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Assessment of Variability Based on Morphometric Characteristics in the Core Set of Soybean Germplasm Accessions

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ABSTRACT

The present study aimed at assessing the extent and pattern of genetic diversity within a core set of soybean germplasm comprising of 98 accessions. A total of thirty-one morphometric traits were studied, among them qualitative traits *viz*., leaf shape, flower color, seed coat color, and hypocotyl colour showed a higher genetic diversity with higher diversity indices. The variability parameters like mean, range of variation, GCV, PCV, heritability and genetic advance were estimated for 18 quantitative traits. The differences between GCV and PCV estimates were narrow for most quantitative traits indicating less contribution of environmental factors in traits expression. High estimates of heritability coupled with high genetic advance were observed in all quantitative traits except for days to maturity. The traits with higher heritability and GA value may indicate their variability and high selective value. Expression of lines in biplots using the first four principal components explains 79.10% of total variation and says black and yellow seeded genotypes have higher and lower variability to exploit, respectively. Hence, selection pressure could profitably be applied to these traits for their improvement.

Key words: Core set, GCV, Genetic advance, Heritability, PCA, PCV, Range.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is the world's most important seed legume, which contributes about 25 per cent of the global edible oil (Rai *et al*., 2016). It is commonly known as a wonder crop (Husain and Shrivastav, 2011), cow of the field, gold from soil and golden bean (Horvath, 1926) belongs to the family *Leguminacae* and derived from a wild progenitor *Glycine ussuriensi* (Robert, 2010). Sub species of soybean are *Glycine gracilis* and *Glycine soja* they are generally considered as the undomesticated progenitors of the domesticated soybean (*Glycine max*) (Chen and Nelson, 2004). Cultivated soybean has a genome size of 1.1 to 1.15 Gb with chromosome pair of twenty (2n=40).

The remarkable progress made in plant genetic resource management in recent days has resulted in the collection of a huge set of plant germplasm that hinders the very purpose for which they exist (Odong *et al*., 2013). Frankel (1984) proposed the concept of core collection that could be established from an existing collection for better management and utilization of plant genetic resources. Characterization of a core set is an efficient approach for exploring and capturing the genetic diversity of large populations, as it represents the maximum genetic diversity of the whole collection and become a powerful tool for evaluation and identification of trait-specific accessions in germplasm (Gireesh *et al*., 2015). Therefore, an investigation was carried out to assess the variability for morpho-metric traits among germplasm accessions conserved at the University of Agricultural Sciences (UAS), Bangalore which in turn helps in understanding the limitations of the domesticated germplasm and potential use of its wild relatives in crop improvement.

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MATERIALS AND METHODS

The material for the study comprised a core set of 98 germplasm accessions which included indigenous and exotic germplasm accessions of soybean along with five check entries (DSB-21, MAUS-2, KB-79, JS-335, KBS-23) procured from All India Coordinated Research Project (AICRP) on Soybean, UAS, GKVK, Bengaluru. A core set of 98 germplasm accessions (12 % of the whole collection), which captured ≥ 90 percent variability of the whole collection (#825) was developed using Power Core, a program that applies advanced M-strategy with a heuristic search (Kim *et al*., 2007).

All germplasm accessions along with five check entries were sown in Augmented design (Federer, 1956) in 4 compact blocks during *Kharif* 2015 and 2016. Each block consisted of 25 germplasm accessions and five checks (replicated twice). Each entry was sown in a single row of 2.5 meters length with a row spacing of 0.45 m and 0.2 m between

plants within a row. A basal dose of 25:50:25 Kg NPK ha^{-1} was applied to the experimental plot. Recommended management practices were followed during the crop growth period to raise a healthy crop.

Observations on different qualitative (hypocotyl pigmentation, anthocyanin colouration of stem, stem pubescence, growth type, leaf shape, leaf colour, flower colour, pod pubescence, pod pubescence colour, seed shape, seed colour, seed luster and hilum color) and quantitative characters (shoot length, root length, hypocotyl length, epicotyl length, plant height at 30, 45 days and at harvest, days to 50% flowering, days to maturity, pod length, number of branches per plant, number of pods per plant, seed size and 100seed weight) were recorded on five randomly selected plants from each germplasm accession and check variety following DUS (2009) and UPOVA (2017) descriptors. The number and per cent accessions belonging to each class were counted and computed, respectively.

Statistical analysis

The average data of five random plants were used for statistical analysis and interpretation. Data on morpho-metric characters on seed, seedling and plant were analyzed using an Augmented design (Federer, 1956) (SAS version 9.3) with non-replicated germplasm accessions and replicated check entries.

Phenotypic diversity for each qualitative traits was estimated using Shannon weaver index (Shannon and Weaver, 1949) was computed as:

$$H' = \{ - \sum_i p_i \times \ln (p_i)\},$$

where p_i is the frequency of the ith genotype.

The exploratory analysis was used to group the genotypes based on quantitative traits and to quantify variability and compare across traits. The principal component analysis (R software version 3.4.3) was used to extort maximum variability present in the data set. Principle components replace the original variables $X_1, X_2, \ldots, Xp$ by few variables $Y_1, Y_2, \ldots, Y_k$ that are linear combinations of the x variables preserving essentially all the information in the x variables and which are un correlated with each other.

Formally the first PC is

$$Y_1 = a_{11}x_1 + a_{12}x_2 + \cdots + a_{1P}x_P$$

Where,

the coefficients a1j, (j = 1, 2 . . . p,) are defined such that

$$V(y_1) = \max [\text{Var} \{a_{11}x_1 + a_{12}x_2 + \cdots + a_{1p}x_p\}]$$

The second PC is

$$Y_2 = a_{21}x_1 + a_{22}x_2 + \cdots + a_{2p}x_p$$

With a_{2j} defined such that

$$V(Y_2) = \max [\text{Var} \{ a_{21}x_1 + a_{22}x_2 + \cdots + a_{2p}x_p \}]$$

All together there are p principal components but not all of them are important so the principle components which have Eigenvalues are more than one will consider observing the variation (Halagunde Gowda, *et al.* 2018). A PCA biplot graph used to display the component scores and the variable loadings obtained by PCA in two or three dimensions and it assesses the data structure to detect groups, extreme values and patrons. The phenotypic component variance (PCV), genotypic component variances (GCV), Heritability in a broad sense and genetic advance were computed according to Falconer and Mackay (1996).

RESULTS AND DISCUSSION

An attempt was made to study the variability in the core set of soybean germplasm accessions by using 18 morpho-metric characteristics and substantial variability was documented for both qualitative and quantitative traits.

Qualitative traits

The results revealed dominance of determinant types (95.91%) with green colored leaves (92.8%) and pointed ovate shaped leaf (68.36%) compared to indeterminate types (4.08%), dark green colored leaves (7.14%) with rounded ovate (26.53%) and lanceolate (5.1%) shaped leafs. This may due to continued domestication and selection in the direction of determinant types during the course of evolution (Vaijayanthi *et al*., 2016).

Genotypes with a pubescent stem (92.85%) and anthocyanin coloured stem (59.1%) were highest compared to non-pubescent (7.14%) and non-anthocyanin pigmented (40.8%) stems. Hypocotyls pigmentation of the accessions was either present (61.22%) or absent (38.77%). Purple flowers (73.46%) were more frequent in the collection compared to white (26.53%) flowers (Table 1). Most of the accessions were found monomorphic for leaf colour and stem pubescence, while traits like leaf shape, anthocyanin pigmentation on stem and hypocotyl were found polymorphic with higher variability. High frequency of ovate leaf perhaps due to continuous evolutionary spectrum exists in soybean germplasm accessions based on leaf length to width ratio (Dong *et al*., 2001). In the evolutionary spectrum, wild soybean accessions with linear leaves and lanceolate leaves are primitive types, while accessions with round leaves have evolved more recently (Xuefei Yan *et al*., 2014).

Accessions bearing pubescent (93.87%) pods which of tawny (87.75%) colored pubescence were higher compared to non-pubescent pods (6.12%) and grey colored pubescence (12.24 %).Genotypes bearing yellow colored seed coat (58.2%) with elliptical seed shape (94.9%) and of dull luster (89.79 %) were found to be prominent over black (18.4%), green (16.3%), brown (4.1%), variegated (2%) and yellow-green (1%) seeded accessions with spherical (5.1%) seed shape and shiny (10.2%) luster. Further, seeds with brown colored (51.02%) hilum were higher followed by black (47.95%) and variegated (1.02%) hilum coloured genotypes (Table 1). The dominance of yellow seeded genotypes in the collection may be due to higher directional selection for yellow seeded ones owing to their high yield potential and

consumer preference (AkitoKaga *et al*., 2012). The predominance of genotypes with elliptical shaped seeds along with brown coloured hilum and dull luster may due to genotypic variability (Jain *et al*., 2017 and Zhou *et al*., 2015).

Phenotypic diversity of the collection

The Shannon-Weaver diversity index was computed for 13 qualitative traits and it was found to have significantly higher phenotypic diversity with average diversity index (H') of 0.544. Traits like leaf shape (0.69), flower color (0.83), seed coat color (0.67), hypocotyl coloration (0.96) and anthocyanin color on stem (0.97) found to have a higher variability with higher diversity indices of more than 0.67 and lower diversity was observed in traits like growth type (0.24), pod pubescence (0.33) and seed shape (0.18) with least diversity indices of less than 0.33. The predominance of characters with higher diversity indices can be considered as an effective descriptor for variability assessment in any population (Table 1).

Quantitative characters

Analysis of variance represents the variability among the germplasm accessions and it showed a highly significant mean sum of square values for all quantitative traits. Mean squares due to accessions, checks and 'accessions *vs* check varieties' were significant for all characters studied. However, blocks showed nonsignificant mean squares for all quantitative characters (Table 2 and 3) suggesting adequacy of the experimental layout. Highly significant mean square values indicated considerable variability not only among the germplasm accessions but also their significant differences with check varieties for most of the quantitative traits as indicated by ANOVA which is a diagnostic tool for detection of variability.

Table 1: Variability for qualitative characteristics and their frequency in soybean germplasm accession.

Traits	Score	Classified as	Percentage	Shannon weaver index (H)	Dominant traits	Diversity
Hypocotyl pigmentation	1	Absent	38.77	0.963	1	High
	9	Present	61.22			
Anthocyanin colouration of stem	1	Absent	40.8	0.97	2	High
	9	Present	59.1			
Stem pubescence	1	Absent	7.14	0.37	1	Moderate
	9	Present	92.85			
Growth type	1	Determinate	95.91	0.246	2	Low
	3	Indeterminate	4.08			
Leaf shape	1	Lanceolate	5.10	0.6952	9	High
	2	Pointed ovate	68.36			
	3	Rounded ovate	26.53			
Leaf colour	1	Green	92.8	0.371	2	Moderate
	2	Dark green	7.14			
Flower colour	1	White	26.53	0.835	2	High
	2	Purple	73.46			
Pod pubescence	1	Absent	6.12	0.332	1	Low
	9	Present	93.87			
Pod pubescence colour	1	Grey	12.24	0.536	9	Moderate
	2	Tawny	87.75			
Seed shape	1	Spherical	5.1	0.183	3	Low
	2	Elliptical	94.9			
Seed colour	1	Yellow	58.2	0.671	9	High
	2	Yellow green	1.0			
	3	Green	16.3			
	4	Black	18.4			
	5	Brown	4.1			
	8	Variegated	2.0			
Seed lusture	1	Shiny	10.20	0.475	9	Moderate
	9	Dull	89.79			
Seed hilum colour	3	Brown	51.02	0.461	9	Moderate
	4	Black	47.95			
	5	Variegated	1.02			

Table 2: Analysis of variance of soybean germplasm accessions for plant characteristics.

Source	df	Mean sum of squares								
		Days to flowering	Days to maturity	Epicotyl length (cm)	Hypocotyl length (cm)	Shoot length (cm)	Root length (cm)	Plant height at 30 days (cm)	Plant height at 45 days(cm)	Plant height at harvest (cm)
Blocks	3	0.026	0.15	0.022	0.012	0.07	0.07	0.055	0.01	0.02
(Accessions+ Checks)	102	37.16**	54.22**	1.07**	3.29**	24.48**	7.20**	124.16**	197.99**	409.55**
Accessions	97	30.87**	17.95**	1.03**	3.36**	24.83**	7.11**	46.82**	83.75**	161.01**
Checks	4	64.02**	272.80**	1.55**	1.23**	4.00**	1.67**	395.33**	590.61**	1739.32**
Accessions VS Checks	1	540.11**	2697.82**	2.69**	4.48**	72.16*	38.05**	6541.12**	9708.97**	19199.28**
Error	12	0.008	0.02	0.009	0.006	0.01	0.04	0.15	0.04	0.02

Descriptive statistics of quantitative characters

It indicated the components of genetic variability, heritability and genetic advance and it was computed by using first and second-degree statistics. Among 17 characters, higher mean and range values were recorded for plant height at harvest (29.9 and 91.4) and number of pods per plant (34.1 and 58.2), respectively. This higher range value indicates the higher variability of the characters and their efficiency in discriminating germplasmaccessions.The genotypes showed high variability for epicotyl length (53.37 %), hypocotyl length (47.46 %), number of branches per plant (33.37 %), number of pods per plant (39.69 %), plant height at 30 days (42.27 %), 45 days (38.63 %) and at harvest (42.42 %), root length (46.41 %), shoot length (43.70 %), seed yield (40.64 %) and hundred seed weight (21.24 %) as indicated by the estimates of PCV (>20%). The genotypes showed moderate variability for days to flowering (12.89 %), pod length (10.74 %), seed thickness (10.77 %) and seed size (18.06 %) since, their PCV estimates lie between 10 to 20 %. The accessions were least variable for days to maturity (4.26 %), seed length (9.15 %) and seed width (8.53 %) as the PCV < 10 %.

The higher estimates of PCV and GCV suggested considerable variability among the accessions. The differences between GCV and PCV estimates were narrow for all the traits (Table 3) indicating less contribution of environmental factors in character expression (Karnwal and Singh, 2009; Aditya *et al*., 2011). Thus selection based on the phenotypic performance of these characters would be an effective way to bring about considerable improvement of these characters (Akram *et al*., 2016).

Broad-sense heritability was higher (>60%) for all the characters (Table 3). Among the various characters understudy, plant height at harvest (99.99%) followed by days to flowering (99.97%) and days to maturity (99.87%) were highly heritable. The estimates of GAM was found higher (>20%) for all the characters except for days to maturity (7.21%). Heritability values are helpful in predicting the expected progress to be achieved through the process of selection. The genetic coefficient of variation along with heritability estimate provides a reliable estimate of the amount of genetic advance to be expected through phenotypic selection.

In the present study, the expected genetic advance was fairly higher for all most all characters except days to maturity. Thus higher estimates of expected genetic advance which takes into account of variability and heritability are conformity evidence for scope and effectiveness of a selection of genotypes (Patil *et al*., 2011). One of the major applications of estimating heritability and genetic parameters that compose the heritability estimate is to compare the expected genetic gains from selection based on alternative selection strategies and different experimental designs (Falconer and Mackay, 1996).

The source of morphometric trait variation extracted by principal component analysis. The Eigenvalues related to

Table 3: Analysis of variance of soybean germplasm accessions for pod and seed characters.

Source	df	Mean sum of squares								
		Number of branches per plant	Number of pods per plant	Pod length (cm)	Seed length (mm)	Seed size (mm^3)	Seed thickness (mm)	Seed width (mm)	Seed yield (g)	100 seed weight (g)
Blocks	3	0.001	0.12	0.02	0.02	0.29	0.02	0.05	0.08	0.09
(Accessions+ Checks)	102	1.61**	202.34**	0.26**	0.55**	1.09*	0.27**	0.28*	154.47**	7.59**
Accessions	97	1.54**	182.35**	0.21**	0.40**	1.05*	0.26**	0.23**	118.84**	4.95**
Checks	4	3.37**	395.44**	1.37**	2.99**	2.35**	0.52**	1.43**	37.73**	49.74**
Accessions v/s Checks	1	1.46**	1368.53**	0.67**	5.44**	0.04^{NS}	0.37**	0.17^{NS}	4077.14**	95.06**
Error	12	0.002	0.054	0.01	0.01	0.29	0.01	0.05	0.07	0.04

*Significant at P= 0.05 level, **Significant at P= 0.01 level.

Table 4: Descriptive statistics for 18 quantitative characters in core set of soybean germplasm accessions.

Characters	Mean ± SE	Max	Min	Range	GCV (%)	PCV (%)	Heritability (%)	GAM (%)
Days to flowering	43.1 ± 0.58	58.90	30.70	28.2	12.88	12.89	99.97	21.10
Days to maturity	99.6 ± 0.43	107.0	86.00	21.0	4.25	4.26	99.89	7.21
Epicotyl length (cm)	1.90 ± 0.11	4.88	0.51	4.4	53.14	53.37	99.13	95.79
Hypocotyl length (cm)	3.86 ± 0.19	8.96	0.56	8.4	47.42	47.46	99.82	95.05
Number of branches per plant	3.72 ± 0.13	7.80	1.50	6.3	33.35	33.37	99.87	75.44
Number of pods per plant	34.1 ± 1.39	68.23	10.00	58.2	39.68	39.69	99.97	60.75
Plant height at 30 days (cm)	16.2 ± 0.70	55.30	4.30	51.0	42.20	42.27	99.68	67.80
Plant height at 45 days (cm)	23.7 ± 0.94	68.50	6.00	62.5	38.62	38.63	99.95	60.50
Plant height at harvest (cm)	29.9 ± 1.29	98.40	7.00	91.4	42.42	42.42	99.99	65.14
Pod length (cm)	4.27 ± 0.05	5.34	3.06	2.3	10.48	10.74	95.24	37.29
Root length (cm)	5.74 ± 0.27	14.70	0.97	13.7	46.28	46.41	99.44	85.08
Shoot length (cm)	11.4 ± 0.51	28.90	1.12	27.8	43.69	43.70	99.96	72.45
Seed length (cm)	6.91 ± 0.07	8.66	5.53	3.1	9.04	9.15	97.50	27.26
Seed size (mm^3)	5.67 ± 0.05	7.22	4.74	2.5	15.37	18.06	72.38	39.74
Seed thickness (mm)	4.74 ± 0.05	6.11	3.44	2.7	10.56	10.77	96.15	35.50
Seed width (mm)	5.62 ± 0.05	7.18	4.80	2.4	7.55	8.53	78.26	25.15
Seed yield (g)	26.8 ± 1.11	56.39	7.85	48.5	40.63	40.64	99.94	71.22
Hundred seed weight (g)	10.5 ± 0.23	19.01	6.20	12.8	21.15	21.24	99.19	47.84

Where, GCV: Genetic co-efficient of variation, PCV: Phenotypic co-efficient of variation, GAM: Genetic advance as percentage of mean.

each principal component represents the variance associated with the particular principal component. The first Eigenvalue (6.82) captures maximum variability (37.90%) and hence identified as the first principal component. The second Eigenvalue (4.00) explained about 22.20 % of the variability of original data and the third one (2.07) captures third-highest variability (11.5%) and the fourth one (1.35) captures fourth-highest variability (7.50) and it continues up to 18 component. The first four Eigenvalues are more than 1.0 and they explain a total of 79.10% variability present in the data so the first four PCs are selected such that it describes 79.92 percentage of the total variability present in the original transformed data. Using these four components we tried to plot the biplot graph to express variation.

Biplot (Fig 1) revealed the contribution of each character for the observed phenotypic variation among soybean germplasm accessions. Chief characters impending jointly in different principle components have the propensity to remain together, which may be kept into consideration during the breeding program to bring about improvement for production and quality associated traits. Fig 1 explains PCA biplot of PCA1 which includes traits like plant height at 30, 40 days and at harvest, shoot length, root length and hypocotyl length were strongly influencing PC1, while seed length, seed width, seed thickness, seed size and test weight have more say in PC2 Biplot of PCA3 and PCA4, where number pods per plant majorly influence PC3, while days to flowering and days to maturity has more say in PC4. Fig 1 also confer clarity regarding the relationship between the variable based on the angle between the variable where seed length, seed width, seed thickness and seed size has a very little angle between them so they possess strong relationship. Plant height at 30, 40 days and harvest, shoot length, root length, hypocotyl and epicotyl length have a strong relationship. Ellipse in Fig 1 explains the cluster of

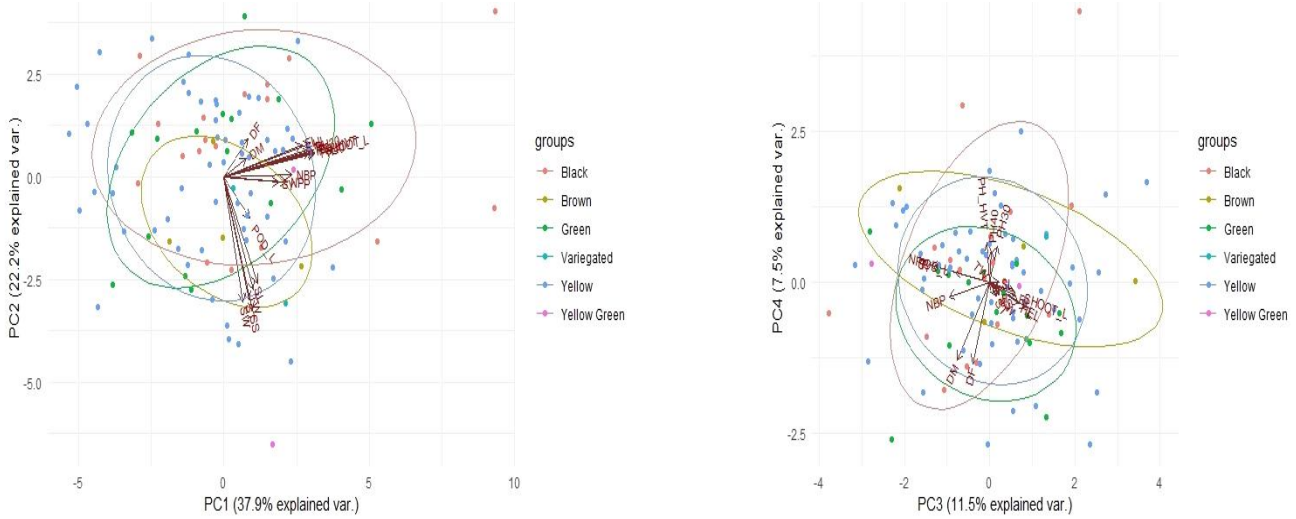

Fig 1: Biplotgrouping of 98 soybean accessions across PC1vs PC2 and PC3 vs PC4.

germplasms based on seed colour classification, black colour germplasms dispersed more so because they are exhibits maximum variation and yellow colour genotypes concentrate together so we say they express the least variation.

CONCLUSION

The study revealed the existence of higher genetic variability in the core collection of soybean indicated their usefulness in the crop improvement programme. Majority of the traits (number of pods per plant, pod length, seed yield, days to flowering, hypocotyl and epicotyl length) under study showed high heritability coupled with high genetic advance stating simple additive selection could be effective in the handling of this traits. The study also suggests the exploitation of genotypes with high genetic diversity as parental lines to have transgressive segregants.

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