currently available commercial varieties (Brown speckled and Cranscop) in the country for most of the agronomically important characters.

In the present study, high genotypic coefficient of variation (GCV) was recorded for grain yield, while high phenotypic coefficient of variation (PCV) number of seeds pod^{-1}, grain yield and common bacterial blight resistance score. Genotypic coefficient of variation was less than its corresponding estimates of PCV for all the characters indicated the significant role of the environment in shaping these traits. Though the calculated PCV was higher than GCV values for all the traits, the magnitude of the differences was low. The narrow magnitude of differences between GCV and PCV values indicating the influence of environment was low in the expression of these characters. This suggested higher chance of improving these traits through selection. Moreover, the characters that exhibited high GCV (grain yield) was most likely improved through selection. The high GCV indicated that the expression is more due to genetic factor than environment (Correa et al., 2015; Rafi and Nath, 2004). The relative wider variation between PCV and GCV for common bacterial blight severity score, number of seeds plant^{-1}, and pods plant^{-1} indicated greater influence of environment in shaping these traits. The result of this research for these traits is in agreement with the observations of other researchers (Chand, 1999; Rafi and Nath, 2004). Correa et al. (2003, 2015) also reported low GCV for days to 50% flowering, days to maturity, number of seeds pod^{-1} and high for seed yield in common bean experiments. Similarly, low PCV and low GCV for days to flowering and days to maturity, high PCV and GCV for seed yield, hundred seed weight and seeds plant^{-1} were reported by Rafi and Nath (2004).

In the present study, broad sense heritability (H^2) estimates were more than 30% for all the characters studied and considered as medium to high. The utility of heritability estimate is, therefore, increased when it is used along with estimate genetic advance (GA) (Johnson

et al., 1955). In this study, high heritability was coupled with high genetic advance for grain yield, while high H^2 and moderate GA were recorded for days to flowering and 100 seed weight. High H^2 with medium to high GA indicated that the character is governed by additive gene action and selection breeding method can be used to improve the traits. For genetic improvement, the selection of parents should be based on the genetic diversity besides *per se* performance. Intercrossing of divergent groups would lead to a wide genetic base in the base population and greater opportunities for crossing over to occur (Thody, 1960). The minimum inter-cluster distance recorded between clusters I and II (D^2= 16.09) indicated close relationship among genotypes included in these clusters. The maximum inter-cluster distance observed between clusters IV and V indicated that genotypes included in these clusters were genetically diverse and if chosen for hybridization program may give broad spectrum of variability in segregating generations.

The principal component analyses (PCA) was used as data reduction tool to summarize the information from phenotypic data so that the influence of noise and outliers on the clustering results is reduced. In this study, the first four PCs explain 85.74% of the total variation. In agreement with this investigation, Vasic et al. (2008) evaluated common bean population and reported that six PCs explained 80% of the total variability. However, other investigators observed low variation in the first two components in common bean (Machado et al., 2002; Rodrigues et al., 2002; Chiorato et al., 2007; Lima et al., 2012) using 12,51, 220, and 100 genotypes, respectively.

In this study, differentiation of the genotypes into different clusters was because of a cumulative effect of a number of characters rather than the contribution of specific few characters (±0.01-0.83). Characters having relatively higher values in each principal component, contributed more to the total variation than lower values in each principal component and they were the ones that most differentiated the clusters. Number of pods plant^{-1} and number of seeds plant^{-1} in PC2; plant height in PC3 and seeds pod^{-1} in PC4 were the major contributors in each PC.

Conclusions

The research result indicated the higher chance of obtaining better genotypes from the newly introduced breeding materials than the currently available commercial varieties (Brown speckled and Cranscop) in Ethiopia for most of the agronomically important characters. This indicated that there is a higher chance of releasing better varieties after multi location trial. The widest inter-cluster distance observed between clusters IV and V indicated that genotypes included in these clusters were genetically diverse and if chosen for hybridization program may give broad spectrum of

variability in segregating generations. The presence of a highly significant genetic distance between the clusters suggests desirable genetic recombination and variation in the subsequent generation from crosses that involve parents from those clusters. Thus, this genetic distance could maximize opportunities for transgressive segregation as there is high probability that genetically wide genotypes would contribute to unique desirable alleles at different loci. Speckled type common bean has a shattering and pollen infertility problem such characters should be considered in the future breeding activity.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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