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Seed Morphometric characterization and Multivariate Analysis of French Bean Germplasm

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ABSTRACT: Germplasm collection, evaluation and minimizing the duplications are key consideration for crop improvement, conservation and exploitation in French bean as wide variation existed in gene pool due to geographical separation. Genetic resources comprised of twenty-seven diverse French bean genotypes including primitive, commercial cultivars, local types collected from different parts of India. Seven qualitative and quantitative seed traits of genotypes were subjected to DUS morphometric test and PCA to disclose its genetic diversity present in them. The screened panel revealed wide variations among genotypes for seed testa colour, seed shape, seed coat pattern, 100 seeds weight, seed length and single seed weight. Six types of seed testa colour was observed in which brown colour was predominate (40.74%) and cuboid seed shape (44.45%) had dominance over five shapes i.e., cuboid, oval, truncate fastigate, kidney shape and truncate. The first four principal components explained 99.27% of total variability. Cluster analysis plotted 27 genotypes into five clusters based on Euclidean distance. Cluster C3 comprised of maximum genotypes (12) whereas, C1, C2, C4 and C5 contained 6, 3, 4 and 2 genotypes respectively. Seed weight contributed maximum to total variation. These findings on seed traits can be used for developing elite French bean cultivar(s) with quality seeds. Furthermore, the study will be advantageous for identifying elite genotypes useful for forthcoming cop improvement in French bean for seeds traits.

Keywords: DUS, PCA, Euclidean, 100 seed weight, cuboid.

INTRODUCTION

The French bean is christened as common bean, snap bean, salad bean, kidney bean, haricot bean and string bean (Purseglove, 1968; Gepts and Debouck 1991; Wortmann, 2006). It is a member of the Fabaceae family with a chromosome number of 2n = 2x = 22. French bean is native to Central and South America (Vavilov, 1950). Its genesis is from the wild species, *Phaseolus aborigineus* L. (Smartt, 1969). It is cultivated for its tender, green pods which are either consumed fresh or processed into canned, frozen, or freeze-dried goods. As a food source, the crop offers essential vitamins like folic acid and thiamine (Petry *et al*., 2015), proteins, fibers, vitamins and minerals (Thamburaj and Singh 2016; Broughton *et al*., 2003; Mora- Avilés *et al*., 2007), flavonoids, antioxidants, and beneficial phytochemicals (FAO, 1999). Additionally, the stems are useful for livestock feed (Wondatir and Mekasha 2014).

The most crucial aspect of any crop production programme is variability, which serves as the foundation upon which all other crop improvement is based Goutam *et al*. (2001). Therefore, it is extremely difficult to develop desirable cultivars or varieties without adopting a specific genotype with good genetic potential. A significant morpho-physiological variations are observed in French beans with regards to plant physiology and stature, seed traits (such as shape, size and colour), comparative timeframe of the breeding cycle, and numerous additional quantitative and qualitative characters which allow farmers to choose best genotypes for cultivation and helps breeders for further crop improvement. A diverse characteristic is observed in French bean seeds (colours, shapes, and sizes) with an average test weight of 150-900 g (Wortmann, 2006). Singh *et al*. (1991) revealed that Andean groups in French bean are characterized with large or medium seed morphology (> 40g/100seeds) and Mesoamerican group genotypes have mostly small seeds (< 25g/100seeds).

In order to improve crops, germplasm evaluation is crucial. The DUS (Distinctness, Uniformity, and Stability) assessment mandated under the Plant Varieties and Farmers Right Act (PPV & FRA 2001), is crucial for varietal registration. It is a useful method for identifying and preventing duplication is DUS characterization of crop genotypes (Das and Kumar, 2012). The morphological characterization of genotypes facilitates in the development of a database that can be helpful for identification and assessment of the genetic variation present in the genotypes. High genetic diversity in French bean is available in India. Many local varieties and landraces have not been fully utilized in genetic improvement programme. Thus, the objective of the study was to collect elite French bean genotypes from various parts of India and to evaluate them for seed characteristics which could be applicable for future breeding and varietal identification.

MATERIAL AND METHODS

The plant genetic materials for investigation comprised of twenty-seven French bean genotypes collected from different parts of India (Table 1) trough exploration trips. The genotypes were conserved and subjected to preliminary characterization based on fourteen seed morphometric traits at Central Horticulture Experiment Station (CHES), Indian Institute of Horticulture Research- ICAR during *rabi* of 2019-2020 and 2020-2021. The site is located at 20.015°N latitude, 85.053°E longitude and 25.5 m above mean sea level. The characterization and evaluation were conducted as per international French bean descriptor of the International Board for Plant Genetic Resources (IBPGR, 1982), guidelines for the conduct of test for Distinctness, Uniformity, and Stability on French bean (PPV & FR authority, GOI, 2007) and other descriptors were considered from literatures. In total seven qualitative (Table 2) and seven quantitative seed traits were recorded (Table 3). Statistical analysis was performed as per Panse and Sukhatm (1985) using the online software; KAU GRAPES version 1.1.0. Cluster Analysis based on quantitative seed traits was done using the online software PBSTAT-CL 2.1.1.

RESULTS AND DISCUSSION

Seed morphometric traits. A preliminary characterization of the genotypes was conducted for important seed qualitative traits *viz*., testa colour, seed shape, testa variegation, seed coat pattern, hilum color, brilliance of seed, seed veining (Table 2) and quantitative traits *viz*., 100 seed weight (g), Single seed weight (g), seed length (mm), seed width (mm), seed thickness (mm), seed length to width ratio and seed width to thickness ratio as per DUS guidelines and French bean plant descriptor (IBPGR, 1982).

Table 1: List of collected genotypes considered for investigation.

Acronym	Genotypes/ Variety	Type	Site of collection
G1	IC 632961	Local type	Raikia, Kandhamal, Odisha
G2	IIHR-B-PV-26	Primitive	Araku Velly, Andhra Pradesh
G3	IIHR-B-PV-16	Primitive	Guptakashi, Uttarakhand
G4	IIHR-B-PV-4	Primitive	
G5	IIHR-B-PV-5	Primitive	
G6	IIHR-B-PV-9	Primitive	
G7	IIHR-B-PV-11	Primitive	
G8	IIHR-B-PV-12	Primitive	
G9	IIHR-B-PV-15	Primitive	
G10	Arka Sukomal	Commercial cultivar	IIHR, Bengaluru, Karnataka
G11	IIHR-B-PV-17	Primitive	Guptakashi, Uttarakhand
G12	IIHR-B-PV-20	Primitive	
G13	IIHR-B-PV-21	Primitive	
G14	IIHR-B-PV-22	Primitive	
G15	IIHR-B-PV-24	Primitive	
G16	IIHR-B-PV-25	Primitive	
G17	Arka Arjun	Commercial cultivar	IIHR, Bengaluru, Karnataka
G18	IIHR-B-PV-27	Primitive	ArakuVelly, Andhra Pradesh
G19	IIHR-B-PV-29	Primitive	
G20	IIHR-B-PV-30	Primitive	
G21	Anupam	Commercial cultivar	Bhubaneswar, Odisha
G22	Ranar	Commercial cultivar	
G23	Phulbani local	Local type	Phulbani, Kandhamal, Odisha
G24	Ayoka	Commercial cultivar	
G25	Phalguni	Commercial cultivar	Bhubaneswar, Odisha
G26	Baisnavi	Commercial cultivar	Phulbani, Kandhamal, Odisha
G27	Angul local	Local type	Angul, Odisha

Seed testa colour. Seed testa colour is a crucial identifiable visual trait which is utilized to distinguish and establish distinctness of different genotypes. In the present investigation, different French bean genotypes observed with varied seed testa colour (Table 2; Fig. 1a and Plate 1). The genotypes are grouped under five categories based on seed testa colour *viz.*, white, black, brown, dark brown, maroon, pale to dark. Majority of the genotypes were observed with brown seed testa colour with higher frequency (40.74%) among other colours. The recorded observation corroborated with findings of many researchers. French bean genotypes show high phenotypic variation in seed testa colour due to broad genetic base (Beebe *et al.*, 2000; Serna *et al.*, 2001; Corte *et al.*, 2010; Bode *et al.*, 2013; Gill *et al.*, 2014; Singh *et al.*, 2014; Kanwar and Mehta 2018;

Kalauni *et al*., 2020). Prashanth (2003) screened French bean varieties for seed testa colour and inferred that Black, brown, and white seed coats are the most common. Silva and Costa, (2003) categorized bean seeds as per single colour i.e., primary color like red, black, beige, white etc. and a secondary color with stripes, spots and streaks on seed surface. Neupane *et al*. (2008); Ashok *et al*. (2008) also utilized seed testa colour to distinguished French bean genotypes. Blair *et al.* (2009) reported predominant black and cream seed colour among French bean genotypes belongs to Mesoamerican and Andean centers of origin of Central and South America respectively. Red seed testa colour in French bean is abundant and preferred by most of consumer as reported by many scientists (Asfaw *et al*., 2009; Blair *et al.*, 2010; de Albuquerque *et al*., 2011; Ghafoor and Arshad 2011; Sultan *et al*., 2014; Tsutsumi *et al*., 2015; Jan *et al*., 2021) and some scientists reported white seed color as predominant (Piergiovanni and Lioi 2010; Stoilova *et al*., 2013; Rana *et al*., 2015). Caldas and Blair (2009) correlated tannins contents in seed with its seed testa color. Delfini *et al*. (2018); Pereira *et al*. (2019); Nogueira *et al*. (2021) correlated the seed testa colour with yield potential of French bean genotypes.

Seed shape. Seed shape is an important trait which drives the consumer preference in the market as well as farmer choice for raising crop for which it is essential to categories French bean genotypes to tune of market demand and choice of farmer by vivid screening and characterization. The French bean genotypes under study were categorized as per seed shape *viz*., cuboid, oval, truncate and kidney shaped (Table 2; Fig. 1b and Plate 1). The study revealed twelve genotypes with cuboid seed shape with higher frequency (44.45%), four genotypes with oval seed shape, two genotypes with truncate fastigate seed shape, eight genotypes with kidney seed shape and one genotype with cuboid and truncate seed shape. Seed shape is an important trait for morphological characterization of French bean germplasm as adopted by many scientists (Rodino *et al*., 2003; Rai *et al*., 2006; Rodino *et al*., 2006; Logozzo *et al*. 2007; Ashok *et al*., 2008; Cabral *et al*., 2010; Lioi *et al*., 2012).

Table 2: Characterization based on qualitative seed traits of French bean genotypes.

Sr. No.	Genotypes	Testa colour	Seed shape	Testa variegation	Seed coat pattern	Hilum color	Brilliance of seed	Seed veining
1.	IC 632961	Brown	Cuboid	Absent	Absent	White	Shiny	Strong
2.	PV-26	Maroon	Oval	Absent	Absent	White	Shiny	Weak
3.	PV-16	Brown	Cuboid	Present	Circular mottling	White	Shiny	Weak
4.	PV-4	Brown	Truncate fastigate	Absent	Absent	White	Matt	Weak
5.	PV-5	Dark brown	Oval	Absent	Absent	White	Shiny	Weak
6.	PV-9	Black	Oval	Absent	Absent	White	Shiny	Medium
7.	PV-11	Brown	Cuboid	Present	Stripped	White	Shiny	Weak
8.	PV-12	Brown	Cuboid	Present	Broad stripped	White	Shiny	Weak
9.	PV-15	Brown	Cuboid	Present	Constant mottled	White	Medium	Weak
10.	Arka Sukomal	White	Kidney shaped	Absent	Absent	Yellow	Shiny	Strong
11.	PV-17	Dark brown	Cuboid	Present	Stripped	White	Shiny	Strong
12.	PV-20	Brown	Truncate fastigate	Present	Rhombobatic spotted	White	Matt	Medium
13.	PV-21	Brown	Cuboid	Absent	Absent	White	Shiny	Medium
14.	PV-22	Dark brown	Cuboid & truncate fastigate	Absent	Absent	White	Matt	Weak
15.	PV-24	Dark brown	Cuboid	Present	Rhombobatic spotted	White	Shiny	Weak
16.	PV-25	Brown	Kidney shaped	Present	Circular mottling	White	Shiny	Medium
17.	Arka Arjun	White	Kidney shaped	Absent	Absent	Yellow	Shiny	Medium
18.	PV-27	Maroon	Oval	Absent	Absent	White	Shiny	Weak
19.	PV-29	Brown	Kidney shaped	Present	Circular mottled	White	Medium	Weak
20.	PV-30	Brown	Kidney shaped	Absent	Absent	White	Shiny	Weak
21.	Anupam	Pale to dark	Cuboid	Absent	Absent	White	Shiny	Weak
22.	Ranar	Black	Kidney shaped	Absent	Absent	White	Medium	Medium
23.	Phulbani local	White	Cuboid	Absent	Absent	White	Shiny	Medium
24.	Ayoka	Black	Kidney shaped	Absent	Absent	White	Shiny	Weak
25.	Phalguni	White	Cuboid	Absent	Absent	White	Shiny	Strong
26.	Baisnavi	White	Cuboid	Absent	Absent	White	Medium	Medium
27.	Angul local	Black	Kidney shaped	Absent	Absent	White	Shiny	Medium

Plate 1: Seed characteristics of French bean genotypes collected from different parts of India.

Table 3: Statistics of quantitative seed traits of French bean (*Phaseolus vulgaris* L.) genotypes

Characteristics	Range	Mean ± SD	CV (%)
100 seed weight (g)	14.95-46.52	26.63 ± 3.06	14.11
Single seed weight (g)	0.15-0.47	0.53 ± 0.03	14.2
Seed length (L) (mm)	8.51-14.1	11.05 ± 1.01	11.14
Seed width (W) (mm)	4.62-8.35	6.52 ± 0.46	11.46
Seed thickness (T) (mm)	4.31-5.82	4.91 ± 0.52	9.81
L/W	1.35-2.13	1.66 ± 0.19	14.1
W/T	0.91-1.67	1.36 ± 0.17	15.59

SD= standard deviation, CV= coefficient of variation, L/W= seed length/ seed width ratio, W/T= seed width/seed thickness ratio

A wide phenotypic variability is seen in French bean with respect to seed shape due to its broad genetic base (Thomas *et al.*, 2002; Prashanth, 2003; Magloir, 2005; Corte *et al.*, 2010; de Albuquerque *et al.*, 2011; Ghafoor and Arshad 2011; Bode *et al*. 2013; Kanwar and Mehta 2017; Tsutsumi *et al.*, 2015; Kalauni *et al.*, 2020). Stoilova *et al*. (2013) witnessed three dominant seed shapes *viz.*, kidney shaped, cuboid and oval.

Similarly, Boros *et al*. (2014) also categorized 22 common bean genotypes as round, oval, kidney and cuboid. Sultan *et al*. (2014) found red seed colour and cuboid seed shape (Okii *et al.*, 2014) are dominant traits in French bean. Oblong seed shape is important for breeding in carioca-type beans (Pereira *et al*., 2019). Nawaz *et al*. (2020) observed significant variation in French bean for seed shape and inferred that most of accessions are predominant with cuboid seed shape whereas other accessions were oval, truncate fastigiated and kidney shape. Sinkovic *et al*. (2019) evaluated 953 accessions of French bean as per seed shape and grouped the accessions into two groups namely, Mesoamerican and Andean gene pool. Jan *et al*. (2021) categorized 109 French bean genotypes into kidney (26.6%), cuboidal (28.4%), circular to elliptical seed shapes (28.4%) which were found most predominant.

Seed coat pattern. The present investigation revealed nine French bean genotypes with presence of seed coat variation i.e., circular mottling, stripted, broad stripted, constant mottled, rhombobatic spotted whereas other genotypes show no seed coat variation (Table 2, Plate 1). The finding is corroborated with Nawaz *et al*. (2020) who inferred that most of accessions are predominant with absent in seed coat pattern whereas other accessions are varied with five different seed coat patterns i.e., constant mottled, stripped, circular mottling, rhomboid spotted and speckled.

Hilum colour. The hilum functions as the point of attachment of seed to the pod. The hilum colour has no agronomical significance but it is taken into consideration to distinguished French bean genotypes. The present study witnessed all genotypes with white hilum colour (Table 2). Islam *et al*. (2006) evaluated 1105 French bean accessions for hilum coloration. Ashok *et al*. (2008) adopted hilum colour to screen and group seven French bean germplasms. The finding corroborated with Nassar *et al*. (2010); Kanwar and Mehta (2017) in French bean.

Hypocotyl pigmentation. The study revealed seven genotypes with presence of purple hypocotyl colour and other twenty genotypes observed with creamish white hypocotyl pigmentation (Table 2). According to Chandrashekhar (2005), the distinct French bean genotypes exhibit purple, light green, light purple, and pale green hypocotyl colour expression. Neupane *et al*. (2008) grouped 100 local and exotic French bean germplasms for hypocotyl colour which includes 63 accessions green, 25 purple and 6 have other pigments in hypocotyls. Ashok *et al*. (2008); Okii *et al*. (2014); Kanwar and Mehta (2017) screened French bean genotypes and found substantial differences in hypocotyls pigmentation among genotypes.

100 seed weight (g). The present investigation revealed wide variation in seed traits measured quantitatively among all French bean genotypes. Seed weight is a vital yield associated trait which also influences the germination, vigour and performance of the seedling after sowing. The statistical studies on 100 seed weight of 27 French bean genotypes are mentioned in Table 3. Perusal of the data revealed maximum 100 seed weight (46.52g) was recorded in PV-17 genotype whereas, minimum 100 seed weight (14.95 g) was recorded in Phalguni variety with grand mean for the population was 26.63 g. The average values of the genotypes for the studied traits were significantly different ($p \leq 0.05$) as presented in Figure 2(a). Based on 100 seed weight, twenty-three genotypes were grouped as light seed weight (<32.5g), three as medium weight (32.5 to 42.5g) and one as heavy seed weight(>42.5g) as presented in Figure 1d (Murube *et al*., 2021). Singh (1989) divided the seed size into three categories, small (<25g/100seeds), median (25-40g/100seeds) and large (> 40g/100seeds). Kwak and Gepts (2009) reported two main gene pools of French bean *i.e.,* Mesoamerican beans are small seeded (< 25g/100seeds) and Andean beans have larger seed size (> 40g/100seeds). Many scientists witnessed a substantial variability for 100 seed weight in screening of French bean genotypes (Stoilova *et al*., 2013; Kumar *et al*., 2014; Rana *et al*., 2015; Kanwar and Mehta 2017; Saba, 2017; Kalauni *et al*., 2020; Sinkovic *et al*., 2019; Kanwar *et al*., 2020). Katuuramu *et al*. (2020) reported 100 seed weight varied from 19–63 g in 15 French bean genotypes and also inferred that most of the determinate genotypes are large kidney seed (Andean type) weighing over 35g per 100 seeds.

Single seed weight (g). Studied French bean genotypes were observed with wide variation for average single seed weight ranges from 0.15-0.47g (Table 3). Maximum seed weight was recorded in the genotype PV-17 whereas, minimum seed weight in variety Phalguni and the mean values were statistically significant (Fig. 2b). Thomas *et al*. (2002) witnessed a wider phenotypic variation in French beans for seed weight on genetic variability evaluation. Singh *et al*. (2014) emphasized seed weight as important morphological markers for characterization of French bean genotypes. Caproni *et al*., (2019) reported that seed weight recorded significant difference among 192 genotypes where Mesoamerican genotypes characterized by lighter seeds than Andeans.

Seed size. Screening genotypes based on seed size is important to lay out future breeding programme for fulfilling the selective market needs of concerned community. The statistical data on seed size of twenty-seven French bean genotypes (Table 3) depicted maximum seed length (14.10 mm), seed width (8.35 mm), seed thickness (5.82 mm) in genotype PV-30, 15, 20 whereas, minimum seed size (SL × SW × ST) (8.51 mm) × (4.62 mm) × (4.31 mm) recorded in genotype PV-27, Phulbani local, PV-5 respectively with statistically significant indicated the genotypes were highly variable for the studied character (Fig. 2c, d, e). The genotypes were categorized in three classes i.e., 14 genotypes as small (<11.0 mm), 9 as medium (11.0 to 12.5 mm), 3 as large (>12.5 mm) for SL, 23 as medium (4 to 5.5 mm), 4 as large (>5.5 mm) for SW and 8 as small (<6 mm), 15 as medium (6 to 7.5 mm), 3 as large (>7.5 mm) for SH (Figure 1e, 2f, 2g). Maass and Usongo (2007) observed that seed sizes ranged from 5.7 to 14.3 mm in length and 4.0-8.6 mm in width in 18 different germplasm accessions of hyacinth bean

(*Lablab purpureus*). Majority of panels studied and revealed genotypic differences on seed length, width and thicknes straits in French bean (Logozzo *et al*., 2007; Giurca, 2009; Nassar *et al*., 2010; Mazhar *et al*., 2013; Kumar *et al*., 2014; Rana *et al*., 2015; Kanwar and Mehta 2017; Saba, 2017; Sinkovič *et al*., 2019). Pandey *et al*. (2011) revealed that pole type French bean genotypic differs based on seed length.

Multivariate analysis. A statistical method for multivariate analysis called principal component analysis (PCA) is used to estimate and decompose complicated and large datasets. The Fisher's least significant difference (LSD) test was performed for seven quantitative seed traits to enumerate the relation between the traits and which seed characteristics are significantly different from others. The findings revealed that 100 seed weight and single seed weight had statistically significant correlation with all seed characteristics. The PCA analysis was used to obtain specific number of linear combinations for variables seed traits. As per Eigen value (≥1) two components were obtained for 27 French bean genotypes which together comprised 81.83% of the variability of original data (Table 4).

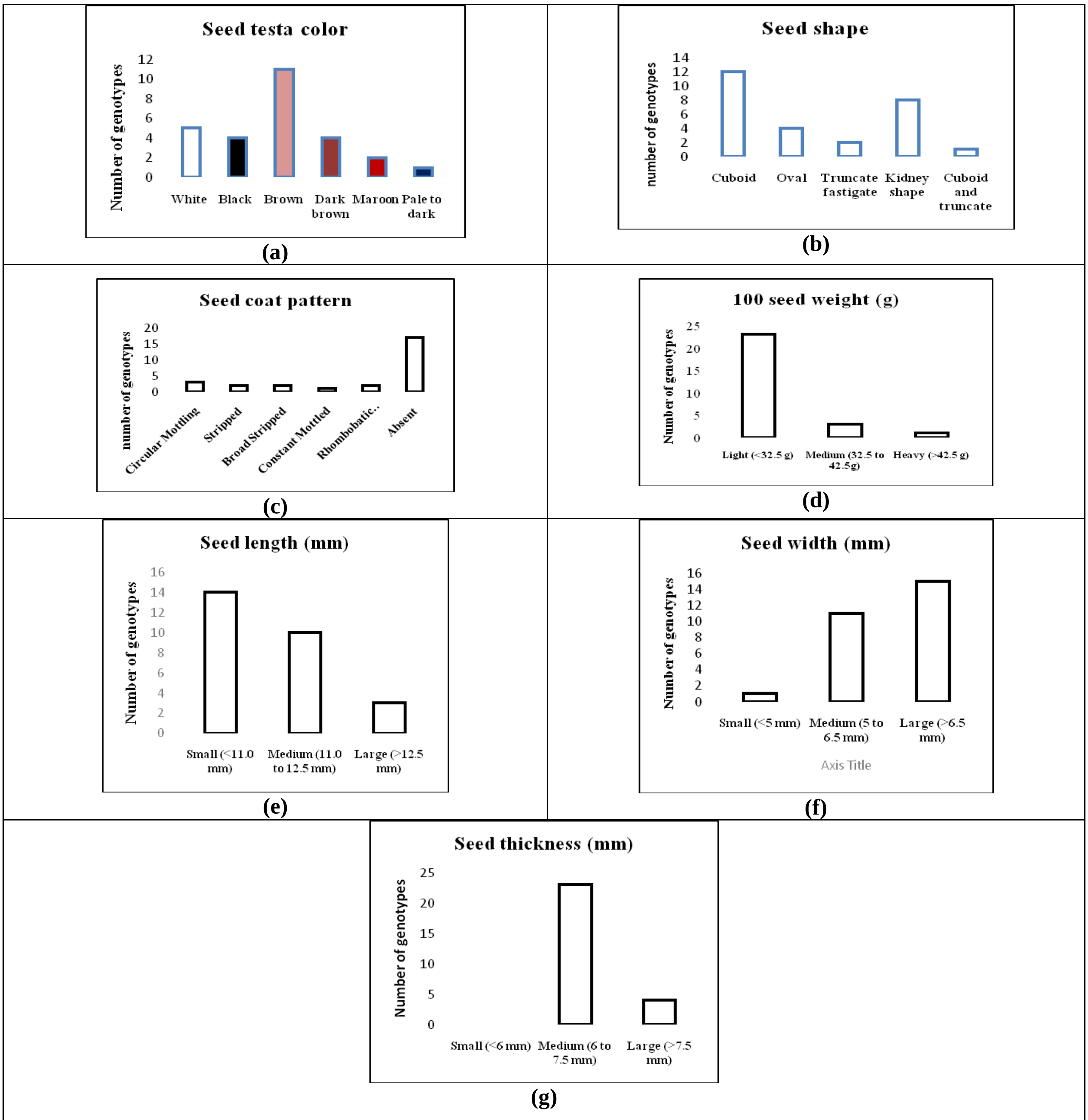

Fig. 1. Frequency distribution of French bean genotypes for different seed traits.

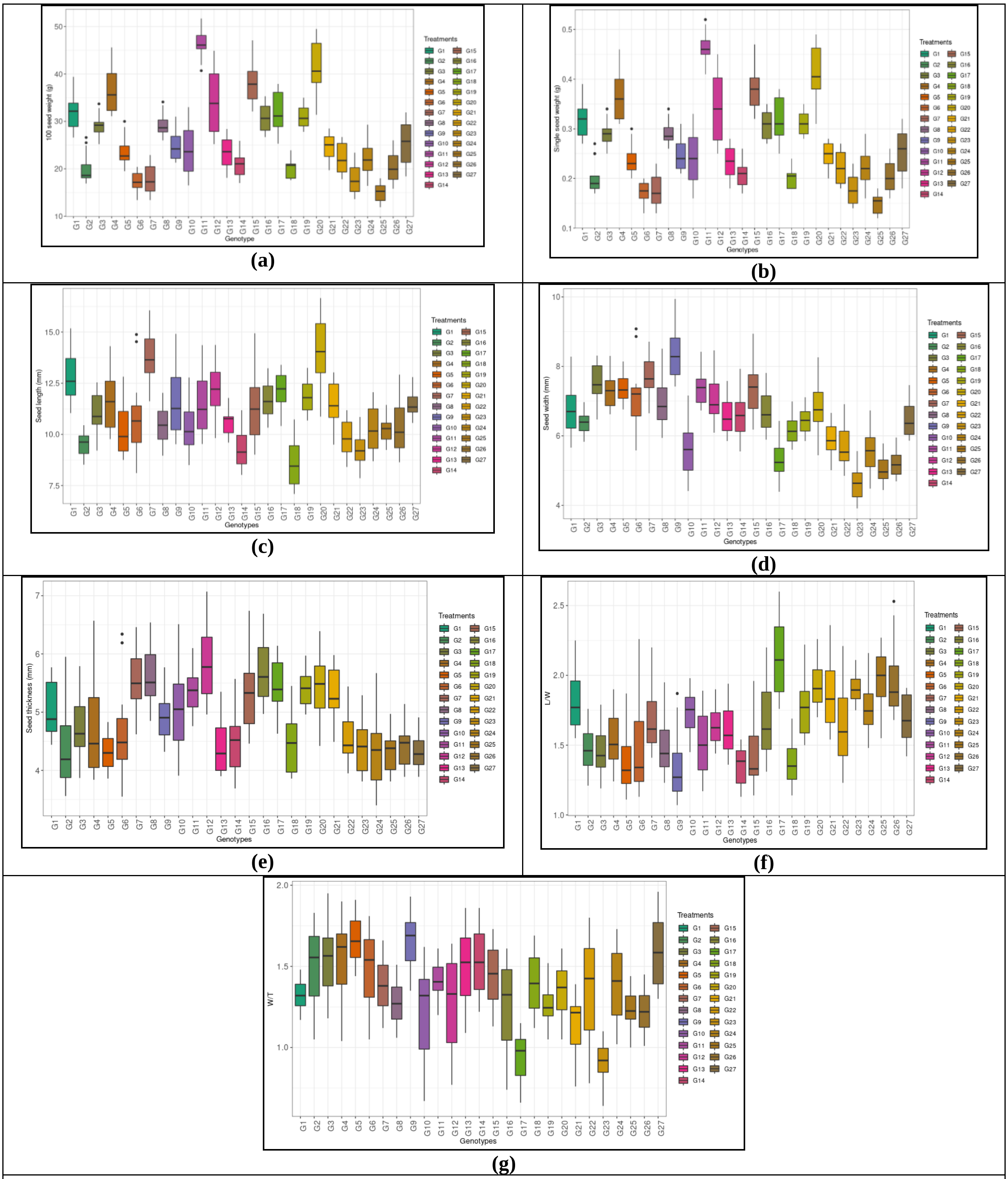

Fig. 2. Box plots (a-g) showing the distribution seed traits of 27 French bean genotypes according to genetic structure groups.

The PCA biplot (Fig. 3) depicted Component 1 (PC1) and 2 (PC2) which explained 81.8% of the total variance for the variable seed traits in the germplasm. The Component 1 explained 47% and Component 2, 34.8% of the total variance. 100 seed weight, single seed weight, seed length, seed height contributed more to Component 1 whereas, seed width, L/W and W/T contributed to Component 2 (Fig. 4). The genotypes were well separated into 2 major group (Fig. 3).

Cluster analysis presented 27 genotypes into five cluster (Fig. 4). The Cluster C3 comprised of highest 12 genotypes whereas, C1, C2, C4 and C5 contained 6, 3, 4 and 2 genotypes respectively. Perusal of correlation of variables on principal components revealed that 100 seed weight and single seed weight were significantly correlation on Component 1 along with seed length and seed thickness contribute more towards Component 1 (Fig. 5).

Table 4: Extracted Eigenvalues and correlation values for quantitative seed traits with the first three principal components.

Variables	Principles components		
	PC1	PC2	PC3
Extracted Eigen values	3.29	2.44	0.73
Explained variance (%)	47.04	34.79	10.36
Cumulative variance (%)	47.04	81.83	92.19
Quantitative seed traits			
HSW	24.93	0.31	21.09
SL	17.66	3.20	38.69
SW	13.15	19.29	10.18
SH	18.52	5.41	3.55
SSW	24.95	0.36	21.01
L/W	0.24	35.72	3.76
W/T	0.54	35.71	1.71

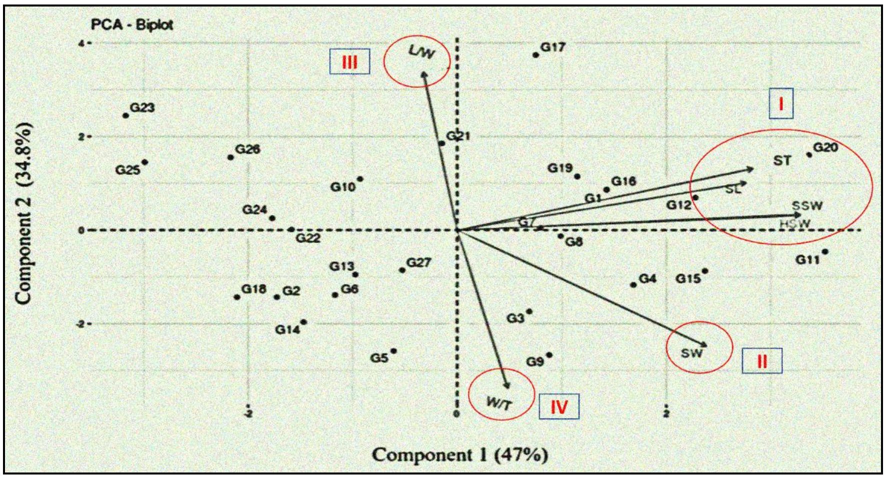

Fig. 3. PCA biplot for quantitative seed traits, HSW: 100 seed weight (g), SSW: single seed weight (g), SL: seed length (mm), SW: seed width (mm), ST: seed thickness (mm), L/W: seed length to width ratio, W/T: seed width to thickness ratio. G1 to G27 as per Table 1.

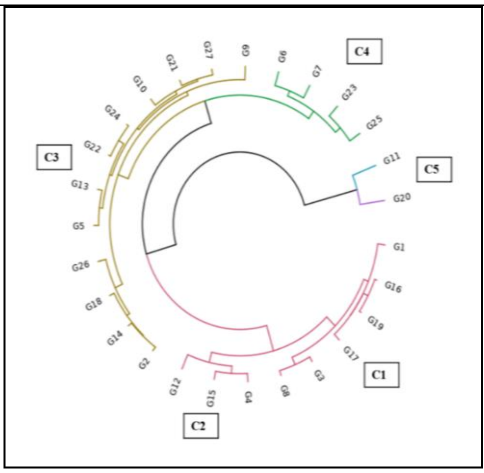

Fig. 4. Clustering of 27 French bean genotypes for seven quantitative seed traits as per eucldian distance.

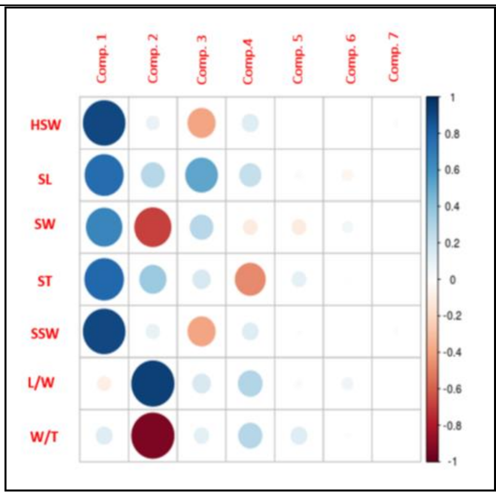

Fig. 5. Correlation between seed traits and PCs.

CONCLUSIONS

Collection and characterization of germplasm for preliminary evaluation with seed traits is most crucial aspects before initiating any breeding programme. The study depicted presence of wide diversity among French bean genotypes for fourteen seed characters including quantitative and qualitative traits. Evaluation of qualitative seeds traits inferred that brown seed testa colour (40.74%), cuboid seed shape (44.45%) and absence in seed coat pattern (62.96%) were the dominant seed traits among studied genotypes. The genotypes were varied greatly for quantitative seed traits; HSW (14.95-46.52g), SSW (0.15-0.47g), SL (8.51-14.1g), SW (4.62-8.35g), ST (4.31-5.82g), L/W (1.35-2.13), W/T (0.91-1.67). The reported genetic diversity in the studied germplasm can be conserved for future crop improvement in French bean for the concerned traits.

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Conflict of Interest. None.

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