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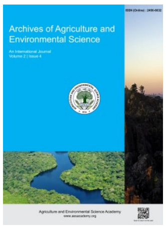

ORIGINAL RESEARCH ARTICLE

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Screening of *Sesbania* accessions based on seed germination and seedling biomass

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ABSTRACT

Seedling emergence and germination percentage of seeds play a vital role for optimum plant population and biomass yield maximization of a crop in the field. An experiment was carried out at Plant Systematics Laboratory of the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh, during the month of April to May 2016 for the screening of *Sesbania* accessions based on seed germination, vigour index and initial biomass yield. The experiment was laid out in a completely randomized design with four replications. One hundred and ten *Sesbania* accessions were used as experimental materials. Four hundred healthy seeds, 100 seeds as one replication, of each accession were spread uniformly on containers for the germination test. Cumulative germination percentage of seeds was counted daily up to 10 days. After 10 days, 40 seedlings (10 from each replication) were taken from each accession and measured. Quantitative descriptors *viz.* emergence (%) and germination (%) of seeds, vigour index, shoot length, root length, base diameter and biomass yield, varied significantly. Based on quantitative descriptors, Agglomerative Hierarchical Clustering (AHC) and Principal Component analyses (PCA) were conducted. In PCA, the first three principal components explained 82.64% of the total variations. In AHC, three individual clusters were developed and six accessions always remained in the same cluster. These six accessions could belong to same species. Based on their seed germination and initial seedling growth, five accessions performed better and selected for further study. Field trials of these accessions are needed to recommend one or more accessions as cultivar(s).

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INTRODUCTION

Quality seed is the prime source of good crop production and human needs, the ultimate goal of food and nutritional security. Seeds quality is a rather broad term and encompasses several factors: seed viability, seed health, varietal and physical purity, germination, however, the further essential factor is the more mysterious trait of seed vigour (Finch-Savage and Bassel, 2016). Seed viability, emergence and germination (%) can affect the optimal plant population in the field; and lack of seedling vigour in surviving plants cannot achieve the maximum biomass yield and yield components of crops (Dornbos, 1995).Vigorous seeds germinate easily, establish rapidly and plants may be uniform in favorable environmental conditions (Finch-Savage and Bassel, 2016). Seedling survival or establishment depends on root growth potential; shoot-root ratio, nutrients status of soil and it's availability to plant as well as environmental conditions (Larsen *et al*., 1986). Moreover, high vigorous seeds should be used in all instances to ensure good standard establishment under field condition. Seed emergence, germination and seedling vigour has a vast domain feature of genetic purity and seed longevity; however, they may contribute to some differentiation in seedling growth like plant height, base diameter, and root and shoot development as lead to biomass yield (Rezapour *et al*., 2013). All these yield contributing descriptors will enhance total biomass production of a crop in the field. If the germination percentage is lower than its optimum value then total biomass production will be lower (Srivastava and Kumar, 2013).

The unavailability of quality seeds is one of the major constraints of the popularization of green manure crops in Bangladesh. Commercially available seeds of green manure crops commonly show lower seed germination (%) as well as seedling vigour (Dhaka *et al*., 2014). Three indigenous species of *Sesbania viz. S. sesban* (L.) Merr., *S. bispinosa* (Jacq.) W. Wight [syn. *S. aculeata* (Wild.) Poir.] and *S. cannabina* (Retz.) Poir., are commonly known as *dhaincha* and used as green manure crop in Bangladesh (Ahmed *et al*., 2009; Sarwar *et al*., 2015). *Dhaincha* is a quick growing plant and easily decomposable in soil at the early stage growth (Pandey *et al*., 2013). Moreover, an exotic species *S. rostrata* Bremek. & Oberm., commonly known as African *dhaincha*, is also used as green manure crop; although the germination percentage of African *dhaincha* seeds is comparatively lower than that of indigenous *dhaincha* species. Hitherto, the National Seed Board of Bangladesh has not released any *dhaincha* cultivar as a green manure crop (Sarwar *et al*., 2015). This is another major constraint of green manure crop cultivation in Bangladesh. Apart from taxonomic research, a little work has been done on the characterization of *dhaincha* species/accessions available in Bangladesh (Sarwar *et al*., 2015; Chanda *et al*., 2017b, 2018). Selection of available genetic resources or hybridization or mutation breeding is one of the conventional methods of genetic improvement and high yielding cultivar development. Among these, selection is one of the oldest methods for high yielding cultivar development (Chahal and Gosal, 2002). Therefore, the objective of the present study was screening of *Sesbania* accessions based on seed germination, seedling vigour and their initial biomass yield with a long time goal of a cultivar development/release.

MATERIALS AND METHODS

Experimental design and site

The present experiment was carried out at Plant Systematics Laboratory of the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh, during the month of April to May, 2016 for the screening of *Sesbania* accessions based on seed germination, seedling vigour and initial biomass yield. One hundred and ten accessions of *Sesbania* spp. were collected through field survey in 19 districts of Bangladesh (detail collection information available upon request). A seed multiplication programme was carried out with uniform field and storage conditions, and the harvested seeds were used as experimental materials. The experiment was laid out as a complete randomized design with four replications. Germination test was conducted in the sterile sand medium in plastic containers. Four hundred seeds of each accession were tested. One hundred seeds were placed in a container and considered as one replication.

Data collection

The number of normal and abnormal seedlings, dead and/or non -germinated seeds were counted daily up to 10 days of seed sowing. Cumulative germination (CG) percentage of seeds was determined following Bewley and Black (1994).

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

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

The seedlings were harvested at 10 days after sowing (DAS). Forty seedlings from each accession, ten seedlings from each replication, were randomly selected and measured. Length of shoot and root were measured using a ruler, the base diameter was measured using digital slide callipers with a precision of 0.1 mm to 150 mm. Seedling vigour index (VI) was determined following Bewley and Black (1994).

Vigour index= (Root length + Shoot length) × Germination (%)

Fresh weight and biomass of shoot and root were measured by electric digital balance (Model #Shimadzu ATY 224). A fresh sample was oven dried at $72^0 \pm 2^0$ C for 72 hrs.

Data analysis

The quantitative parameters *viz*. shoot length, root length, base diameter, fresh weight & biomass of shoot and root, and total biomass yield, were analyzed and calculated with Excel application such as arithmetic mean, standard deviation, the range of variation. Agglomerative Hierarchical Cluster (AHC) and Principal Component Analyses (PCA) were done based on the quantitative descriptors *viz*. germination (%), vigour index, shoot length, root length, base diameter and total biomass, by using XLSTAT 2009.3 and "R" software program, respectively.

RESULTS AND DISCUSSION

Germination and seedling descriptors

In general, the emergence percentage of seeds (100 to 35) is comparatively higher than percent germination (100 to 33) (Table 1). Among 110 *Sesbania* accessions, seeds of 18 accessions (#5, 58, 59, 66, 68, 73, 77, 78, 98, 101, 102, 103, 112, 114, 116, 120, 121 and123) attain at the maximum emergence (100%); but only eight accessions (#59, 101, 102, 103, 116, 120, 121 and123) showed the highest percentage (100%) of germination (data not shown here). The higher seed germination (%) is a good syndrome for quality seeds and produces a maximum number of healthy plant populations in the field. It may be occurred due to less seed dormancy and/or genetic effects of collected *Sesbania* accessions (Bentsink and Koornneef, 2008). The highest emergence and germination percentage of seeds will produce optimum plant population when seeds are sown in the field. Chanda *et al*. (2017a) observed that seed germination and seedling survival depends on seed size and micro site requirements. The percentage of abnormal seedling and dead seed were 4.92 and 9.67, respectively (Table 1).

On average, the shoot length of seedlings was 10.0±0.97 cm and the root length was 4.0±0.95 cm (Table 1). Accessions #40, 47, 70, 79, 81, 82, 85, 93, 94, 95, 97, 98, 121 and 122 produced longer shoot (>11 cm) compare to that of other accessions (data not shown here). On contrary, accessions #26, 35, 40, 42, 43, 45, 47, 52, 56, 102 and 113 showed higher root length (>5 cm) than other accessions (data not shown here). Plant survival depends on root length and root weight as well as contents of root nutrients (Larsen *et al*., 1986). Base diameter varied from 0.08-0.03 cm. The base diameter may be correlated with biomass yield or *vice versa* (Verlinden *et al.*, 2013). The shoot-root length ratio ranged from 5:1 to 2:1. The shoot-root ratio is important for establishment, growth and survival of seedling. Mishra *et al.* (2014) reported that the root-shoot ratio is used to assess the overall health of plants. Nevertheless, the vigour index ranged from 1731–384 with an average value of 1217 (Table 1). These differences may be due to the genetic make-up of collected *Sesbania* accessions (Joshi-Saha and Gopalakrishna, 2007; Sarwar *et al.*, 2015). Riaz *et al.* (2013) observed similar result in cotton germplasms and found significant variability among genotypes. Accession #26, 45, 47, 93, 97, 98, 101, 102, 116 120, 121 and 122 were performed better in terms of seedling vigour index (above 1440) (data not shown here). The higher vigour index indicates that the crop has wider adaptation and better growth potentiality compared to lower ones (Shreelalitha *et al.*, 2015).

Fresh weight and biomass

The average shoot fresh weight was 129.0±13.1 mg/seedling and the average root fresh weight was 31.0±5.6 mg/seedling (Table 2). On the other hand, shoot biomass ranged from 13.0 to 5.0 mg/seedling and root biomass varied from 7.0 to 1.0 mg/ seedling. The highest biomass was 20.0 mg/seedling and the lowest was 6.0 mg/seedling (Table 2). The accession #7, 10, 47, 49, 70, 79, 81, 82, 85, 93 and 107 produced higher biomass (11.0 mg/seedlings and above) compared other accessions (data not shown here). The best morphological indicator of survival potential depends on the shoot-root length ratio (Larsen *et al.*, 1986). The seedling survival may depend on per cent germination and vigour index. The shoot-root biomass ratio ranged from 9:1 – 2:1 (Table 2). In shoot-root ratio, the shoot length was negatively correlated with root growth potential (Larsen and Boyer, 1986) which ultimately showed their effect on root biomass.

Principal component analysis

In Principal Component Analysis (PCA), the first three principal components explained 82.64% of the total variation in original data. The first component (37.23%) was mainly related to the shoot length, root length, germination percentage and vigour index of seedlings. The second component (29.43%) was related to shoot length, base diameter and total biomass. The third component (15.98%) was related to root length and base diameter. All the accessions had distinct positions on PCA graph, although some over lapping was observed (Figure 1). Among the accessions, only accession #47 and #105 were the most distantly positioned (the upper most right and the lower most left quadrate, respectively) taxa on the PCA graph (Figure 1). It may be happened due to the highest and lowest value in factor score of first component. The accession #47 secured the highest value (+3.07) and accession #105 showed the lowest value (-6.57) among 110 accessions (data not shown here). Accessions #66, 70, 79, 81, 82, and 85 constituted a closer separate group in the upper left quadrate of the graph (Figure 1) and these accessions could belong to one species. Accession #47 and #105 may belong to two different species and the rest of accessions to one or more species of *Sesbania*.

Agglomerative hierarchical cluster analysis

The dendrogram constructed from shared scores showed that morphological distance and relationship of 110 accessions. In agglomerative hierarchical cluster (AHC) analysis, the accessions were divided into three clusters (Figure 2). Cluster 1, 2 and 3 contained 100, six and four accessions, respectively. Accession #66, 70, 79, 81, 82 and 85 constituted a cluster (Cluster 2), accession #105, 117, 118 and 119 (Cluster 3) and rest of the accessions constituted the Cluster 1 (Figure 2). Accessions moved to different clusters due to differences in quantitative values of morphological descriptors, which might be influenced by their genetic make-up (Sarwar *et al.*, 2015). Multivariate analysis of agro-morphological traits of accessions formed separate clusters in dendrogram (Joshi-Saha and Gopalakrishna, 2007). The accession #66, 70, 79, 81, 82 and 85 may belong to same species; accession #105, 117, 118 and 119 in another and the rest of accessions may be included in one or more species (Figure 2). This result of AHC may confirm the result obtained from the PCA. All accessions were positioned closer to each other, as they had almost the similar characteristics (Ameera *et al.*, 2014). The AHC analysis may be considered as the first step for identification of *Sesbania* accessions. A detail floral morphological and/or molecular characterization is necessary to confirm the identification of *Sesbania* species. Joshi-Saha and Gopalakrishna (2007) reported that although two *Sesbania* spp. *viz. S. bispinosa*and *S. cannabina*, are morphologically similar, these are distinct at the molecular level.

Morphological description of selected *Sesbania* accessions

On the bases of germination behaviour, vigour index and biomass yield, five superior accessions (#7, 10, 47, 49 and 93) have been selected (Table 3). These accessions will be used as experimental materials for further studies on other qualitative and quantitative descriptors e.g., biomass yield at later stages, decomposability, organic matter accumulation & N_2-fixing ability in soil, etc. to recommend as a cultivar. Although some other accessions performed better in either germination behaviour or biomass yield, those were not selected for further studies for different reasons. For example, accessions (#66, 70, 79, 81, 82 and 85) produced higher shoot length and biomass; these accessions have not been considered for further study due to their poor seed germination behaviour (data not shown here). The germination ability of seeds of these accessions was relatively lower (below 65%), so field performance of these accessions may be questionable. On contrary, accessions #59, 101, 102, 103, 116, 120, 121 and 123 showed higher germination percentage and accessions #26, 45, 97, 98, 101, 102, 116, 120, 121, 122 and 123 showed higher vigour index, however, produced lower (<10.0 mg/seedling) biomass yield (data not shown here). Therefore, these accessions have also not been considered for further study.

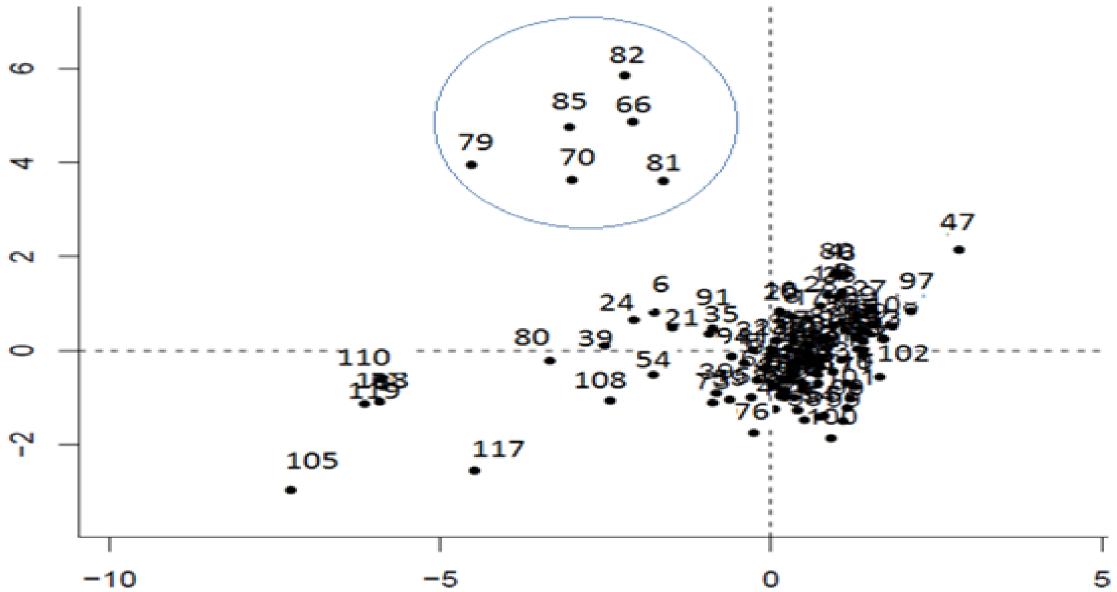

Figure 1. *Two dimensional graph representing Sesbania accessions in the principal component analysis.*

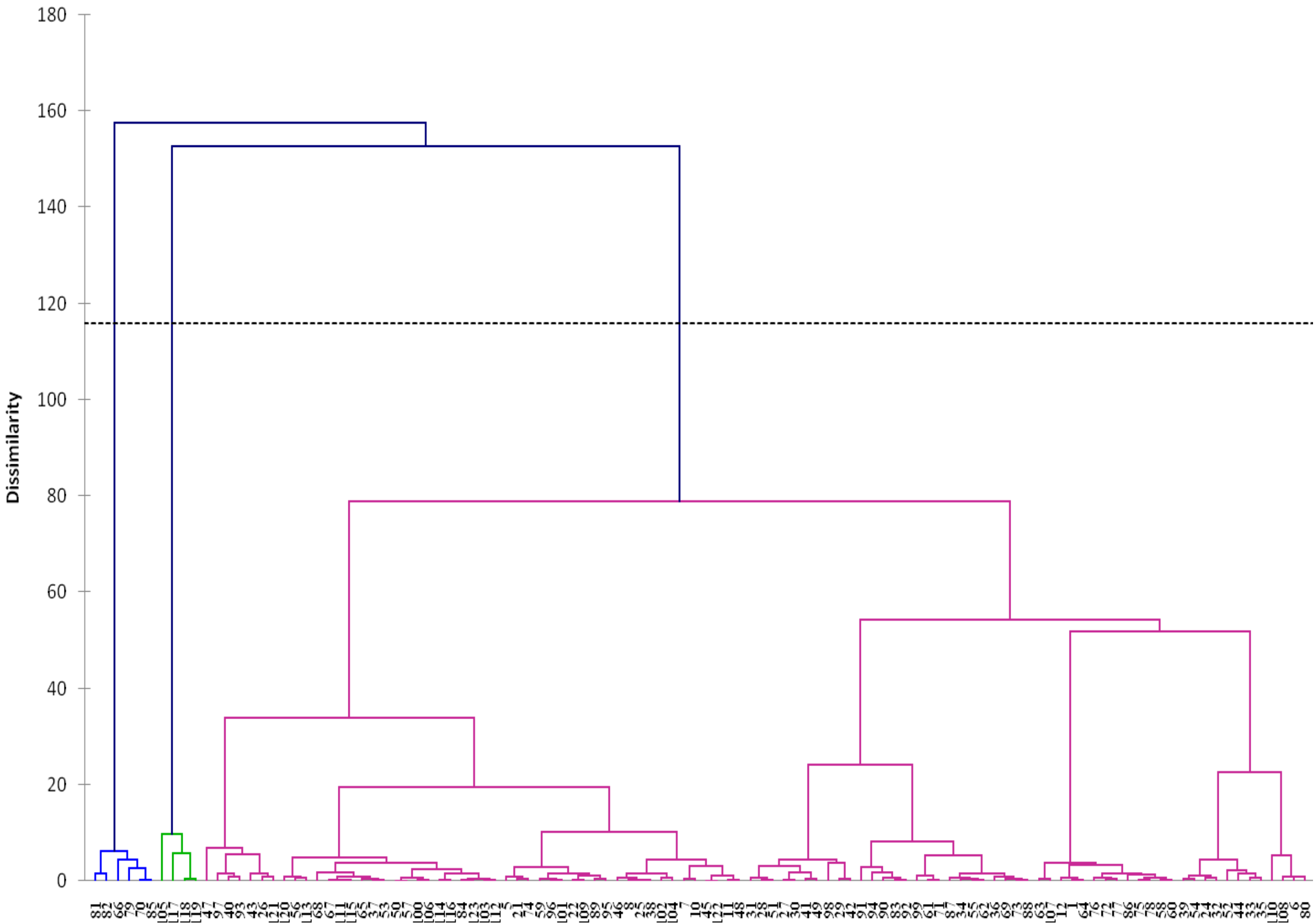

Figure 2. *Dendrogram obtained from quantitative data by agglomerative hierarchical clustering analysis.*

Table 1. Germination and seedling descriptors of 110*Sesbania* accessions.

Parameter	Range	Mean	Sd.
Emergence (%)	100.0-35.0	90.22	14.28
Germination (%)	100.0-33.0	85.31	14.11
Abnormal seedlings (%)	15.0-0.00	4.87	3.64
Dead/un-germinated seed (%)	65.0-0.00	9.95	15.57
Shoot length (cm)	15.0-3.00	10.0	0.97
Root length (cm)	9.00-1.00	4.00	0.95
Base diameter (cm)	0.08-0.03	0.05	0.01
Shoot-Root length ratio	5:1-2:1		
Vigour index	1731-384	1217	254.82

Table 2. Fresh weight and biomass of 110*Sesbania* accessions.

Parameter	Range	Mean	Sd.
Shoot fresh weight (mg/seedling)	169.0-96.0	129.0	13.1
Root fresh weight (mg/seedling)	52.0-16.0	31.0	5.60
Shoot biomass (mg/seedling)	13.0-5.00	7.20	1.20
Root biomass (mg/seedling)	7.00-1.00	1.60	1.00
Total biomass (mg/seedling)	20.0-6.00	9.00	1.92
Shoot-Root fresh weight ratio	9:1-2:1		

Table 3. Seed and seedling morphological descriptors of 5 selected *Sesbania* accessions.

Accession no.	Germination percentage	Vigour index	Shoot length (cm)	Root length (cm)	Shoot weight (mg/seedling)		Root weight (mg/seedling)		Total biomass (mg/seedling)
					Fresh	Biomass	Fresh	Biomass	
7	92	1334	10.80	3.70	132.0	8.00	30.0	2.00	10.0
10	93	1335	10.85	3.72	144.0	8.00	35.0	3.00	11.0
47	88	1350	10.85	4.49	152.0	9.00	39.0	2.00	11.0
49	99	1731	11.00	6.48	154.0	10.0	44.0	3.00	13.0
93	97	1372	10.63	3.51	139.0	8.00	36.0	3.00	11.0

Conclusion

Based on seed germination percentage, seedling vigour index, and biomass yield, five better performed *Sesbania* accessions (#7, 10, 47, 49 and 93) were selected for further studies. In PCA, the first three principal components explained 82.64% of the total variations in original data. In AHC analysis, based on different quantitative descriptors the accessions were divided into three major clusters with a few sub-clusters. Both in PCA and AHC analyses, *Sesbania* accessions #66, 70, 79, 81, 82 and 85 remained closer/in the same cluster, and these accessions could belong to same species. Rest of the accessions may belong to three or more different species of *Sesbania*.

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