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MORPHOLOGICAL DESCRIPTORS OF SEED AND SEEDLING FOR IDENTIFICATION OF *DHAINCHA* (*SESBANIA* SPP.) ACCESSIONS

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Abstract

Seed and seedling morphology of 110 *dhaincha* accessions were studied to search new characters that could add information pertinent to their species identification. Four seed coat colours - red, brown, grey and black, and two seed shapes - cylindrical and rectangular, were observed. Grey coloured, rectangular seeds produced seedlings with swollen radical with violet coloured hypocotyls. Wide variations were observed in the values of other morphological descriptors. Agglomerative hierarchical cluster analysis using quantitative descriptors showed that 110 accessions clustered in to four groups. The first two components (Factor 1 and 2) of principal component analysis explained 62.01% of the total variance of the collected *dhaincha* accessions. Seedlings with swollen radical and violet coloured hypocotyls, produced from grey coloured and rectangular seeds, were constantly positioned together in a cluster. Seed coat colour, shape of seed and radical, and hypocotyls colour may be used as important morphological descriptors for species identification of *dhaincha* accessions at the early growth stage.

Introduction

Floral morphology of *dhaincha* (*Sesbania* spp.) plants shows a continuous variation in the field which makes the identification of *Sesbania* species difficult to the beginners. Three species of *Sesbania viz*. *S. sesban* (L.) Merr., *S. bispinosa* (Jacq.) Wight and *S. cannabina* (Retz.) Poir., are commonly known as *dhaincha* in Bangladesh (Ahmed *et al*. 2009). Seed and seedling characters have emerged as very important morphological descriptors for identification of a species (Sanyal and Paria 2015). Seed colour and shape also help in identifying genetic variability in seed (Sajjad *et al*. 2012). Seed and seedling growth are governed by morphology and other derivatives. Source of seed and its genetic behavior denote seedling variability. Seedling morphology of flowering plants can help for taxonomic identification of concerned to morphology and others purposes (Ahammed and Paria 1996), and seedling characters are as important and reliable as that of floral one in the delimitation of species, genera and even families (Sanyal and Paria 2015). Seeds and seedlings also reveal much information about ecological as well as evolutionary profile of higher plants (Sousa and Sousa 1981). The morphological observation of *Sesbania* seedlings has been carried out by many workers (Sousa and Sousa 1981, Monteiro 1984, Srivastava and Kumar 2016). Monteiro (1984) described five principal characteristics of *Sesbania* seedlings: (i) Epigeal germination with a long hypocotyls and short epicotyls, (ii) foliaceous cotyledons, very short petiolate and escaping from the testa, (iii) first eophyll simple and resembling the cotyledons, (iv) second eophyllparipinnate with fewer leaflets than the metaphylls, and (v) epicotyls longer than the internodes between the first metaphylls. Although the characters of seedlings are limited in number, their diversity is large and thus their assemblages serve the purpose of identification (Welling and Laine 2000). Therefore, the present study was undertaken to investigate new characters of morphological importance for identification of *dhaincha* accessions at the early stage of growth.

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Materials and Methods

An experiment was conducted at the Laboratory of Plant Systematics, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh, to identify new character pertinent to identification of *Sesbania* species. Seeds of 110 *dhaincha* accessions were collected from different agro-ecological zones of Bangladesh (detailed collection information available upon request). The external morphological features of seeds *viz*., seed coat colour, shape, size and 1000-seed weight were carefully studied. Randomly selected 30 seeds were used for measurement from each accession. Seed coat colour and shape were determined under magnifying glass and photographed using digital camera (Shariq *et al*. 2015). Length and width were measured using a digital slide calipers with a precision of 0.1 to 150 mm (Ribeiro *et al*. 2015). Seed length was measured from base to top and width was taken in the middle line of seed.

For seedling morphology study, the experiment was laid out completely randomized design with four replications. Two hundred (50 × 4) healthy seeds of each accession were used for seedling morphology. Forty seedlings, 10 from each replication, from each accession were randomly taken two weeks after sowing for morphological study. The morphological descriptors *viz*. length of shoot, root, epicotyls and hypocotyls, cotyledon length and width, number of foliage leaves per plant, length and width of leaves, number of leaflets pairs per leaf were studied. The quantitative characteristics e.g., seedling height, base diameter, number of leaves/plant, leaf length and width, etc. were analyzed and calculated with Excel application (Aziagba-Bibian *et al*. 2015) such as arithmetic mean, standard deviation, range of variation.

Agglomerative hierarchical clustering (AHC) analysis was conducted using the XLSTAT 2009.3 program (https://www.xlstat.com/en/), based on the quantitative characters *viz*. seed length, 1000-seed weight, shoot length and base diameter. A dendrogram was built by AHC analysis (Sneath 1969). Principal component analyses (PCA) were conducted using the “R” software program based on the quantitative characteristic *viz*. seed length, seed width, seed length: seed width, 1000-seed weight, shoot length, root length and base diameter.

Results and Discussion

Based on seed coat colour, *dhaincha* accessions could be divided into four major groups (with many intermediates) *viz*. red, brown, grey and black (Fig. 1). Red seed coat was found in 49, brown in 48, grey in 9 and black in 4 accessions representing 44, 44, 8 and 4% of total collected *dhaincha* accessions, respectively (data not shown here). Heering *et al*. (1996) observed 7 types of seed coat colour *viz*. orange-brown, brown-mottled black, yellow-green, light green-mottled black, dark green-mottled black, black-mottled green and black, in *S. sesban* ssp. *sesban*. Mahmoud *et al*. (2015) observed that seed colour of *S. bispinosa* is pale brown, olive-green or greenish-black. The grey coloured seeds were rectangular in shape and produced seedlings with swollen radical with violet coloured hypocotyls; it may belong to another species. On the other hand, two distinct types of seed shape - cylindrical and rectangular, were observed in collected *dhaincha* accessions (Fig. 1). Cylindrical shaped (CS) seeds were observed in 104 accessions and rectangular shaped (RS) seeds in 6 accessions, respectively representing 95 and 5% of total collected accessions (data not shown here). Seed shape may differ due to genetic variability of the collected accessions (Sarwar *et al*. 2015). Different seed shapes - cylindrical vs. rectangular, may be produced by plants of different *Sesbania* species. Salimpour *et al*. (2007) reported great variability in seed shape among different *Trifolium* species. Ghamdi (2011) has conducted similar study in *Indigofera* (Fabaceae), where dissimilarity was also observed in seed surface and seed shapes within traditional species.

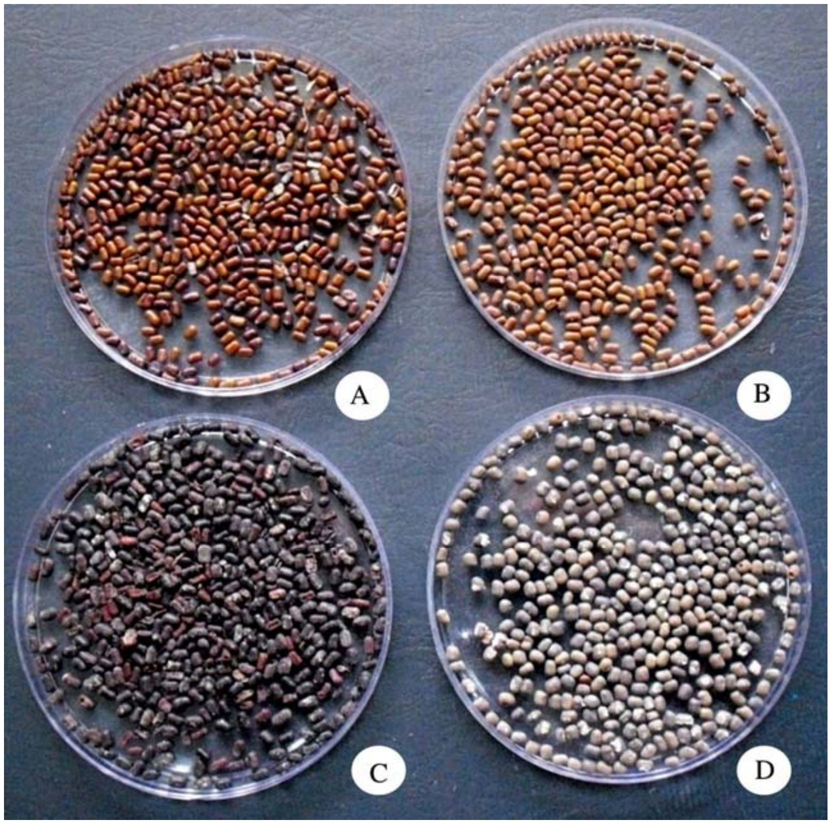

Fig. 1. Seed shape and seed coat colour of different *dhaincha* accessions (*Sesbania* spp.). A. Red colour and cylindrical seed shape. B. Brown colour and cylindrical seed shape. C. Black colour and cylindrical seed shape. D. Grey colour and rectangular seed shape.

Variations in seed length, seed width, seed length-width ratio, seed length × seed width, and 1000-seed weight are presented in Table 1. A wide range of variations was observed in seed length and width among the *dhaincha* accessions. In CS seeds, average seed length and width was 3.83 and 2.26 mm, respectively. In RS seeds, seed length and width varied from 4.07 to 3.42 mm and 2.93 to 2.20 mm, respectively. In CS seed, average length-width ratio was 1.69, while average seed area (length × width) was 8.64 mm^2. On the other hand, the value of average length-width ratio of RS seed was 1.43, while average seed area was 9.61 mm^2 (Table 1). Salimpour *et al*. (2007) reported that seed length, seed weight and seed length: seed weight of *Trifolium* species showed the greatest diversity among the species. The highest 1000-seed weight of CS seed was found 21.1 g and lowest was 13.3 g (Table 1). However, highest 1000-seed weight of RS seed was 22.0 g and lowest was 16.1 g. Results may vary due to genetic make-up (Sarwar *et al*. 2015) and/or the effect of collection locations of different *dhaincha* accessions. Bakhah *et al*. (2006) studied on shape, size and weight of certain weed seeds and found similar results.

Different morphological descriptors of *dhaincha* seedlings are presented in Table 2. Quantitative descriptors of seedlings showed significant variations. The seedlings from CS seed produced longer root compared to the RS seed produced seedlings. On the other hand, RS seed produced longer shoot compared to the CS seed produced shoot length. However, RS seed produced comparatively larger base diameter over than CS seed produced base diameter. It may be due to genetic make-up of the species. Grey coloured RS seeds produced swollen radical and violet colour hypocotyls (Fig. 2). These distinct morphological descriptors may help to separate and identify the *Sesbania* species. The seedling morphological descriptors may suggest that CS and RS seed producing plants belong to at least two or more different *Sesbania* species. Seedlings, from both types of seed, produced long hypocotyls and short epicotyls. Hypocotyls length was 94% and epicotyls length was 6% at two weeks aged in both RS and CS seed producing seedlings. It may occur due to the genetic make-up of *Sesbania* species (Sarwar *et al*. 2015). Monteiro (1984) opinioned that seedling of *Sesbania* having long hypocotyls and short epicotyls are suitable for the

Table 1. Seed length, width and 1000-seed weight of *dhaincha* accessions.

Parameter	Cylindrical seed shape (CSS)			Rectangular seed shape (RSS)		
	Range	Mean	Sd	Range	Mean	Sd
Seed length (mm)	4.18 - 3.44	3.83	0.10	4.07 - 3.42	3.70	0.22
Seed width (mm)	2.62 - 1.93	2.26	0.13	2.93 - 2.20	2.60	0.23
SL: SW	1.92 - 1.24	1.69	0.13	1.73 - 1.24	1.43	0.19
SL × SW	10.21 - 7.12	8.64	0.60	10.65 - 8.39	9.61	0.85
1000-seed wt. (g)	21.1 - 13.3	16.5	1.45	22.0 - 16.1	20.0	2.06

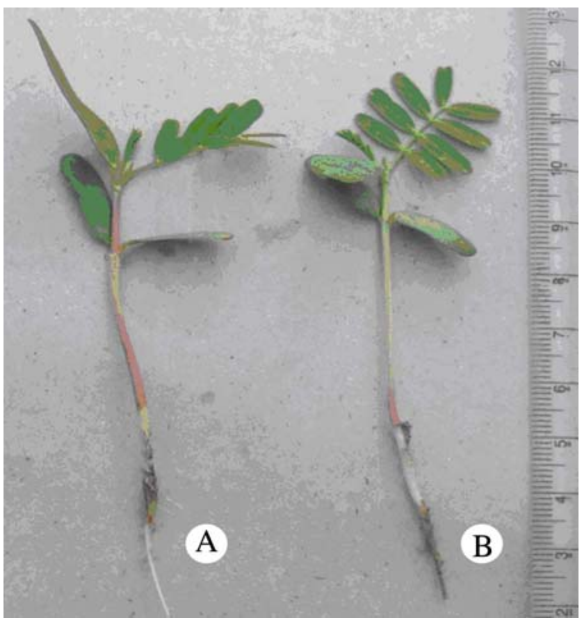

Fig. 2. Fourteen days old seedlings produced from rectangular shaped seed (A) and cylindrical shaped seed (B).

species of *S. tetraptera* and *S. grandiflora*. However, Veasey *et al.* (1999) observed that shoot length (hypocotyls length + epicotyls length) of *S. sesban* was longer than that of *S. grandiflora* but shorter than those of *S. exasperata*, *S. tetraptera* and *S. virgata* at 17 days aged seedling. Two opposite cotyledons were found both in CS and RS producing seedlings. Later, two types of leaves, the first leaf was a simple leaf and the second one was a compound leaf (paripinate), produced on epicotyls (Fig. 3). Cotyledons were relatively larger in CS seed produced seedlings compared to RS seed produced seedlings. However, uni-foliate leaf, leaflets and compound leaves were larger in size in RS seed produced seedlings than CS seed produced seedlings (Fig. 3). These results confirm the observations of Veasey *et al*. (1999) and Sanyal and Paria (2015). They found that first simple leaf was alternate, unifoliate and stipulate. Compound leaves were alternate, paripinate, stipulate, petiolate and even leaflets (in both CS and RS seed produced seedlings). Veasey *et al*. (1999) reported that leaf length of *S*. *sesban* was longer than that of *S*. *exasperata* but shorter than those of *S*. *grandiflora*, *S*. *tetraptera* and *S*. *virgata* at 17 days old seedling. However, leaf width of *S*. *sesban* was smaller than those of *S*. *grandiflora* and *S*. *virgata* but wider than *S*. *tetraptera* at 17 days old seedling. From the above discussion, it may be concluded that CS seed produces seeding belong to one (or more) *Sesbania* species and RS seed produces seedling to other species.

Table 2. Seedling attributes of *dhaincha* accessions.

Parameter	Cylindrical shaped (CS) seed			Rectangular shaped (RS) seed		
	Range	Mean	Sd	Range	Mean	Sd
Root length (mm)	90.0 - 10.0	37.8	11.9	70.0 - 20.0	30.0	8.64
Shoot length (mm)	130.0 - 30.0	101.3	11.5	150.0 - 80.0	119.0	15.3
Base diameter (mm)	0.90 - 0.30	0.53	0.17	1.10 - 0.05	0.67	0.16
Hypocotyls length (mm)	120.0 - 28.0	95.6	12.1	139.0 - 75.0	111.42	14.41
Epicotyls length (mm)	10.0 - 2.00	5.76	1.89	11.0 - 5.00	7.88	1.28
Cotyledon length (mm)	17.0 - 10.0	14.2	1.46	18.0 - 10.0	14.2	1.16
Cotyledon width (mm)	7.00 - 4.00	5.44	0.62	6.00 - 4.00	5.37	0.61
Simple leaf length (mm)	26.0 - 10.0	18.9	3.90	28.0 - 10.0	19.2	3.40
Simple leaf width (mm)	12.0 - 4.00	6.39	1.52	10.0 - 4.00	6.27	1.46
No. of foliage leaves/plant	3.00 - 2.00	2.04	0.19	3.00 - 2.00	2.03	0.18
Compound leaf length (mm)	16.0 - 4.00	9.65	2.62	15.0 - 4.00	9.00	2.30
Compound leaf width (mm)	10.0 - 2.00	4.81	1.18	8.00 - 2.00	4.63	1.10
No. of leaflets (pair)/leaf	5.00 - 4.00	4.99	0.08	5.00 - 4.00	4.97	0.19
Leaflets length (mm)	11.0 - 2.00	5.45	1.22	10.0 - 2.00	5.43	1.70
Leaflets width (mm)	4.00 - 1.00	2.19	0.68	4.00 - 1.00	2.17	0.65

In AHC analysis, truncation at the level of 94.56 resulted in 4 clusters (Fig. 4). The similarity and dissimilarity pattern of clustering analyses were done in order to assess the species grouping. The dendrograms showed that every cluster has some sub-clusters and every sub-cluster contain several accessions. Clusters 1, 2, 3 and 4 contains 24, 59, 4 and 23 accessions, respectively (Fig. 4). Accessions of the same species might be grouped together in a cluster based on morphology and agronomic characteristics (Veasey *et al*. 2001). The accession numbers 70, 79, 81 and 82 always remain in the same cluster 3 and the accession number 105 alone. Rest of the accessions goes to the different clusters (Fig. 4). It may occur due to genetic variability (Sarwar *et al*. 2015) and/or collection location (/origin) of the accession (Rani *et al*. 2006). Heering *et al*. (1996) reported that different cluster separated based on their leaflet number and size, seed size and colour as well as growth habit. The clustering pattern of accessions may also differ due to their location effect and genetic behavior. Each accession has typical characteristics and showed similarity or dissimilarity. Grouping of accessions into clusters may be rather sensitive to the particular potion of the descriptors used in clustering. A different option of descriptors, apparently similar reasonable, may give different clusters. Above discussion and dendrograms analysis revealed that accession numbers 70, 79, 81 and 82 may belong to same species and accession number 105 may also be another species, and rest of accessions may be other one or two species. The AHC analyses could be considered as the first step of identification of accessions.

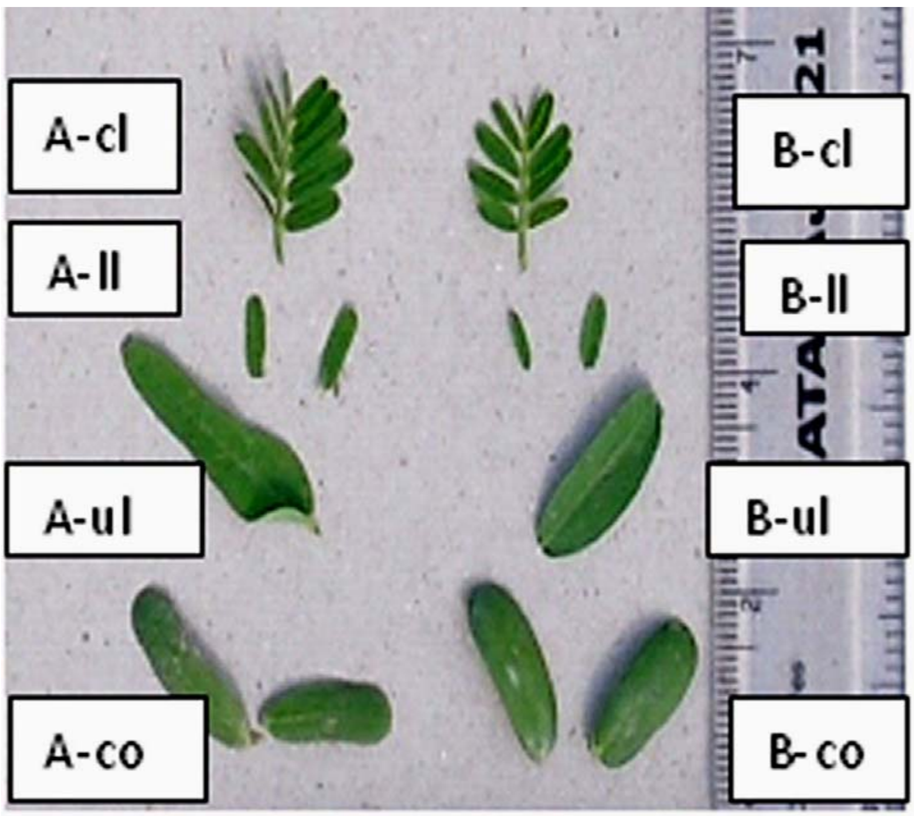

Fig. 3. Rectangular shaped seed produces cotyledons (A-co), uni-foliate leaf (A-ul), leaflet (A-ll), compound leaf (A-cl), cylindrical shaped seed produces cotyledons (B-co), uni-foliate leaf (B-ul), leaflet (B-ll) and compound leaf (B-cl).

In PCA using the seven quantitative characters, the first and second principal components explain 62.01% of the variance of the sample, 43.80% for the first component - Factor 1; and 18.20% for the second - Factor 2 (Fig. 5). The maximum number of *dhaincha* accessions were located on the right part of the PCA graph, while the accession number 70, 79, 81, 82 and 85 were located very closely on the left part and accession number 105 was alone and showed in lower part of the graph (Fig. 5). This separation was mostly caused by the differences in shoot length, 1000-seed weight, seed width and base diameter effect. The accessions number 70, 79, 81, 82, and 85 may belong to same *Sesbania* species; similarly accession number 105 may be another species.

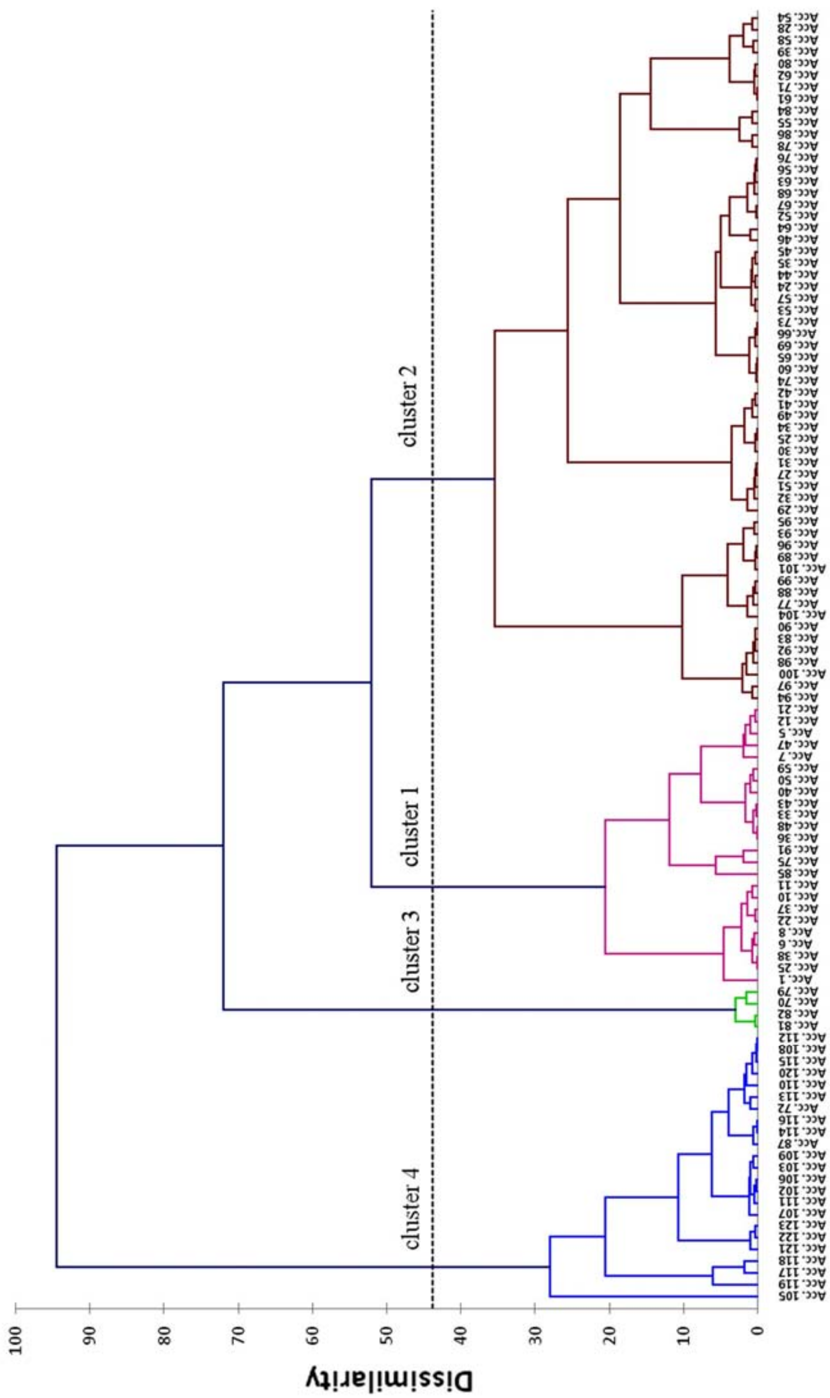

Fig. 4. Dendrogram obtained from quantitative data by Agglomerative Hierarchical Clustering Analysis.

Results of PCA were supported by dendogram resulted from AHC analysis (Fig. 4). Moreover, accession number 117 was also positioned in distinct location lower left part and other accessions also were situated other place at the right part due to the effect of seed length, root length and seed length: seed width. Accessions distantly located in PCA might be the separate species of *Sesbania* (Fig. 5). The descriptors used for accessing the variability among *dhaincha* accessions were efficient in discriminating the110 accessions and might be *Sesbania* species as well. The principal component scatter plot of *dhaincha* accessions depicted that they are close together and being similar when related on seven variables. The collected accessions were not more diverse from each other as they were overcrowded on the same area. It indicates that the accessions having almost similar quantitative characters with respect to seed length and base diameter, may belong to same species.

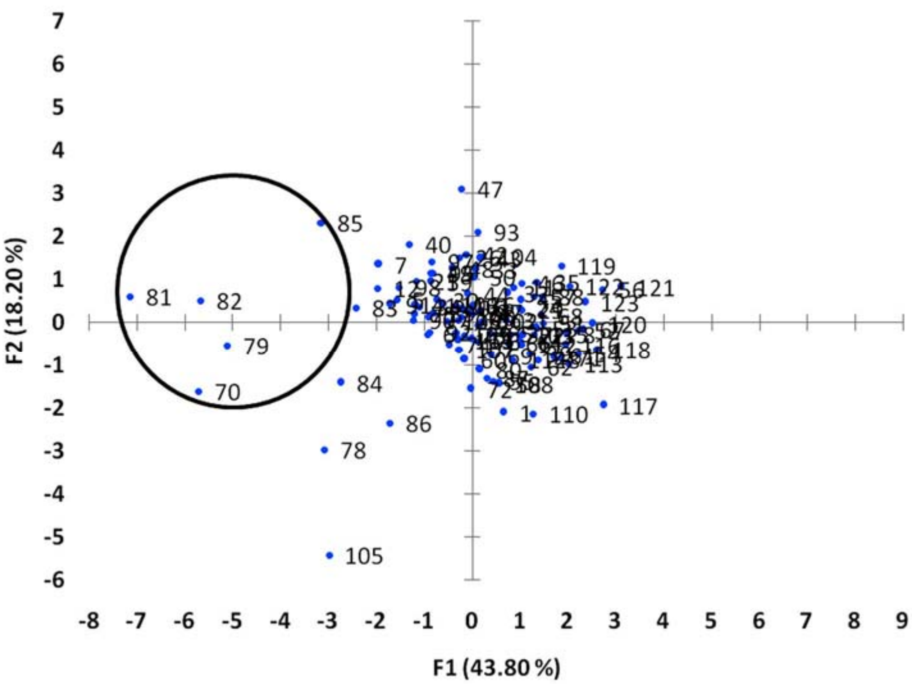

Fig. 5. Two dimensional graph representing accessions in the principal component analysis.

The comparison between the AHC analysis and PCA, the dendrogram has identified some relationships among the collected *dhaincha* accessions (Fig. 4), however, the PCA detect these relationships more precisely (Fig. 5). Several quantitative and qualitative differences were observed in seed and seedling morphological descriptors *viz*. seed width, shoot length, root length, hypocotyls length, colour of shoot, and swollen radical, which were very helpful to distinguish among the *dhaincha* accessions and might also be to identify *Sesbania* species. From the results of this study, the collected *dhaincha* accessions may belong to at least three different *Sesbania* species. A detailed characterization of *dhaincha* accessions by floral morphological descriptors should be done for accurate identification (/confirmation) of *Sesbania* species.

Seed and seedling morphological descriptors *viz*. seed shape, seed coat colour, radical shape and hypocotyls colour, could be useful for the identification of *dhaincha* accessions (*Sesbania* species) at the initial and/or early stage of growth.

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