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Multivariate Analysis for Seed and Seedling Attributes of *Sesbania* Genotypes

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Abstract: An experiment was conducted at Laboratory of Plant Systematics of the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh during April/2017 to evaluate morphological traits of seed and seedling attributes and to estimate genetic diversity of *Sesbania* genotypes using multivariate statistics. The experiment was laid out as a complete randomized design with four replications. The highest coefficient of variation was observed in root length (RL) followed by total dry matter (TDM), vigor index (VI) and base diameter (BD) of seedling. There was significant, either positive or negative, relationship among different morphological traits for seed and seedling. The positive correlation was found for TDM with seed width (SW), 1000-seed weight; shoot length (ShL) and BD. However, the VI was positively correlated with seed length (SL), ShL, RL and germination percentage (GP) and negatively correlated with BD. First three principal components (PCs) *viz.* PC1, PC2 and PC3 contributed 32.03, 26.31 and 12.54% of total variance with the discriminating value of 2.88, 2.37 and 1.13, respectively. The highest contribution for total genetic divergence was attributed by 1000-seed weight followed TDM, SW, ShL and RL. The 1000-seed weight played the highest contribution towards the total divergence followed by TDM, SW, ShL and RL. In cluster analysis, significant genetic diversity was found for the studied genotype of seed and seedling traits. The cluster-1 exhibited the highest average values for SL, RL, GP and VI, and cluster-3 showed the highest values SW, 1000-seed weight, ShL, BD and TDM. Thus, indicated that there was significant genetic diversity for seed and seedling morphological traits of studied *Sesbania* genotypes. Considering all of the studied traits for seed and seedling would be helpful for selection of diverse genotypes and these diverse genotypes could be the good genetic materials for improvement of *Sesbania* genotypes.

Keywords: *Sesbania*, seed and seedling morphology, PCA, AHC analysis.

INTRODUCTION

The genus *Sesbania* belongs to the cosmopolitan family Papilionaceae with *ca.* 60 species. Three species of *Sesbania* namely *S. sesban* (L.) Merr.,*S. bispinosa* (Jacq.) W. Wight [former *S. aculeata* (Wild.) Poir] and *S. cannabina* (Retz.) Poir, are locally known as *dhaincha* in Bangladesh [1]. The origin of the crop is not clear but it is cultivated all over the world [2, 3]. The major use of *dhaincha* is as green manure. The other diversified usages are as animal fodder and feed, medicinal purposes, fuel, bio-energy sources, fiber source, weed control and live support fencing as well [4- 6]. It is quick growing and easily decomposable in soil. Due to rapid growth, various nodule formation ability and high level of atmospheric nitrogen fixation capability, the crop is widely used as an excellent improver of soil fertility [7, 8]. The germplasms collection is required to maintain the genetic diversity in growth habit and yield potential of *Sesbania*, to develop higher crop production [9]. Characterization of *Sesbania* germplasms may play a great opportunity in maintaining and utilization of genetic resources as well as initiating a sound breeding program for improving these species. However, very limited information is available in this aspect of *Sesbania* genetic resource [10].

The multivariate analyses, especially the Principal Component Analysis (PCA) and Agglomerative Hierarchical Clustering (AHC) analysis have been utilized to measure the genetic diversity of germplasms. The purpose of PCA and AHC analyses is to knockdown the time large number of accessions with smaller sets of components and summaries the data presentation [11, 12]. It saves time and provides the accuracy data which may use for future breeding programmer. The PCA is helpful to select the morphological traits which are responsible for higher crop yields. Clustering analysis denotes the similarities or dissimilarities among the traits. It seeks to minimize within group variance and maximize between group variance. It is also helpful for parental selection in the breeding program [12]. This study was, therefore, conducted with the objectives of characterization and identification of morphological traits for seed and seedling attributes of *Sesbania* species using multivariate analysis.

MATERIALS AND METHODS

Experimental material and site

An experiment was conducted at the Laboratory of Plant Systematics, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh, to select the morphological traits of seed and seedling attributes and to estimate genetic diversity of *Sesbania* genotypes using multivariate analysis. Seeds of one hundred and ten *dhaincha* accessions were collected from different agro-ecological zones which cover 19 districts of Bangladesh (detail collection information available upon request). The data on seed morphological descriptors *viz*. seed length, seed width and 1000-seed weight, were carefully taken. Randomly selected 30 seeds were used for measurement from each accession. Length and width were measured using a digital slide calipers with a precision of 0.1mm to 150mm [13]. Seed length was measured from base to top and width was taken in the middle line of seed.

Experimental procedure

For seedling characterization, the experiment was laid out as a complete randomized design with four replications. From each accession, 50 healthy seeds were spread uniformly on a standard Petridis. Cumulative germination (CG) percentage of seeds was counted daily up to 10 days. According to Bewley and Black [14] the CG percentage of seeds was calculated the following formula.

$$CG\% = (\sum n / N) \times 100$$

Where, n is the number of seeds germinated at each day and N is the total number of seeds sown.

Vigor index (VI) was calculated based on the mean shoot and root length; multiplied by the percentage of seed germination on 10 days[22].

$$VI = (\text{Mean shoot length} + \text{Mean root length}) \times \text{germination percentage}$$

DATA COLLECTION

From each accession randomly 10 seedlings were selected and measured. Shoot length, root length, base diameter, total dry matter (shoot + root) weight were measured by electric digital balance on ten days aged. Fresh sample were oven dried at $72^0 \pm 2^0$ C for 72 hrs.

DATA ANALYSIS

Agglomerative hierarchical clustering (AHC) analyses were conducted using the XLSTAT 2009.3 program, based on agro-morphological traits *viz*. seed length, seed width, 1000-seed weight, germination percentage, vigor index, shoot length, root length, base diameter, total dry matter weight. A dendrogram was built by AHC analysis. Principal component analyses (PCA) were conducted using the "R" software program based on the above agro-morphological traits.

RESULTS AND DISCUSSIONS

Morphological variability for quantitative traits

Nine morphological traits were consideration to characterize and measure the diversity of 110 accessions of *Sesbania* species. The morphological variability among the accessions was obtained in all of the studies parameters. The mean seed length was 3.82±0.12 mm and mean seed width was 2.28±0.16 mm (Table 1). The highest 1000-seed weight was 21.95 g and lowest was 13.53 g. Seed germination varied from 26 to 100% and mean vigor index was 1207±255. The highest shoot length and root length produced 13.30 cm and 6.48 cm, respectively and lowest was 6.20 cm and 1.86 cm, respectively. Whereas mean base diameter was 0.05±0.01 cm. The highest total dry matter(TDM) was obtained 0.19 g/10 plant and lowest was 0.06 g/10 plant (Table 1). The highest coefficient of variation was obtained for the root length (24.00%) followed by TDM (22.00%), vigor index (21.13%) and base diameter (20.00%) (Table1). The coefficient of variation expresses as a percentage of the overall mean, the amount of genetic variation among accessions. Information of this coefficient is significant in a genetic improvement program because it indicates the amplitude of genetic variation in a variable concerning the opportunity of its improvement [12]. According to Rocha *etal*. [23], the coefficient of genetic variation indicates the presence of variability among the accessions, with higher values matching to greater heritability and accordingly the probability of finding superior individuals during the process of selection.

Matrix correlation of quantitative traits

The matrix correlation among the nine morphological variables was significant at 5% level (Table 2). Both positive and negative correlations were found among the morphological variables. The relationship between seed length with germination percentage, vigor index and root length were significantly positive. The results revealed that seed length enhance root length, germination percentage and vigor index. Seed width showed positive and significant relationship with 1000-seed weight, shoot length, and total dry matter production, however, negatively and significant correlation with root length. It indicated that seed width influences 1000-seed weight, shoot length, and total dry matter production but negative influence in root length. On the other hands, 1000-seed weight was positively correlated with shoot length and total dry matter production but negatively correlated with root length (Table 2). Germination percentage showed highly correlation values with vigor index and root length. It revealed that germination percentage enhances vigor index and root length of

*Sesbania*species. However, negative correlation was observed in base diameter with germination percentage. Vigor index significant and positively correlated with shoot length and root length but negative and significant correlation was observed with base diameter (Table 2). Shoot length showed positive and significant correlation with TDM production and base diameter. Base diameter positively correlated with TDM production (Table 2). Morphological traits were positively correlated with each other indicated that selection may be positive direction based on these traits towards crop improvement program [12].

Principal component of quantitative traits

The Eigen value showed more than 1 and first three principal components (PC1, PC2, and PC3) accounted for 70.87% of the total variation (Table 3). First three PC1, PC2 and PC3 contributed 32.03, 26.31 and 12.54% of total variance with the discriminating values of 2.88, 2.37 and 1.13, respectively. Zhou *etal*. [15] reported that the first three principal components are often the most important in reflecting the variation patterns among the accessions. Pla [16] suggests at least 80% total variability as needed. Nevertheless, the first three canonical variable did not reach the 80% of the total variation, the forth variable was included in the study and that was sufficient explain 80.94% of total variation.

The coefficient value was considered more than 0.3 for important traits selection [17]. The PC1 showed highest contributing from 1000-seed weight (0.81) followed by seed width (0.76), total dry matter (0.76), shoot length (0.60) and base diameter (0.39) (Table 3). The PC2 revealed that the highest loading of vigor index (0.92) followed by germination percentage (0.80), shoot length (0.59), seed length (0.41) and root length (0.37). The PC3 showed that highest loading of base diameter (0.69) lowest root length (0.56). The first and the second PCs with a cumulative of 58.34% revealed that most variation among the populations, screening a high degree of correlation among the traits. Overall, the PCA analysis under this study represents that phenotypic and genotypic characters are useful to identify *Sesbania* accessions from the largest variability [18, 12].

Major contributions of quantitative traits

The maximum contribution towards the total divergence was observed in 1000-seed weight (22.92%), followed by total dry matter production (19.85%), seed width (19.83%), shoot length (12.37%) and root length (9.50%) (Table 4). The minimum percentage of contribution was observed in seed length (1.24%), followed by vigor index (3.61%), base diameter (5.30%) and germination percentage (5.39%). The traits 1000-seed weight, total dry matter, seed width, shoot length and root length values contributed 84.47% and remaining characters had very less contribution toward genetic divergence. Highest contributing characters should be given importance during selection in the population for improvement of yield contributing traits. Kumar *etal*. [19] and De *etal*. [20] opined that the traits contributing maximum towards of the total divergence should be considered during selection. The first component (32.03%) was mainly related to the 1000-seed weight, seed width, shoot length, total dry matter and base diameter of seedlings. The second component (23.31%) was related to vigor index, germination percentage, seed length and root length (Fig. 1).

Mean value for clustering of quantitative traits

Cluster 1 showed highest seed length, germination percentage, vigor index and root length (Table 5). However, cluster 3 exhibited highest seed width, 1000-seed weight, shoot length, base diameter and total dry matter production and cluster 2 showed lowest values for all parameters. The clustering pattern showed that there was significant genetic variability among the *Sesbania* accessions. Cluster-3 remains promising genotype if possible to improve the germination percentage through hybridization or crossing or any technique.

Hierarchical clustering of quantitative traits

Sesbania accessions were grouped into 3 clusters by the AHC analysis based on nine agro-morphological descriptors (Fig. 2). The main clusters were further sub-divided into some sub-clusters. Cluster 1 consisted of 92 accessions, cluster 2 consisted in of 12 accessions and cluster 3 consisted of 6 accessions. Different clustering patterns were done based on different agro-morphological patterns (Fig. 2). Within a cluster the genotypes showed a high homogeneity and high heterogeneity [12]. Endang *etal*. [12] stated the clustering pattern could be utilized in choosing parents for cross combination which likely to be generate the highest possible variability for the effective selection of various economic purposes [24]. The accessions number 66, 70, 79, 81, 82 and 85 always remains in a same cluster. So these six accessions may belong to same species.

Table -1: Descriptive statistics of morphological traits among 110 *Sesbania* accessions

Character	Minimum	Maximum	Mean	Std. deviation	CV(%)
Seed length (mm)	3.37	4.18	3.82	0.12	3.14
Seed width (mm)	1.93	2.93	2.28	0.16	7.02
1000-Seed Wt. (g)	13.53	21.95	16.79	1.67	9.95
Germination (%)	26.00	100.00	85.17	15.83	18.59
Vigor Index	210	1731	1207	255	21.13
Shoot length (cm)	6.20	13.30	10.10	0.98	9.70
Root length (cm)	1.86	6.48	4.00	0.96	24.00
Base diameter (cm)	0.02	0.08	0.05	0.01	20.00
Total Dry Matter (g/10Plant)	0.06	0.19	0.09	0.02	22.22

Significantly different at $p < 0.05$

Table-2: Matrix correlations among the seed and seedling variables

Variables	Seed Length (mm)	Seed Width (mm)	1000-Seed Wt. (g)	Germi-nation (%)	Vigor Index	Shoot length (cm)	Root length (cm)	Base Diameter (cm)	Total Dry Matter (g/10Plant)
Seed Length (mm)	1								
Seed Width (mm)	-0.10	1							
1000-Seed Wt. (g)	0.01	0.66*	1						
Germination (%)	0.22*	-0.11	-0.05	1					
Vigor Index	0.27*	-0.05	0.00	0.92*	1				
Shoot length (cm)	0.05	0.45*	0.50*	0.15	0.35*	1			
Root length (cm)	0.26*	-0.31*	-0.37*	0.24*	0.49*	-0.07	1		
Base Diameter (cm)	-0.12	0.13	0.12	-0.32*	-0.22*	0.20*	-0.03	1	
Total Dry Matter (g/10Plant)	0.05	0.44*	0.63*	-0.17	-0.02	0.59*	-0.13	0.26*	1

Values in * mark significantly different at $p < 0.05$

Table -3: Eigenvector coefficients of PCA and factor loading values of the first four PC

Character	PC1	PC2	PC3	PC4
Seed Length (mm)	-0.19	0.41	0.28	0.80
Seed Width (mm)	0.76	0.20	-0.22	0.00
1000-Seed Wt. (g)	0.81	0.30	-0.19	0.15
Germination (%)	-0.39	0.80	-0.28	-0.20
Vigor Index	-0.32	0.92	-0.02	-0.22
Shoot length (cm)	0.60	0.59	0.14	-0.18
Root length (cm)	-0.52	0.37	0.56	-0.05
Base Diameter (cm)	0.39	-0.19	0.69	-0.34
Total Dry Matter (g/10Plant)	0.76	0.28	0.26	0.12
Eigen value	2.88	2.37	1.13	0.91
Percentage	32.03	26.31	12.54	10.07
Cumulative percentage	32.03	58.34	70.87	80.94

Table -4: Percent contribution of different variables of the total diversity in *Sesbania* accessions

Variable	Contribution (%)	Cumulative Contribution
Seed Length (mm)	1.24	1.24
Seed Width (mm)	19.83	21.07
1000-Seed Wt. (g)	22.92	43.99
Germination (%)	5.39	49.38
Vigor Index	3.61	52.99
Shoot length (cm)	12.37	65.36
Root length (cm)	9.50	74.86
Base Diameter (cm)	5.30	80.16
Total Dry Matter (g/10Plant)	19.85	100.00

Table -5: Mean value of the 9 quantitative traits in 3 clusters of different accessions of *Sesbania* species

Class	Acc. No.	Seed Length (mm)	Seed Width (mm)	1000-Seed Wt. (g)	Germi-nation (%)	Vigor Index	Shoot length (cm)	Root length (cm)	Base Diameter (cm)	Total Dry Matter (g/10Plant)
Average value										
1	92	3.83	2.27	16.72	91.10	1292.57	10.11	4.08	0.05	0.09
2	12	3.80	2.19	15.49	52.08	701.81	8.94	4.06	0.06	0.08
3	6	3.70	2.60	20.53	60.50	911.97	12.38	2.67	0.07	0.15

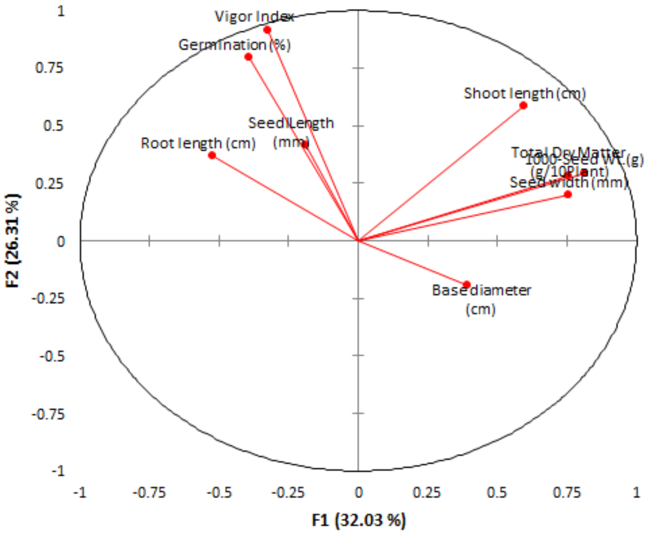

Fig-1: PCA based on nine seed and seedling attributes of *Sesbania* species

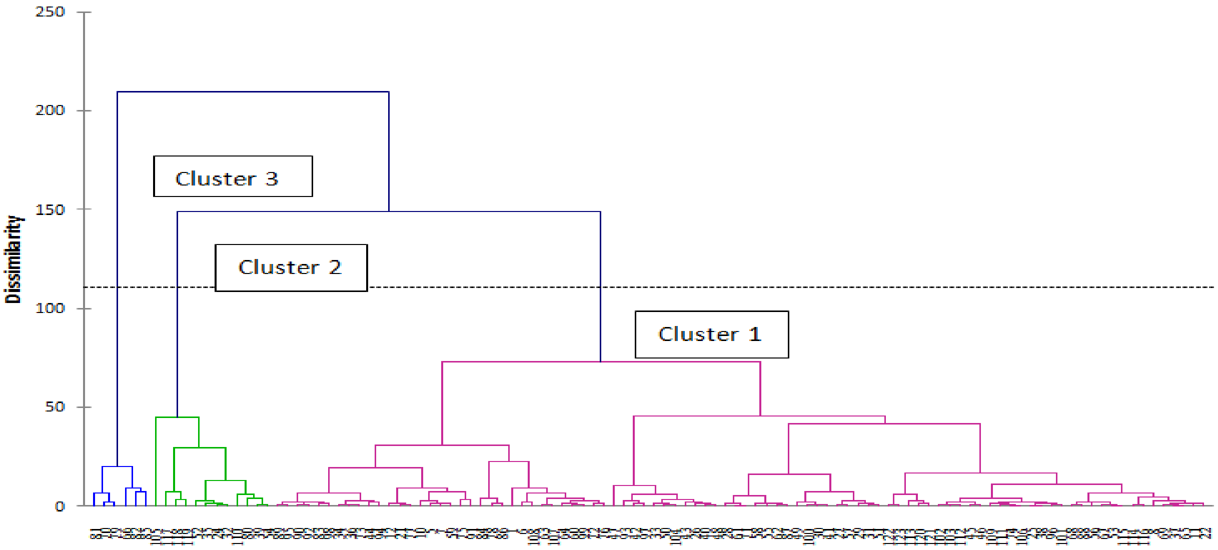

Fig-2: Dendrogram based on nine seed and seedling attributes of *Sesbania* species

CONCLUSION

Out of one hundred and ten accessions, only eight accessions showed 100% germination and seventy two accessions showed above mean value (1207) of vigor index. Nevertheless, sixty three accessions showed above mean value (14.11 cm) of total length (shoot length + root length). Twenty one accessions produced above mean (0.09 g/10plant) value of total dry matter. Vigor index high positive and significantly correlated with germination percentage, shoot length and root length. Total dry matter production significant and high positive correlation showed with seed width, 1000-seed weight, shoot length and base diameter. The PCA indicated seed width, 1000-seed weight, shoot length, total dry matter production are most important traits for selection of genotypes. The highest contribution towards the total divergence was observed in 1000-seed weight and lowest was seed length. Mean value of the nine quantitative traits goes to three cluster and cluster 1 performed better in terms of germination percentage and vigor index. However, cluster 3 showed high value of 1000-seed weight, shoot length and total dry mass production. The accessions number 66, 70, 79, 81, 82 and 85 always remains in a same cluster. So these six accessions may belong to same species. Diverse germplasms material may help to create existence of diversity among *Sesbania* accessions and would help to characterize them for further study at genotypic, phenotypic and molecular level.

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