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MORPHOLOGICAL DIVERSITY OF TROPICAL COMMON BEAN GERMPLASM

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) landraces and varieties grown by farmers in the tropics are a major source of genes and genetic diversity for bean improvement. These materials are, however, threatened by genetic erosion. In this study, we sought to understand the current state of genetic diversity of common bean in Uganda, using the available collection consisting of 284 bean accessions. A field experiment was conducted at the National Crops Resources Research Institute in Namulonge, Uganda. The level of morphological variation estimated with the Shannon Weaver diversity index (H), ranged from 0.47 to 0.58, with an overall mean of 0.56±0.19, an indicator of moderate genetic diversity. Principal component analysis (PCA) clustered the germplasm into three major groups (G1, G2 and G3). The genotypes differed mostly for growth habit, pod cross-section, pod curvature, hypocotyl colour, days to flowering, node number on the main stem, number of flower buds, and 100 seed weight.

Key Words: *Phaseolus vulgaris*, Principal Component Analysis, Shannon Weaver diversity index

RÉSUMÉ

Les cultivars et variétés de haricot commun (*Phaseolus vulgaris* L.) cultivés par les fermiers dans les tropiques son tune source majeur de ènes et diversité génétique pour l'amélioration du haricot. Ce materiel est, par ailleurs, handicapé par une érosion génétique. Le but de cette étude est de comprendre la situation courante de la diversité génétique du haricot commun en Ouganda, en utilisant la collection disponible de 284 accessions de haricots. Un essai était conduit au National Crops Resources Research Institute à Namulonge, Ouganda. Le niveau de variation morphologique estimé à l'aide de l'indice de diversité de Shannon Weaver (H),variait de 0.47 à 0.58, avec une moyenne générale de 0.56±0.19, un indicateur de diversité génétique modéré. L'analyse de la composante principale (PCA) a groupé le germplasme en trois groupes majeurs (G1, G2 et G3). Les génotypes differaient plus par l'habitude de croissance, la section des gousses, la courbature des gousses, la couleur de l'hypocotyle, les jours à la floraison, le nombre de nodes sur la tige principale, le nombre de bourgeon des fleurs et le poids de 100 graines.

Mots Clés: *Phaseolus vulgaris*, Analyse de la Composante Principale, indice de diversité de Shannon Weaver

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a multipurpose diploid (2n = 2x = 22) self-pollinated crop (Stoetzer, 1984) and the most widely grown pulse in eastern and southern Africa (CIAT, 2005). There are two major commercial classes of bean; snap and dry beans (Singh, 2001a; 2001b) with nine types of dry bean majorly grown in the Uganda (Buruchara, 2006). These include: Calima (red flecked) and reds (large and small) accounting for about 50% and with a high market demand. Others are navy, creams, brown-tan, yellow, purple, white and black beans. Morphologically common beans differ in growth habits (Singh, 1982), vegetative characters, flowers, pods and seed traits (Purseglove, 1976; Singh *et al.*, 1991a; 1991b) which are useful for selection in breeding programmes.

Landraces of common bean grown by farmers in the eastern and southern Africa, Uganda inclusive are valuable sources of genetic variation (Blair *et al*., 2010) for breeding. Unfortunately, in Uganda this genetic diversity is threatened by pests and diseases (CIAT, 2005; Mukankusi, 2008) and adoption of elite varieties by farmers is at the expense of the un-popular landraces (Sekabembe, 2010) leading to genetic erosion, consequently narrowing the genetic base of beans in Uganda. Moreover, there is no documented information on current bean genetic diversity in the whole country to guide conservation and breeding priorities.

The objective of this study, was to characterise common bean germplasm collections in Uganda and evaluate phenotypic diversity to inform strategies for effective *In situ* and *Ex situ* conservation and utilisations in bean breeding.

MATERIALS AND METHODS

Plant samples and study location. The materials used in this study were 284 bean accessions, including 15 lines from Colombia, one line from Rwanda and 268 landraces, currently maintained at the National Crops Resources Research Institute (NaCRRI) at Namulonge, Uganda (data not shown). Seven of the accessions were released varieties (Six in Uganda and one in Rwanda). The study was conducted on-station at NaCRRI, located: 0° 321 N, 32° 371 E and 1150 meters above sea level. NaCRRI has an annual mean temperature of 27 °C, relative humidity of 65%, and deep loamy clay soils. The soils are weakly acidic, with pH ranging from 5 to 6, and organic matter levels of 2 to 3% in the surface horizons. The rainfall shows a bimodal pattern, with a tropical wet and mild dry climate (Yada *et al*., 2010).

Experimental design. The experiment was arranged in a randomised complete block design (RCBD), with three replications. Experimental units consisted of two rows of each genotype, measuring 4 meters long with intra- and inter-row spacing of 15 cm and 50 cm, respectively. With each block measuring approximately 284 m by 4 m, we were able to fit all the 284 accessions within each homogenous block. This experimental site was quite flat, with fairly uniform soil fertility. This made it possible to get blocks which could fit several treatments, especially for small size plots as those used in evaluation of germplasm accessions. The genotypes were evaluated in 2 consecutive rainy seasons, first in September 2010 and April 2011.

Morphological traits. Twenty two descriptors of common bean were evaluated according to the International Board for Plant Genetic Resources descriptor list - IBPGR (1982) for *P. vulgaris.* The data were recorded at different stages, from plant emergency to seed harvest on randomly selected plants from each field plot.

The traits studied were: days to emergence (ED), days to flowering (DTFLO), hypocotyl colour (HYPCLR) and emerging cotyledon colour (COTCLR). The flower and plant growth traits included, colour of standard petal (CLRSTD) and colour of wing petals (CLRWG) and plant type (PTP). The pod traits were: pod colour (PDCLR); pod cross section (PDXSC); pod curvature (PDCUV); pod colour at physiological maturity (PDCLRPM). The seed traits were recorded after harvest, and included: seed coat patterns (SDCTPTN); seed coat darker colour (SDCTDC); seed coat lighter colour (SDCTLC); Brilliance of seed (BRLSD); and seed shape (SDSHP). Six quantitative traits studied were: leaflet length (LFL); node number on main stem from base to

first inflorescence (Nodeno); flower buds per inflorescence (FLB/INFLO); pod length (PDLG); locules per pod (LOC/PD); and 100 seeds weight (100Ws).

Data analysis. Numerical values for the categorical traits from the two seasons were coded according to IBPGR descriptor list (1982) for subsequent analysis. Frequency distributions and correlations among traits were elucidated using the PROC CANCORR subprogram of (SAS Institute, 2011). The phenotypic diversity of the traits was analysed with the Shannon-Weaver (1949) Diversity Index (H), given as:

$$H = -\sum_{i=1}^{s} pi \ln (pi)$$

Where:

s is the number of phenotypic classes for a character and *pi* is the relative proportion of the total number of entries (N) in the ***i***th class.

Principal component analysis (PCA) was conducted as reported by Burle (2008) on ranged data with linear dimensionality reduction using SAS (2011) to project the data into lower dimensions and to display genetically related genotypes in clusters (Mohammadi and Prasanna, 2003). The PCA was also used to show the traits which accounted for significant variation in the common bean germplasm.

RESULTS

Distribution of phenotypic characters. All genotypes emerged five days after planting. The mean, maximum and minimum values, of seven quantitative traits among the germplasm for two seasons are shown in Table 1. The traits were significantly different ($P<0.01$) among the genotypes. Mean Leaflet length (LFL) was 8 cm and mean number of nodes on the main stem (Nodeno) was eight. More than 50% of the genotypes flowered after 37 days; while the mean number of flower buds (FLB) was three. Mean length of the pods (PDLG) was 10 cm; number of locules per pod (LOCPOD) was five while the weight of 100 seeds (100Ws) varied widely with mean of 29 grammes.

Qualitatively, most accessions (59%) had green hypocotyl (HYP.CLR), 39% had purple; while 2% were green with purple stripes. The predominantly emerging cotyledon colour (COTCLR) was green (49%), with 28% of the accessions having purple COTCLR, 15% of the genotypes were very pale green, 4% red and only 3% were green purple striped. The predominant plant type (PTP) was indeterminate bush - types II (49%), followed by determinate bush - type I (34%), then semi-climbing - type III (12%) and climbing - type IV least (5%).

In freshly opened flowers, the predominant colour of standard petals (CLRSTD) was white (43%). Others were carmine red (31%), purple (14%) with equal proportions of white with lilac edge and white with red stripes (each with 1%). Most accessions (41%) had white flower wings (CLRWG), while 28% were plain red to dark lilac, and 22% purple. Six percent had white with purple stripes, and very few accessions (0.3%) had strongly veined wings in red to dark lilac.

The predominant fully expanded immature pod colour was green (55%). Others were purple

TABLE 1. Mean, maximum and minimum values of seven morphological traits of 284 tropical common bean germplasm studied

Morphological traits	Mean	Minimum	Maximum	CV%
Leaflet length (LFL)	8	6	11	27.3
Number of nodes on the main stem (Nodeno)	8	4	14	9.5
Days to flowering (DFLO)	37	26	51	11.5
Number of flower buds (FLB)	3	1	6	13.8
Length of pods (PDLG)	10	4	12	23.5
Locules per pod (LOCPOD)	5	2	7	7.8
Weight of 100 seeds (100Ws)	29.0	13.3	51.3	5.3

Length of leaflet and pods were measured in centimeters (cm) and weight of 100 seeds in grammes

stripe on green (17%), carmine stripe on green (11%), red stripe on green (8%), dark purple (7%) and carmine red or pink (2%). The cross-section of fully expanded immature pod (PDXTION) was round elliptic (63%), whereas 35% were pear-shaped, and 2% either very flat or had figure of eight. Most accessions (45%) had yellow pod colour at physiological maturity (PDCLRPM), followed by pale yellow mottling (40%), with a small number of red (7%), pink (5%) and dark purple pods (2%). Fifty six percent (56%) of the germplasm had curved pods (PDCUV), 14% had straight pods, 27% were slightly curved and few recurved (3%). A total of 45.3% of the germplasm had no seed coat pattern. The dominant seed coat colour was maroon (18%). The dominant seed shape was cuboid with a frequency of 46%.

Phenotypic trait correlations. The results of pair wise correlations among traits are shown in Table 2. The most strongly correlated traits were colour of standard petal (CLRSTD) with colour of wing petal (CLRWG) (r = 0.96). Plant type (PTYP) was correlated (r = 0.61) with number of nodes on the main stem (Nodeno). Seed coat darker colour (SDCTDCLR) had moderate positive correlation with seed coat lighter colour (SDCTLCLR) (r=0.52). Correlations were moderate (r = 0.48) between weight of 100 seeds (100Ws) and pod length (PDLG), but negative (r = -0.60 and -0.59) with days to flowering (DFLO) and locules formed per pod (LOCPOD), respectively.

Morphogical diversity. Results from the Principal components analysis (PCA) and Shannon-Weaver diversity index (H) values for the 22 traits studied are presented in Table 3. The H value ranged from 0.47 - 0.58, with a mean of 0.56±0.19. The PCA reduced the data to a few dimensions and explained 35.3% of total phenotypic variation in the germplasm. The first 2 Principal components (PCs) with eigen-value (latent roots) greater than 2.0, contributed most of the total variation in the germplasm.

The 284 accessions occurred in three major clusters (eclipses: G1, G2 and G3), with some genotypes occurring as sub-groups, for example genotypes U369 and U40, positioned between the three eclipses (Fig. 1). The G1 group was predominated by beans with medium to large seed size and plant types I and II (short bushes). Some large seeded released varieties such as NABE 12C and NABE14 were, however, positioned in eclipse G1.

The G2 group was predominated by genotypes with low 100 seeds weight for example U126 (22.03 g), U112 (27.43 g) and plant types II and III. The G3 group was dominated by semi-climbers and climbers (types IV) without a clear distinction in categories of seed size. For example some genotypes like R5U410 was small seeded while U369 was large seeded.

DISCUSSION

Common bean germplasm in Uganda has broad genetic diversity and is morphologically diverse (Table 1). This is due to the inherent genetic differences among the accessions and variations in light, temperature and moisture regimes (Morakinyo and Ajibade, 1998) which caused assorted performance of bean germplasm across the two seasons.

Phenotypic trait correlations. There were strong correlations between some traits (Table 2), which allows for simultaneous selections and use of the related traits interchangeably in selection. The strongly correlated traits are possibly under the influence of the same genes or pleiotropic effects (Miko, 2008). Practically, during bean improvement, if two strongly correlated traits are desired, they can both be selected simultaneously basing on one of the traits. For example, the positive correlations between 100 seed weight and pod length indicates that the length of the pod can be used to determine grain density and yield. On the other hand, the selection for 100 seed weight would lead to late flowering and low locules per pod since these traits were negatively correlated. The near to unit correlations (0.96) of wing and standard petal colours suggests that these traits are controlled by one gene or are very closely linked.

Morphogical diversity. PCA results illustrated the overall picture of the pattern of genetic diversity of the common germplasm based on morphology. The eigen value formed the basis

TABLE 2. Correlation coefficients among 22 morphological traits of tropical common bean germplasm

Traits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1. HYPCLR	1.00																					
2. COTCL	0.67*	1.0 0																				
3. PTYP	-0.13	-0.12	1.00																			
4. CLRSTD	-0.17	-0.28	-0.12	1.00																		
5. CLRWG	-0.25	-0.32	-0.11	0.96*	1.00																	
6. PODCLR	0.38	0.42	-0.05	-0.16	-0.2	1.00																
7. PODXSC	0.29	0.10	-0.11	0.04	0.00	0.12	1.00															
8. PODCUV	-0.19	-0.10	0.28	-0.19	-0.15	-0.14	-0.18	1.00														
9. PDCLRPM	0.09	0.01	-0.07	0.29	0.27	0.22	0.12	-0.14	1.00													
10. SDCOTP	0.05	0.00	-0.15	0.14	0.13	0.06	0.07	-0.09	0.08	1.00												
11. SDCTDCLR	0.00	0.09	0.09	-0.32	-0.3	0.09	-0.01	0.13	-0.22	-0.11	1.00											
12. SDCTLCLR	0.06	0.04	-0.05	-0.05	-0.06	-0.08	0.18	-0.07	-0.11	-0.26	0.52*	1.00										
13. BRLSD	0.03	-0.08	0.11	0.02	0.01	-0.01	0.10	-0.02	-0.01	0.00	-0.08	-0.09	1.00									
14. SDSHP	-0.17	-0.08	0.11	-0.13	-0.11	-0.01	-0.18	0.09	-0.07	-0.14	0.08	0.02	-0.08	1.00								
15. ED	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00							
16. LFL	-0.17	-0.08	0.11	-0.13	-0.11	-0.01	-0.18	0.09	-0.07	-0.14	0.08	0.02	-0.08	0.00	0.00	1.00						
17. Nodeno	-0.35	-0.22	0.61*	-0.24	-0.18	-0.14	-0.3	0.43	-0.2	-0.19	0.10	-0.1	0.02	0.14	0.00	0.14	1.00					
18. DFLO	-0.29	-0.15	0.35	-0.32	-0.28	-0.05	-0.45	0.35	-0.17	-0.15	0.06	-0.17	-0.05	0.23	0.00	0.23	0.71*	1.00				
19. FLB	0.23	0.19	-0.34	0.02	-0.01	0.18	0.13	-0.26	0.17	0.14	-0.05	0.09	0.01	-0.06	0.00	-0.10	-0.53*	-0.3	1.00			
20. PDLG	0.12	0.09	-0.12	0.05	0.04	0.07	0.15	-0.18	-0.01	-0.06	0.08	0.08	0.09	0.25	0.00	0.25	-0.27	-0.32	0.21	1.00		
21. LOCPOD	-0.3	-0.13	0.28	-0.39	-0.33	-0.17	-0.32	0.41	-0.25	-0.16	0.1	-0.07	-0.04	0.16	0.00	0.16	0.55*	0.61*	-0.20	-0.09	1.00	
22. 100Ws	**0.28**	**0.15**	**-0.15**	**0.24**	**0.22**	**0.06**	**0.42**	**-0.32**	**0.16**	**0.05**	**-0.04**	**0.07**	**0.09**	**-0.05**	**0.00**	**-0.1**	**-0.39**	**-0.6***	**0.12**	**0.48**	**-0.59***	**1.00**

*Significant correlations (P<0.05)

TABLE 3. **E**igen-values of the first two principal component axes (PC) and Shannon-Weaver diversity index (H) estimates for the 22 traits used to classify the bean germplasm

Character	Principal Component Axes		H
	PC1	PC2	
Hypocotyl colour (HYPCLR)	**0.21**	**0.35**	0.57
Cotyledon colour (COTCLR)	0.13	**0.40**	0.57
Plant type (PTYP)	**-0.24**	-0.03	0.57
Standard petal colour (CLRSTD)	**0.2**	**-0.46**	0.55
Wing petal colour (CLRWG)	0.17	**-0.48**	0.55
Pod colour (PODCLR)	0.1	0.28	0.57
Pod crossection (PODXSC)	**0.23**	0.10	0.57
Pod curvature (PODCUV)	**-0.26**	-0.01	0.57
Pod colour at physiological maturity (PDCLRPM)	0.16	-0.12	0.57
Seed coat pattern (SDCOTP)	0.12	-0.09	0.57
Seed coat darker colour (SDCTDCLR)	-0.09	**0.27**	0.47
Seed coat lighter colour SDCTLCLR	0.04	0.17	0.47
Brilliance of the seed (BRLSD)	0.03	-0.04	0.57
Seed shape (SDSHP)	-0.15	0.08	0.57
Days for seedling emergency (ED)	0.00	0.00	0.58
Leaflet length (LFL)	-0.15	0.08	0.58
Number of nodes on the main stem (Nodeno)	**-0.38**	-0.07	0.57
Days to flowering (DFLO)	**-0.38**	0.00	0.58
Number of flower buds (FLB)	**0.22**	0.13	0.57
Length of the pods (PDLG)	0.15	0.11	0.58
Locules per pod (LOCPOD)	**-0.35**	0.05	0.58
Weight of 100 seeds (100Ws)	**0.31**	0.03	0.57
Eigen-value/latent roots for each PC	4.63	2.79	
Variation in Percentage (%) for each PC	22.03	13.28	
Mean diversity index (H)			0.56±0.19

Principal component axes 1 and 2 and traits with eign values set arbitrarily above 0.2 (highlighted), explained 35.31% of total variation in the bean germplasm. Shannon-Weaver diversity index (H) estimates show a high diversity of germplasm in the tropics with quantitative traits more diverse

for identifying component axes (Panthee *et al*., 2006) with scores, cut off level arbitrarily set above 0.2 to show traits, which explained most variations in the germplasm. For instance, considering only PCA eign values in PC score 1, most genotypes, had pigmented hypocotyls and standard petals, curved pods, late flowering, non climbing with short internodes, form few flower buds, large seeded and increased 100 seed weight. This suggests that the traits above are the most important for future common bean characterisation and conservation studies.

According to Koinange *et al.* (1996), plant type and seed size are important for pre- and post-common bean crop's domestication and were employed logically to help interpretation of trait distributions among the genotypes in the PCA plot. The PCA clustering pattern (eclipses: G1, G2 and G3) point to possible existence of a third group or sub-gene pool within the tropical bean germplasm being conserved in Uganda, which could be a result of mutations or gene pool hybridisation. The separation along the first PC axis (eclipses G1 and G2-G3) is a possible gene pool split between Andean and Mesoamerican gene pools, which were reported to differ in morphology, for example in leaf size, shape, plant type and seed sizes (Gepts and Debouck, 1991; Duran *et al*., 2005).

The mean Shannon diversity index (H) estimate of 0.56±0.19 indicates that different classes of traits and genotypes have a balanced

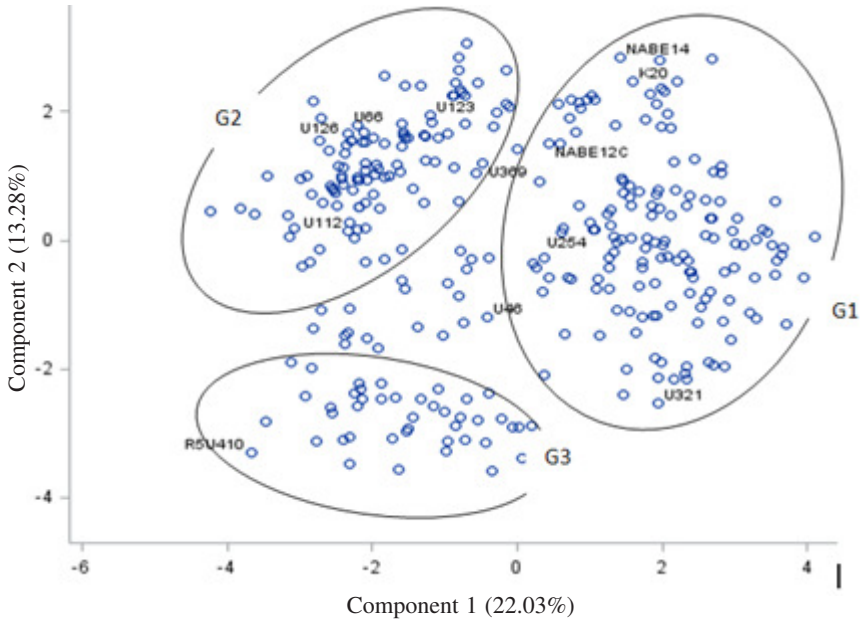

Figure 1. Two dimension plot of Principal Component Analysis (PCA) clustering based on morphological similarity of 284 common bean germplasm in Uganda.

representation from the places of collection. Yada *et al.* (2010) used Shannon-Weaver diversity (H) analysis index on 1256 sweet potato accession using 20 morphological descriptors and reported mean H of 0.71±0.03 and inferred high diversity among the sweet potato clones from Uganda.

The traits observed as critical for bean characterisation in this study like growth habit, seed size and flower colour, were also found to be important in beans from Ethiopia and Kenya (Asfaw *et al.*, 2009), which indicates similar diversity manifestation in the East African region. Blair *et al.* (2010) observed considerable variations in landraces in Central Africa, in seed size and colour predominated by the red mottled types which was very frequent in this study.

In other studies on common bean, Stoilova *et al*. (2005) characterised 30 landraces from Portugal and Bulgaria, using the IPBRI descriptor for *P. vulgaris* and identified suitable accessions for breeding purposes. Duran *et al.* (2005) also phenotyped 56 bean landraces and cultivars from the Caribbean and distinguished Mesoamerican and Andean gene pools.

The high diversity of bean germplasm observed in this study, is in part due to farmer's customary seed exchanges (CIAT, 2005). Blair *et al.* (2010) reported farmer's preference for many landraces, where diversified bean types are used for various agronomic and cultural reasons (David and Sperling, 1999). In addition, varieties preferred for home cooking with unique seed colours are selected for sale in the local markets, hence, maintaining bean diversity in the tropics. Frequent mutations and genetic recombination are the other possible causes of high diversity of the bean germplasm studied.

CONCLUSION

There is a broad genetic diversity of bean germplasm in Uganda. The germplasm clustered into three major groups with most variations attributed to growth habit, pod cross-section, pod curvature, hypocotyl colour, days to flowering, node number on the main stem, number of flower buds and 100 seed weight. The above traits are highly recommended for use in common bean characterisation, conservation and breeding. The selection of parental materials for breeding can be performed on basis of the similarity of the clustering and correlation information of the phenotypic traits.

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