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Arab Univ.
J. Agric. Sci.,
Ain Shams Univ.,
Cairo, 17(1), 19-30, 2009

NUMERICAL TAXONOMY STUDY ON SPECIES OF CLEOMACEAE IN EGYPT

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Keywords: Morphology, Anatomy, Seed protein banding, Numerical taxonomy of Cleomaceae

ABSTRACT

A data matrix comprising 100 characters of morphology, anatomy and seed protein banding recorded comparatively for ten species of *Cleome* and *Gynandropsis* (Cleomaceae) was analyzed under three fundamentally different numerical methods. The PRIMER analysis used the Bray Curtis (SØrensen) distance measure together with the single linkage clustering methods. The SPSS program used Ward'sclustering as a distance measure and the average linkage clustering method distance or complete linkage distance. All three dendrograms agree in grouping the ten species into two major groups: A (including *C. droserifolia* and *C.chrysantha*) and B (including *C. amblyocarpa*, *C. paradoxa, C. arabica, C. viscosa C. brachycarpa, C. scaposa*, *C. hanburyana* and *Gynandropsis gynandra*).This indicates that Gynandropsis *gynandra* ought to be submerged in *Cleome* as *Cleome gynandra*.

INTRODUCTION

Cleome and *Gynandropsis* distributed in tropical and subtropical regions. Only nine *Cleome* species and one *Gynandropsis* are reported from Egypt **Boulos (1999)**.

Different studies are present in regard to the treatments of tribe *Cleomeoideae* as being included within Capparidaceae (Capparaceae) or segregated as distinct family Cleomaceae. On the other hand **Muschler (1912); Post (1932); Pax & Hoffman (1936); Montasair & Hassib (1956); Jafri (1977); Thorne (1992) and Boulos (1999)** classified the genera *Cleome* and *Gynandropsis* under family Capparaceae. While, **Tăckholm (1974); Boulos (1995); El-Hadidi & Fayed (1994/1995) and Hall *et al* (2002)** segregated these two genera under family Cleomaceae.

From a taxonomic treatments *Cleome* and *Gynandropsis* are generally considered as problematic genera for troubled position and the relationships between the two genera are still debatable and not well resolved. **El-Hadidi & Fayed (1994/1995), Al-Gohary (1997), Khafagi & Al-Gohary (1998)** and **Voznesenskaya *et al* (2007)** included *Gynandropsis* under *Cleome* as *Cleome gynandra* while **Boulos (1999)** retained *Gynandropsis* as a distinct genus.

Electrophoretic patterns of seed storage protein have been a useful tool in taxonomy as an additional approach to assess relationships **(Gifford and Chinnappa, 1986).** The protein gel profiles reflect genetic affinities within a taxon and even between different biological entities **(Ladizinsky, 1979).**

Also, the general morphology at higher taxonomic level is phylogentically valuable **(Manson 1997)**, while the combined analysis of molecules and morphology is a powerful tool in low-level taxonomy **(Fjellhein *et al* 2001).**

On the other hand there is no study has been done on the seed protein pattern of *Cleome* and *Gynandropsis* for that reason the present work intended to fling light upon the significance of electrophoretic patterns of seed storage protein in addition to morphological data and using numerical analysis which may prove the importance of these characters in the species delimitation.

MATERIALS AND METHODS

Nine *Cleome* species and one *Gynandropsis* were collected **(Table 1)** either fresh or as herbarium specimens and identified according to keys of **Tăckholm (1974) and Boulos (1999).**

(Received December 24, 2008)
(Accepted January 14, 2009)

Table1.The species and their collection data

Species	Localities and Date
1- *Cleome droserifolia* (Forssk.)Del.	- Gable Elba,23/1/2005
2- *C. amblyocarpa Barratte*&Murb.	-Rafah,Sinai,13/9/1965*
3- *C. paradoxa* R.Br.exDC.	-Gable Elba,24/2/1976*
4-*C. arabica* L.	-Wadi Aber near Suez,15/2/1965* - Wadi Hof, 19/2/ 2002.
5- *C. chrysantha* Decne	-Gable Elba,23/1/ 2005
6- *C. viscosa* L.	-Burg El Arab, Mariut 24/9/1971* -Aswan, 3/2006
7- *C. brachycarpa* DC.	-Gable Elba,23/1/ 2005
8- *C. scaposa* DC.	-Gable Elba,23/1/2005
9- *C. hanburyana* Penz.	-Gable Elba,23/1/2005
10-*Gynandropsis gynandra* (L.) Briq	-Cairo-Inshas road,15/4/1960*

*= Herbarium specimen (CAI, CAIM)

Morphological data was gathered from literature and scored for the ten species of the Cleomaceae included in this analysis. The sources of data for this analysis were **Muschler (1912); Post (1932); Montasir& Hassib (1956); Zohary (1966); Jafri (1977); Khalifa & Al-Gohary (1982); Al-Gohary (1997); Khafagi & Al-Gohary (1998) and Boulos (1999).**

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed for banding of seed proteins **(Table 2)** according to the **Studier (1973).** Gels were photographed, scanned and analyzed using Helena Jonior 24 photoscanner and the data were integrated using scanner software.

100 characters were used for analysis. Characters of morphology, anatomy and seed proteins banding tabulated in **Appendix I & 2**. These were subjected to numerical analysis under two programs: The PRIMER software, version 5.0 analyses used the Bray Curtis (SØrensen) distance measure together with the single linkage clustering methods measure similarity percent and the SPSS version 16 program used Ward's clustering method Agglomeration Schedule measure Euclidean distance, average linkage distance and complete linkage distance(between group).

The relationships between the studied species of *Cleome* and *Gynandropsis* have been demonstrated as dendrograms **(Fig. 1).** The grouping of operational taxonomic units (OTU'S) produced from the analysis were examined and compared with the current taxonomic classification of the two genera of family Cleomaceae.

RESULTS AND DISCUSSION

1- Morphological data

The 100 characters states used in cluster analysis are tabulated in **(Appendix I)** used to construct a data matrix **(Appendix II).**

The morphological characters (including vegetative and floral parts, pollen grain and seed surface scan features in addition anatomical studies of stem, petiole, and blade) were recorded and showed great variations within the studied species as shown in **(Appendix I).**

2- Seed protein electrophoresis

The results of the electrophoresis pattern analysis of the seed proteins of the ten studied species of Cleomaceae are presented in **Table (2).**

The bands were detected with different molecular weights ranged from 205 KDa to 10 KDa. The total number of bands about 23 varied from species to another, ranging between 8 – 21 bands for studied species. The highest number of protein bands (21) was found in *C. hanburyana*, while the lowest number (8) was recorded in *C. viscosa*.

Table 2. SDS-PAGE of total seed protein bands of investigated species

Lanes / Rows	M (mol.w.)	Species 1	2	3	4	5	6	7	8	9	10
R1	205	+	+	+	+	+		+	+	+	+
R2	176	+	+	+				+	+	+	+
R3	128									+	
R4	116	+	+	+					+	+	+
R5	114									+	
R6	106	+	+	+		+				+	+
R7	97	+		+						+	+
R8	89	+		+	+	+	+	+	+	+	+
R9	84	+	+	+	+	+	+	+	+	+	+
R10	70	+	+	+				+	+	+	+
R11	60	+	+	+	+	+	+	+	+	+	+
R12	55	+	+	+	+	+	+	+	+	+	+
R13	51	+	+	+	+	+	+	+	+	+	+
R24	42	+	+	+	+	+	+	+	+	+	+
R15	38	+	+	+	+	+	+	+	+	+	+
R16	36	+	+	+						+	+
R17	29				+	+			+		
R18	27									+	+
R19	24								+		
R20	22	+	+	+					+	+	+
R21	18	+	+	+	+	+			+	+	+
R22	16	+	+	+				+	+	+	+
R23	10	+	+	+	+	+	+	+	+	+	+
Total bands		18	16	18	11	12	8	12	17	21	19

Some of the examined species had a specific band as in *C. hanburyana* (mol. wt 128, 114 KDa), *C. scaposa* (mol. wt 24 KDa), The results also showed that the bands with molecular weights 84, 60, 55, 51, 42, 38 and 10 KDa were common and shared in all studied species and may be taken as the genus specific bands. The bands having mol.wt.89 KDa absent only from *C. amblyocarpa* and present in the remainders. On the other hand the bands having mol.wt.176, 70 and16 KDa absent from *C. arabica*, *C.chrysantha* and *C. viscosa* only.

3- Numerical analysis

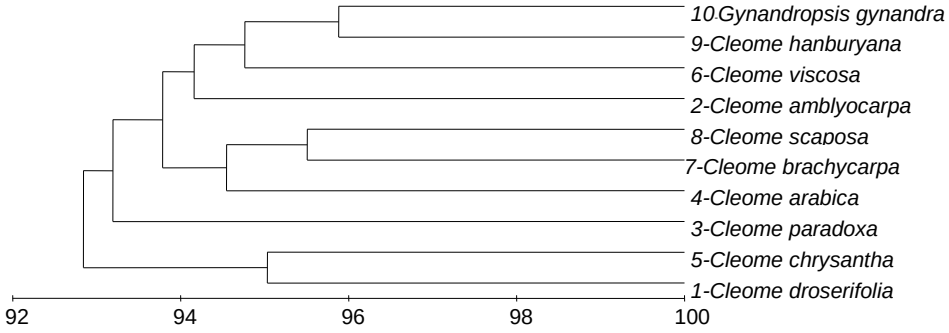
All combined characters from morphological and anatomical characters as well as seed protein banding recorded comparatively for ten species for numerical analysis by using different methods of clustering as a tool in identification of the studied species and in taxonomic relationships among *Cleome* and *Gynandropsis*.

The results of all different methods of clustering particularly Bray Curtis with single linkage measure similarity percent **(Fig.1, A),** WARD linkage Agglomeration Schedule measure Euclidean distance **(Fig.1, B-a)**, average linkage distance **(Fig.1, B-b)** and complete linkage distance show two major clusters.

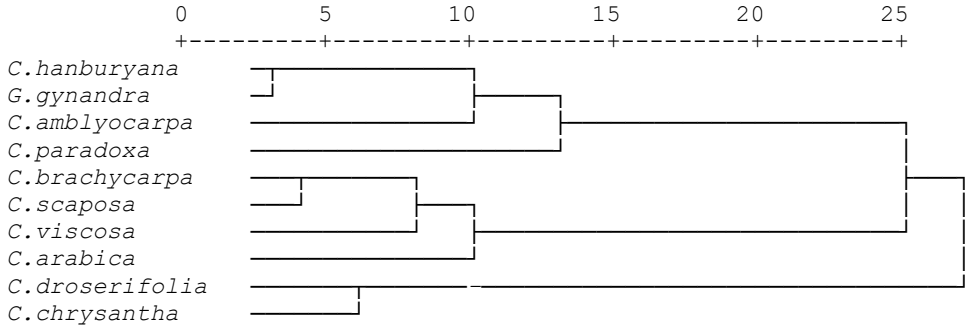
The dendrograms resulting from Bray Curtis and single linkage measure similarity percent **(Fig.1, A)**, average linkage distance and complete linkage clustering showed that: the cluster **"I"** comprises two species; *C. droserifolia* and *C.chrysantha,* while the cluster **"II"** comprises the remainder species which divided into two groups: group **"A"** contains two subgroups, subgroup **"a"** included only one species, *C. paradoxa* while the subgroup **"b"** consists of three species; *C. arabica, C. brachycarpa* and *C. scaposa*. At the same time group **"B"** also separated into two subgroups: subgroup **"1"** incorporated only one species; *C. amblyocarpa* whereas subgroup **"2"** included *C.*

viscosa C. *hanburyana* and *Gynandropsis gynan- dra.*

A **Dendrogram using Bray Curtis with single linkage Clustering Method measure similarity percent**



B **a: Dendrogram using Ward's Clustering Method**
Rescaled Distance Cluster Combine



b: Dendrogram using Average Linkage Clustering Method (Within Group)
Rescaled Distance Cluster Combine

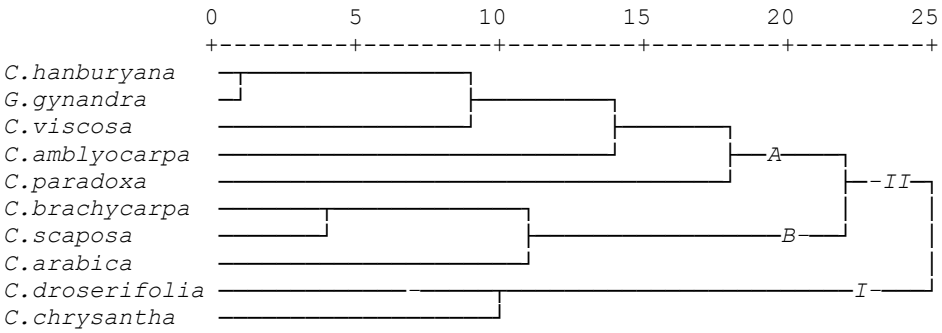


Fig. 1.
Dendrograms showing the interrelationships between 10 species of Cleomaceae based on 100 characters of morphology and seed protein.

A: PRIMER Program; B: SPSS Program

All analysis agree in the creation of three major assemblages of species (*C. hanburyana, Gynandropsis gynandra* and *C. viscosa* in one group, *C. brachycarpa, C. scaposa* and *C. arabica* in second group and *C. droserifolia* and *C. chrysantha* in third group).

The most obvious discrepancy between the four results concerns the placing of *C. amblyocarpa* with group one as well as in singling out *C. paradoxa* in a separate group (in the similarity percent, average linkage distance and the complete linkage distance analysis or with the second group).

The close relationship between *C. droserifolia* and *C.chrysantha* in all clustering methods is supported by 71 characters no.1,2,4-7,9,11-13,15-19,22,24,27-30,34,37-45,48-52,55-58,60-67,69-74,76-78,80,82,83,85,86,88-93,96,97and 100 in **(Appendix I).**

The close relationship between *C. brachycarpa* and *C. scaposa* is supported by 74 characters no.2-4,6,9,10,12-18,20,23-26,29,31,32,35-37,40-42,44-47,51-57,59-68,70-76,78-80,82-94,96, 99and 100 in **(Appendix I).**

The close relationship between C*. hanburyana* and *Gynandropsis gynandra* in all clustering methods is supported by 76 characters no.1-5, 7-11,14-18,20, 22- 23,26-32, 35, 36, 38, 39,41, 43-45, 47-61, 63, 64, 66,69, 70, 73,74, 76-79, 81, 83-100 in **(Appendix I).**

This indicates that these species are forcefully related on the bases of morphology, anatomy and seed protein pattern.

This result agree with **El-Hadidi & Fayed (1994/1995), Al-Gohary (1997), Khafagi & Al-Gohary (1998)** and **Voznesenskaya *et al* (2007)** for retaining *Gynandropsis gynandra* in *Cleome* as *Cleome gynandra.*

The combined analysis of seed proteins and morphological characters resulted in higher degree of confirmation in the species.

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Appendix I. Characters and characters states list used for the numerical analysis of the species

Character	Characters states
Plant	1- Annual [1]/ perennial [2]. 2- Herb [1] /_shrub [2]. 3- Aromatic [1] / not so [2]. 4- Up to 80 cm. [1] / more than 80 cm. [2]. 5-Woody base [1] / not so [2]. 6- Densely hairy [1] / sparsely hairy [2].
Leaf	7-Simple [1]/ compound [2]. 8- Blade shape: ovate [1]/ not so [2]. 9- : obovate [1 10- : orbicular [1] / not so [2].] / not so [2]. 11- : elliptic [1] / not so [2]. 12- Blade apex: obtuse [1]/ not so [2]. 13- : acute [1] / not so [2]. 14- Blade veins: uninerved [1]/ trinerved [2]. 15- Blade length: 0.5- 4.0 cm. [l]/ reach to 8cm. [2]. 16- Blade texture: hairy [1]/ not so [2]. 17- Petiole length: reach to 3cm. [1]/ reach to 10cm. [2].
Flower	18- Terminal [1]/ axillary [2]. 19- Flower across: 1-4mm [1]/ not so [2]. 20- Flower: actinomorphic [1]/ zygomorphic [2]. 21- Bract: leaf like [1]/ not so [2]. 22- : trifoliate [1] / not so [2]. 23- : undifferentiated from leaf [1] / not so [2]. 24- Pedicel: reach to 1.5 cm. [1] / more than 1.5cm. [2]. 25- Sepal: dimorphic [1]/ not so [2]. 26- : as long as petal [1] /shorter [2]. 27- : ovate [1] / not so [2]. 28- : lanceolate [1]/ l not so [2]. 29- : oblong-elliptic [1] / not so [2]. 30- Petal: yellow [1] / not so [2]. 31- : appendiculate [1]/ not appendiculate [2]. 32- : dimorphic [1]/ not dimorphic [2]. 33- : obovate [1] / not so [2]. 34- : elliptic [1] / not so [2]. 35- : oblong [1] / not so [2]. 36- Stamen: 4-8 [1] / 10- 14 [2]. 37- Androphore: present [1]/ absent [2]. 38- Pollen grains: size; 23-46µm. [1]/ 14-2lµm. [2]. 39- Pollen grains: shape; prolate spheroid (1)/ subprolate -prolate [2]. 40- Pollen grains: exine ornamentation; granulate [1]/ reticulate [2]. 41- Ovary. Gynophores: present [1]/ absent [2]. 42- Style: conspicuous [1]/ inconspicuous [2].
Fruit	43- Length: 1-2 cm. [1]/ longer [2]. 44- Shape: flat [1]/ not so [2]. 45- : linear [1]/ not so [2]. 46- : erect (1]/ pendulous [2].

Appendix I. Cont.

Character	Characters states
Seed	47- Size: 0.5-1.0 mm. [1]/ 1.5- 2.0 mm. [2]. 48- Color: brown [1]/ black [2]. 49- Shape: orbicular [1]/ not so [2]. 50- : ovate [2] / not so [2]. 51- : quadrangular [1] / not so [2]. 52- Texture: glabrous [1]/ wooly [2]. 53-Surface: reticulate [1]/ not so [2]. 54- : granulate [1]/ not so [2]. 55- : lanate [1]/ not so [2].
Stem anatomy	56- Outline: terete [1]/ angular [2]. 57- Epidermal cells: one type [1] / mixed [2]. 58- Cortical cells: collenchyma + chlorenchyma + parenchyma [1]/ chlorenchyma + parenchyma [2]. 59- Pericycle fiber: ring [1]/ patches [2]. 60- Cambium ring: regular [1]/ irregular [2].
Petiole anatomy	61- Outline: terete [1]/ crescent [2]. 62-Cuticle: thin [1]/ thick [2]. 63- Cortical cells: parenchyma [1]/ parenchyma +collenchyma [2]. 64- Vasculature: siphonostele [1]/ dictyostele [2]. 65- Vascular stele with crown [1]/ without [2].
Blade anatomy	66- Cutin: thin [1]/ thick [2]. 67- Mesophyll: isobilateral [1]/ isopolylateral [2]. 68- Mechanical tissue: present [1]/ absent [2]. 69- Bundle sheath: present [1]/ absent [2]. 70- Stomata leveling: raised [1]/ sunken [2].
Trichomes	71- Glandular with multicellular head and unicellular stalk: present [1] / absent [2]. 72- Glandular with multicellular head and uniseriate multicellular stalk: present [1]/ absent [2]. 73- Glandular with multicellular head and unbranched multiseriate – multicellular stalk: present [1]/ absent [2]. 74- Glandular with multicellular head and branched multiseriate- multicellular stalk: present [1]/ absent [2]. 75- Unicellular papillose: present [l]/ absent [2]. 76- Non glandular unicellular: present [1]/ absent [2]. 77- Shaggy: present [1]/ absent [2].

Appendix I. Cont.

Character	Characters states
Seedx protein bands	78- Band no.1: present [1]/ absent [2]. 79- Band no.2: present [1]/ absent [2]. 80- Band no.3: present [1]/ absent [2]. 81- Band no.4: present [1]/ absent [2]. 82- Band no.5: present [1]/ absent [2]. 83- Band no.6: present [1]/ absent [2]. 84- Band no.7: present [1]/ absent [2]. 85- Band no.8: present [1]/ absent [2]. 86- Band no. 9: present [1]/ absent [2]. 87- Band no.10: present [1]/ absent [2]. 88- Band no.11: present [1]/ absent [2]. 89- Band no.12: present [1]/ absent [2]. 90- Band no.13: present [1]/ absent [2]. 91- Band no.14: present [1]/ absent [2]. 92- Band no.15: present [1]/ absent [2]. 93- Band no.16: present [1]/ absent [2]. 94- Band no.17: present [1]/ absent [2]. 95- Band no.18: present [1]/ absent [2]. 96- Band no.19: present [1]/ absent [2] 97- Band no.20: present [1]/ absent [2]. 98- Band no.21: present [1]/ absent [2]. 99- Band no.22: present [1]/ absent [2]. 100- Band no.23: present [1]/ absent [2].

Appendix 2. Data matrix of morphology, anatomy and seed protein banding characters listed in Appendix I

Organ \ Species	No.	*Cleome deoserifolia*	*Cleome amblyocarpa*	*Cleome paradoxa*	*Cleome arabica*	*Cleome chrysantha*	*Cleome viscose*	*Clwome brachycarpa*	*Cleome scaposa*	*Cleome hanburyana*	*Gynandropsis gynandra*
Whole plant	1	2	1	1	2	2	1	2	1	1	1
	2	2	1	1	1	2	1	1	1	1	1
	3	1	2	2	1	2	2	2	2	2	2
	4	1	1	2	1	1	1	1	1	1	1
	5	1	2	1	1	1	2	1	2	2	2
	6	1	1	2	1	1	1	1	1	1	2
Leaf morphology	7	1	2	2	1	1	2	2	1	2	2
	8	2	2	2	1	1	1	2	1	2	2
	9	2	1	2	2	2	2	2	2	1	1
	10	1	2	2	2	2	2	2	2	2	2
	11	2	1	1	2	2	2	1	2	2	2
	12	1	1	2	2	1	2	2	2	1	2
	13	2	1	1	1	2	1	1	1	2	1
	14	2	1	1	2	1	1	1	1	1	1
	15	1	1	2	2	1	1	1	1	1	2
	16	1	2	2	1	1	2	1	1	1	2
	17	1	1	2	1	1	2	1	1	2	2
Flower	18	2	1	1	1	2	1	1	1	1	1
	19	2	2	1	2	2	2	2	1	1	2
	20	2	1	2	2	1	2	1	1	2	2
	21	2	1	2	1	1	2	1	2	2	1
	22	2	2	1	2	2	1	2	1	1	1
	23	1	2	2	2	2	2	2	2	2	2
	24	1	1	2	1	1	2	1	1	1	2
	25	1	2	1	2	2	1	2	2	1	2
	26	2	2	2	2	1	2	2	2	2	2
	27	2	1	2	2	2	1	1	2	1	1
	28	1	2	1	2	1	2	2	1	2	2
	29	2	2	2	1	2	2	2	2	2	2
	30	1	1	1	2	1	1	1	2	2	2
	31	1	2	2	2	2	2	2	2	2	2
	32	1	2	1	2	2	1	2	2	2	2
	33	2	2	2	1	1	2	2	1	2	1
	34	2	2	1	2	2	1	1	2	1	2
	35	1	1	2	2	2	2	2	2	2	2
	36	1	1	1	1	2	2	1	1	1	1
	37	2	2	2	2	2	2	2	2	2	1
	38	1	1	1	1	1	1	2	1	2	2
	39	2	2	2	2	2	2	2	1	2	2
	40	2	2	2	2	2	1	1	1	2	1
	41	2	2	1	2	2	2	2	2	1	1
	42	1	2	1	2	1	2	2	2	1	2
Fruit	43	1	2	2	2	1	2	1	2	2	2
	44	1	2	1	2	1	2	2	2	2	2
	45	2	1	2	1	2	1	1	1	1	1
	46	1	2	1	2	2	1	1	1	1	2

Appendix 2. Cont.

Species / Organ	No.	*Cleome deoserifolia*	*Cleome amblyocarpa*	*Cleome paradoxa*	*Cleome arabica*	*Cleome chrysantha*	*Cleome viscose*	*Clwome brachycarpa*	*Cleome scaposa*	*Cleome hanburyana*	*Gynandropsis gynandra*
Seed	47	1	2	1	1	2	2	1	1	2	2
	48	1	1	1	1	1	1	1	2	1	1
	49	1	2	2	2	1	1	2	1	1	1
	50	2	2	2	1	2	2	1	2	2	2
	51	2	1	1	2	2	2	2	2	2	2
	52	1	2	2	2	1	1	1	1	1	1
	53	2	2	2	1	1	1	1	1	1	1
	54	1	2	2	2	2	2	2	2	2	2
	55	2	1	1	2	2	2	2	2	2	2
Stem anatomy	56	1	2	1	1	1	2	1	1	2	2
	57	1	2	1	2	1	1	2	2	1	1
	58	2	1	2	2	2	2	1	2	2	2
	59	1	2	2	2	2	2	2	2	2	2
	60	2	2	2	2	2	2	1	1	2	2
Petiole anatomy	61	2	2	2	1	2	2	2	2	2	2
	62	2	1	2	1	2	1	2	2	1	2
	63	2	2	1	1	2	1	1	1	2	2
	64	2	1	2	1	2	2	2	2	2	2
	65	1	1	2	2	1	2	1	1	2	1
Blade anatomy	66	2	2	2	1	2	2	2	2	2	2
	67	1	2	2	2	1	1	1	1	1	2
	68	1	1	2	1	2	2	2	2	2	1
	69	2	2	1	1	2	1	2	1	1	1
	70	2	1	2	1	2	1	1	1	1	1
Tichomes	71	2	2	2	2	2	2	2	2	1	2
	72	1	2	2	2	1	2	2	2	1	2
	73	1	1	2	1	1	1	1	1	1	1
	74	2	2	2	2	2	1	2	2	2	2
	75	1	1	2	1	2	1	1	1	1	2
	76	2	2	1	2	2	2	2	2	2	2
	77	2	2	2	2	2	2	2	1	2	2

Appendix 2. Cont.

Species / Organ	No.	*Cleome deoserifolia*	*Cleome amblyocarpa*	*Cleome paradoxa*	*Cleome arabica*	*Cleome chrysantha*	*Cleome viscose*	*Clwome brachycarpa*	*Cleome scaposa*	*Cleome hanburyana*	*Gynandropsis gynandra*
Seed protein electrophoresis	78	1	1	1	1	1	2	1	1	1	1
	79	1	1	1	2	2	2	1	1	1	1
	80	2	2	2	2	2	2	2	2	1	2
	81	1	1	1	2	2	2	2	1	1	1
	82	2	2	2	2	2	2	2	2	1	2
	83	1	1	1	2	1	2	2	2	1	1
	84	1	2	1	2	2	2	2	2	1	1
	85	1	2	1	1	1	1	1	1	1	1
	86	1	1	1	1	1	1	1	1	1	1
	87	1	1	1	2	2	2	1	1	1	1
	88	1	1	1	1	1	1	1	1	1	1
	89	1	1	1	1	1	1	1	1	1	1
	90	1	1	1	1	1	1	1	1	1	1
	91	1	1	1	1	1	1	1	1	1	1
	92	1	1	1	1	1	1	1	1	1	1
	93	1	1	1	1	1	1	1	1	1	1
	94	1	1	1	2	2	2	2	2	1	1
	95	2	2	2	1	1	2	2	1	2	2
	96	2	2	2	2	2	2	2	2	1	1
	97	2	2	2	2	2	2	2	1	2	2
	98	1	1	1	2	2	2	2	1	1	1
	99	1	1	1	2	2	2	1	1	1	1
	100	1	1	1	1	1	1	1	1	1	1