SEM of seed coat surface characters and the taxonomic relationships in the genus *Sesbania* Scop. (Leguminosae - Papilionoideae).

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Criteria obtained by SEM of seed coat surface have been used to re-assess the taxonomic relationships between 19 species belonging to three subgenera of the genus *Sesbania*. The produced characters were analyzed by the NTSYS-pc. program package using the UPGMA clustering method. The produced phenogram supported the treatment of the three taxa Agati, Daubentonia and Sesbania as subgenera in the genus *Sesbania*. SEM criteria have revealed that, the genus represent a natural monphyletic group and the subgenus Sesbania is the center of the genus from which the other subgenera could have been evolved.
Key words: Leguminosae – Papilionoidae – *Sesbania* – Seed scan.

Introduction

Species of the genus *Sesbania* have a considerable economic importance in agriculture as manure for food crops; a source of wood, paper and gum; animal fodder and to maintain soil productivity as well as to improve soil structure for wheat and rice cultivation (Evans & Rotor 1987, Arunin *et al.*, 1988; Ladha *et al.*, 1989; Shioya & Ito, 1990). *Sesbania* belongs to tribe Robinieae of the subfamily Papilionoideae and comprises about 50, mostly annual species distributed throughout tropical subtropical regions (Mabberley, 1997).

Taxonomic structure of *Sesbania* has always been different between taxonomists. Adanson (1763), Bailey (1949) and Mabberley (1987) had considered Agati, Daubebtonia, Glottidium and Sesbania as distinct genera closely related to each other. However, Bentham (1859) had treated the former three taxa as sections within the genus *Sesbania*. Siwundla & Stucky (1986; 1989) confirmed the treatment of *Glottidium* as a monotypic genus and retained *Daubentonia* as a section of *Sesbania*. On the other hand, Baker (1876), Gillette (1963), Burbidge (1965), Sachet (1988) and Monterio (1994) considered Agati, Daubentonia and Sesbania as the main subgenera of *Sesbania*. Difficulties in determining the specific relationships have been encountered by the

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workers in the genus because of the insufficient information as well as the conflicting nomenclature of the species (Saraswati *et al.* 1993; Badr *et al.* 1998).

The species relationships within the genus have been delimited based on their vegetative, floral and seed shape characters (Monteiro, 1994); cytological observations (Salimuddin, 1993; Forni-Martins *et al.* 1994; Abou- El-Enain *et al.*, 1998) and SDS-PAGE of seed protein profiles (Saraswati *et al.* 1993; Badr *et al.* 1998). However, scanning electron microscopy (SEM) of seed coat criteria have not been applied at the subgeneric level in *Sesbania*.

SEM of seed coat surface is a useful technique in the identification and classification of various taxa (Barthlott, 1981). A comparison of surface scan patterns of the seed coat has efficiently been used in studying species of some genera including *Vigna* (Kumar *et al.*, 1984), *Cassia* (Ponomarino *et al.*, 1990; Bhattacharya & Saha, 1991), *Sesbania* (Seth &Vijaya-raghavan, 1991) and *Vicia* (Chernoff *et al.*, 1992).

In the present work, the SEM of seed coat surface criteria are used to reassess the taxonomic relationships of 19 species in the genus *Sesbania* in the light of morphological, cytological and SDS-PAGE of seed protein characters.

Materials and Methods

Seeds of the species studied were provided by the gene bank of International Livestock Center for Africa (ILCA), Ethiopia, the plant genetic resources conservation unit of the United States Department of Agriculture (USDA), Gorgia, USA and the Botanical Garden of the Faculty of Education, Ain Shams University (BGFE), Egypt. The source and origin of the examined materials are given in table 1. Plant of almost all taxa have been grown in open ground in the Botanical Garden of the Faculty of Education, Ain Shams University, where voucher specimens are kept at the Department of Biological Sciences and Geology.

For the study of the seed coat surface, seeds were mounted with colloidal silver on copper stubs, coated with a thin layer of gold in Polaron E 5000, the epidermal seed coat were photographed by a Jeol-T- Scanning Microscope at the magnification of 750. Terminology of Stearn (1966);Stant (1973) and Barthlott (1981) has been used to describe the characteristics of the seed coat. For the data analysis, total number of the recorded characters in each taxon were scored and coded as shown in Table 1 for creating the data matrix of computation. The relationships between the studied taxa; have been expressed by average taxonomic distance (dissimilarity); and demonstrated as phenogram based on the analysis of the recorded characters using the NTSYS program package for IBM-pc as described by Rohlf (1993). In the computer analysis, the taxa are numbered as indicated in Table (1).

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Results and Discussion

Investigation of the SEM patterns of seed coat surface in the species of the three subgenera i.e. Agati, Daubentonia and Sesbania (Fig. 1 and Table 1) has revealed the presence of a considerable number of common characters. These include the polygonal reticulate spermoderm cells; wavy shape, medium thickness and the presence of the striation of anticlinal walls as well as the rough highly striated periclinal walls. The speromoderm cells in species of the subgenus Agati were characterized by the polygonal shape, monomorphic pattern, the cell size about 16x11µm and the highly striated periclinal walls, while the speromoderm cells in species of subgenus Daubentonia were characterized by hexagonal, monomorphic and cell size about 12x10 µm.

The phenogram illustrating the relationships between the studied species based on the character analysis using UPGMA clustering (Fig. 2) has revealed that, the species studied are divided into three major groups belonging to the three subgenera Daubentonia, Agati and Sesbania that were distinguished from each other at the distance levels of 0.6, 0.9 & 1.45, respectively, and clustered together at the 1.62 level. The first group (i.e. Daubentonia) included *S. virgata* (19) and *S. speciosa* (17). The second group (i.e. Agati) included *S. grandiflora* (6), *S. tetraptera* (18) & *S. formosa* (4) from which the former species (6) was separated, while the latter two species (i.e.18 & 4) were clustered together. The third group (i.e. Sesbania) comprised three subgroups, one included *S. goetzeii* (5), *S. rostrata* (14), *S. hitistyla* (8), *S. greenwayii* (7) and *S. pachycarpa* (12) from which *S. bispinosa* (1) and *S. cericea* (15) were separated. The second subgroup included *S. exaltata* (2), *S. macrantha* (10) *and S. quadrata* (13). Two species namely *S. microphylla* (11) and *S. exasperata* (3) were distinct from other species in the latter subgroups. The third subgroup included *S. leptocarpa* (9) and *S. sesban* (16).

Badr *et al.* (1998) recorded considerable differences between the sections Agati and Daubentonia and treated them as heterogeneous groups distinguished both from each other as well as from species of the subgenus Sesbania. They also pointed out that, the seed protein data was insufficient for their delimitation in two separate genera as proposed by Adanson (1763) Bailey (1949) and Mabberley (1987). However, the present data confirm the retention of the three taxa Agati, Daubentonia and Sesbania in the genus *Sesbania* and their delimitation as three subgenera as proposed by Baker (1871; 1876), Gillette (1963), Burbidge (1965), Sachet (1988) , Monteiro (1994) and Badr *et al.*(1998).

In the light of the cytological data (Salimuddin, 1993; Forni-Martins *et al.* 1994 and Abou El-Enain *et al.*1998) and SDS-PAGE of seed protein data (Saraswati *et al.* 1993; Badr *et al.* 1998), the subgenus Sesbania was considered as the center of the genus from which the subgenera Agati and Daubentonia were derived. The SEM of seed coat features supports this conclusion. Three closely related SEM seed coat surface patterns (Fig. 1 and Table 1) have been observed in species of the subgenus Sesbania and recorded in species of both subgenera Agati & Daubentonia at the same time. The first pattern is the polygonal polymorphic with a hairy like appendages and recorded in nine species namely *S. exasperata* (3), *S. goetzii* (5), *S. greenwayi* (7), *S. hirtistyla* (8), *S. microphylla* (11), *S. pachycarpa* (12), *S. quadrata* (13), *S. rostratra* (14) and *S. sericea* (15). The second is the reticulate slightly hexagonal pattern and is recorded in *S. bispinosa* (1), *S. leptocarpa* (9) and *S. sesban* (16). The latter two patterns are observed in species of the subgenera Agati & Daubentonia, respectively and can be considered as transition forms between the three subgenera revealing the common origin between them. The third is the ill-defined pattern and is recorded in two species i.e. *S. exaltata* (2) and *S. macrantha* (10).

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Furthermore, these data indicate that, the genus *Sesbania* represents a natural monophyletic group in the tribe Robinieae. The latter view has previously been concluded by Badr *et al.* (1998) based on the similarities in SDS-PAGE pattern; vegetative and floral characters (Montiero, 1994), the uniformity in the basic chromosome number (Goldblatt, 1981) and homogeneity in karyotype symmetry (Salimuddin, 1993; Vijayakumar & Kuriachan, 1995 and Abou-El-Enain *et al.*1998).

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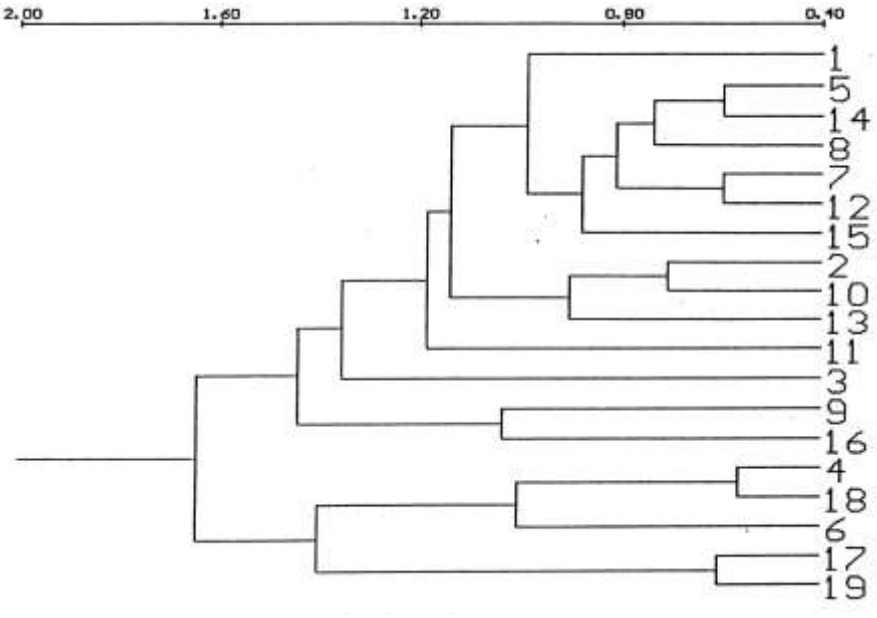

Fig. 2: The phenogram illustrating the relationships between the species studied; numbered as in Table 1; using the UPGMA clustering method.

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Table 1: Source, origin and subgeneric delimitation of the species studied and codes of the produced spermoderm characters for the numerical analysis.

No	Species	Source	Origin	Subgenus	Spermoderm cell characters									
					Shape	Pattern	Size	Anticlinal walls				Periclinal walls		
								Shape	Thick.	Ster.	Level	Shape	Text.	Ster.
01	*S. bispinosa* (Jacq.) Wright	USDA	India	Sesbania	1	1	1	1	2	2	1	1	4	2
02	*S. exaltata* Cory	USDA	Australia	Sesbania	2	1	0	0	2	1	1	1	1	1
03	*S. exasperata* H.B.K.	USDA	Argentina	Sesbania	2	1	0	0	2	0	2	1	0	0
04	*S. formosa* (F. Muell) Burb.	USDA	Australia	Agati	1	0	3	1	2	2	1	0	1	2
05	*S. goetzii* Harms	ILCA	Tanzania	Sesbania	2	1	1	1	2	1	1	1	0	1
06	*S. grandiflora* (L.) Pior.	ILCA	Ethiopia	Agati	1	0	3	0	2	1	1	1	3	2
07	*S. greenwayii* Gill.	ILCA	Tanzania	Sesbania	2	1	1	1	1	2	1	1	0	1
08	*S. hirtistyla* Gill.	ILCA	Tanzania	Sesbania	2	1	1	1	2	1	2	1	2	1
09	*S. leptocarpa* Cronq.	USDA	Afghanistan	Sesbania	1	1	0	1	2	2	0	1	4	1
10	*S. macrantha* Phill. & Hutch.	ILCA	Tanzania	Sesbania	2	1	0	0	2	1	1	0	1	0
11	*S. microphylla* Phill.&Hutch.	ILCA	Tanzania	Sesbania	2	1	0	1	0	1	1	1	0	0
12	*S. pachycarpa* DC.	ILCA	Senegal	Sesbania	2	1	1	1	2	2	1	1	2	1
13	*S. quadrata* Gill.	ILCA	Tanzania	Sesbania	2	1	0	0	1	1	0	1	0	1
14	*S. rostrata* Brem. & Obem.	ILCA	Tanzania	Sesbania	3	1	1	1	2	1	1	1	2	1
15	*S. sericea* (Willd.) Link.	BGFE	Egypt	Sesbania	2	1	1	1	2	1	1	1	2	1
16	*S. sesban* (L.) Merr.	BGFE	Egypt	Sesbania	1	1	1	0	2	2	1	1	4	2
17	*S. speciosa* Taub.	USDA	Ethiopia	Daubentonia	0	0	2	0	0	1	1	0	0	1
18	*S. tetraptera*	USDA	Swaziland	Agati	1	0	3	1	2	1	1	0	1	2
19	*S. virgata* (Cav.) Pers.	USDA	Uruguay	Daubentonia	0	0	2	0	0	1	0	0	0	1

USDA= United States Department of Agriculture; ILCA= International Livestock Center for Africa gene bank ; BGFE = Botanical Garden of the Faculty of Education; Thick. =Thickness, Ster. = Striation, Text. = Texture. Character codes: Cell shape: hexagonal = 0, polygonal = 1, irregular = 2, illdefined = 3; Cell pattern: monomorphic = 0, polymorphic =1; Cell size: ≈ 5x3 µm = 0, ≈ 7x4 µm = 1, ≈ 12x10 µm = 2, ≈ 16x11µm = 3. Anticlinal walls, Shape: straight=0, wavy = 1; Thickness: thin = 0, medium = 1, thick = 2; Steriation: absent = 0, few = 1, many = 2; Level : normal = 0, raised = 1, highly raised = 2. Periclinal walls, Shape: flat = 0, concave = 1; Texture: smooth = 0, rough = 1, hairy = 2, tuberculate = 3, illdefined = 4. Striation: absent = 0, few = 1, numerous = 2.

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