SDS-PAGE of seed proteins and SEM of seed coat surface in *Caesalpinia gilliesii* Wall., *C. pulcherrima* Sw., *C. sepiaria* Roxb. and *Delonix regia* Raf. (Leguminosae - Caesalpinioideae)

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Abou-El-Enain M. M. & Loutfy M. H. 1999. SDS-PAGE of seed proteins and SEM of seed coat surface in *Caesalpinia gilliesii* Wall., *C. pulcherrima* Sw., *C. sepiaria* Roxb. and *Delonix regia* Raf. (Leguminosae - Caesalpinioideae)**. *Taeckholmia* 19(1):** 37-52.

SDS-PAGE of seed protein profiles , SEM of seed coat surface patterns and selected macromorphological characters were studied in the related taxa Caesalpinia gilliesii, C. pulcherrima, C. sepiaria *and* Delonix regia*. The obtained characters were used as one set of data and analyzed using the NTSYS-pc program. The dendrogram produced showed a closer relationship between* D. regia *&* C. pulcherrima*. Taxonomic relationships between the four taxa were discussed in the light of their morphological, anatomical, embryological and phytochemical criteria. Merging of* C. pulcherrima *in the genus* Delonix *has been suggested.*
Key words: *Caesalpinia* – Caesalpinoidae – Electrophoresis – Leguminosae – Seed scan.

Introduction

Caesalpinia gilliesii, *C. pulcherrima*, *C. sepiaria* and *Delonix regia* are known ornamental and firewood plants distributed throughout subtropical and temperate regions (Mabberley, 1987& 1997; Huang & Huang, 1991). They belong to tribe Caesalpinieae and subfamily Caesalpinioideae of the Leguminosae (Polhill & Vidal, 1981). The related taxa *C. gilliesii*, *C. pulcherrima* and *Delonix regia* have been either merged in one genus i.e. *Poinciana* (Bentham, 1865; Taubert, 1894; Melchior, 1964) or delimited in the two different genera *Caesalpinia* and *Delonix* (Pettigrew & Watson, 1977; Polhill & Vidal, 1981; Puy-DJ-du *et al*., 1995). Taxonomic relationship of these taxa; have attracted the attention of taxonomists not only because their classification is limited to a very few

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Received 11 February 1999. Revision accepted 23 April 1999.

characters (Lersten & Curtis, 1994; Rudall *et al.* 1994) but also because of the unclear boundaries and the confusion in nomenclature between them (Kit *et al*., 1994; Shehata, 1997).

Several taxonomic studies have been carried out to discuss relationships of the *Caesalpinia* and *Delonix* either at the specific or at the generic level using different criteria. Nageshwar *et al.* (1984) and Prabha-Choudhary & Choudhary (1987) analyzed the phytochemical structures among a number of species and pointed out the close relationships between *C. pulcherrima* and each of *C. sepiaria* and *D. regia*, respectively. Lersten and Curtis (1996) surveyed secretory structures of leaf in the Caesalpinieae and scored some differences between *C. gilliesii*, *C. pulcherrima* and *D. regia*. Shehata (1997) studied the morphological, anatomical & embryological features in the latter three taxa and pointed out the similarity of their embryological characters and differences in some morphological and anatomical features. However, no previous studies have used the seed protein electrophoresis or seed coat surface criteria to discuss the relationships among the four taxa.

Seed protein banding patterns as revealed by polyacrylamide gel electrophoresis in the presence of Sodium dodecyl sulfate (SDS-PAGE) have provided a valid source of taxonomic evidence for addressing taxonomic relationships at both the generic and specific levels (Ladizinsky & Hymoitz, 1979; Cook, 1984; Badr, 1995). Variations in SDS-PAGE of seed protein profiles have successfully been used to differentiate between species in a number of genera, for example *Vigna* (Paino *et al*., 1993), *Trifolium* (Badr, 1995), *Phaseolus* (Schmit *et al.*, 1996) and *Lathyrus* (El-Shanshoury, 1997). Similarly, scanning electron microscopy (SEM) of seed coat surface was found useful 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 &Vijayaraghavan, 1991) and *Vicia* (Chernoff *et al*., 1992).

On the other hand, macromorphological characters can help in solving taxonomic problems and must not be ignored in reconstructing plant relationships and phylogeny (Werff & Endress 1991; Donoghue & Sanderson, 1992). Macromorphological criteria were used to reassess the relationships among various plant families and genera eg. Rohrer *et al* ., (1991) and Robertson *et al*., (1992) on the Rosaceae, Kadereit *et al* .,(1994) on the Papaveraceae and Sun & Chung,(1986) ; Rohwer,(1994) on the Lauraceae .

In the present work, SDS-PAGE of seed protein patterns, SEM of seed coat surface criteria and selected macromorphological attributes were used to provide more information about the taxonomic relationships between *Caesalpinia gilliesii* Wall., *C. pulcherrima* Sw., *C. sepiaria* Roxb. and *Delonix regia* Raf .

Materials and Methods

Seeds of the examined taxa (Table 1) were collected from plants growing 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. To extract seed proteins, hree replicas of 0.5 g of mature seeds were mixed; each with an equal weight of pure, clean, sterile fine sand and powdered using a mortar and pestle and homogenized with 0.2 M Tris-HCl buffer, pH=8 for 1h at 4 °C. The extract was centrifuged at 12000 rpm for 10 min. The supernatant (protein extract) was transferred to new tubes and immediately used

Received 11 February 1999. Revision accepted 23 April 1999.

Table 1: The taxa studied, source of their seeds, their country of origin, habit, leaf duration, flowering time & present distribution in Egypt.

Taxa studied	Source of seeds	Country of origin	Habit	Leaf duration
Caesalpinia gilliesii (Wall.) D. Dietr = *Poinciana gilliesii* Hook = *Erythrostemon gilliesii* Link, Klotzsch & Otto. English name : Bird of paradise, paradise poinciana, peacock flower, ①③②	BGFE The live specimens introduced in 1986 from the Botanical Garden. Fac. of Science. Alex. Univ.	Argentina, Chile & Uruguay ④①③②	Straggling Unarmed shrub - ①③	Half deciduous or deciduous depending on the locality
Caesalpinia pulcherrima (L.) Swartz. =*Poinciana pulcherrima* L. English name: Barbados pride, Barbados flower fence, Dwarf poinciana, peacock flower. ①③②	BGFE The live specimens introduced in BGFE 1987 from the Gardens of Commonwe-alth Cemeteries. Heliopolis Cairo.	Unknown, widely cultivated in the tropics ①③②⑥	Slightly armed shrub - ①③	Half deciduous
Caesalpinia sepiaria Roxb = . *C. decapetala* (Roth) Alston. English name: Mysore Thorn. ③	BGFE The live specimens introduced in BGFE 1996 from Urman garden.	India ③②	Scrambling heavily armed shrub ③	Half deciduous
Delonix regia (Hook). Raf = . *Poinciana regia* Bojer. English name: Flame tree, Flamboyant, Royal poinciana, peacock flower. ①③ ⑥②	BGFE The live specimens introduced in BGFE 1982 from Zohariya Garden.	Madagascar ⑤①⑥③②	Unarmed tree with a broad spreading crown ①⑥	deciduous

① Bailey (1949)
② Mabberley (1997)
③ Bailey & Bailey (1976)
④ Mondadori (1982)
⑤ El-Hadidi & Boulos (1988)
⑥ Wyman (1977)
BGFE Botanical Garden of Faculty of Education
* Herbarium of Department of Flora & Phytotaxonomy researches
● Herbarium of Cairo Univ. Fac. of Science. Bot. Department

Received 11 February 1999. Revision accepted 23 April 1999.

Table 1: Continued

Taxa studied	Flowering time	Distribution in Egypt
Caesalpinia gilliesii (Wall.) D. Dietr = *Poinciana gilliesii* Hook = *Erythrostemon gilliesii* Link, Klotzsch & Otto. English name : Bird of paradise, paradise poinciana, peacock flower, ①③②	Early spring (March, April) ④	Introduced late 19th century- Now, somewhat rare. Planted mainly in coastal regions, particularly at Alexandria. Several specimens are present in the Botanical Garden of Fac. Science. Alex. Univ. Herbarium specimens: - Alex. Univ., Fac. of Science Garden, Moharram Bey: Adel El Gazzar, 1.6.1975. • - Burg El Arab, The western Mediterranean coastal region (Mma), Vivi Tackholm, 26.5.1963. • - Zohariya Garden, Gezira, Cairo: M. Drar, 7. 7. 1953. • Vivi Tackholm, 30.10.1959.*
Caesalpinia pulcherrima (L.) Swartz. =*Poinciana pulcherrima* L. English name: Barbados pride, Barbados flower fence, Dwarf poinciana, peacock flower. ①③②	Summer & autumn (June-November) ①	Introduced late 19th century. Formerly abundant in Cairo. Now, nearly extinct. Represented by few specimens at the Zoo Garden- Giza and a handful of relict private gardens in all Egypt. Herbarium specimens: - Cairo. Univ., Fac. of Agric. Garden, Giza: Nabil El Hadidi, September 1953. Vivi Tackholm, 2.11.1959. • - Orman Botanic Garden, Giza: N. D. Simpson, 25.8.1927.; M. Drar, 15. 11. 1932.* - Zohariya Garden, Gezira, Cairo: J. R. Shabetai, 24.7.1928.*
Caesalpinia sepiaria Roxb = . *C. decapetala* (Roth) Alston. English name: Mysore Thorn. ③	Early summer June ①	Introduced late 19th century. Now somewhat rare. Several specimens are present at the Delta Barrage, Urman Garden. Herbarium specimens: Barrage, Nile Delta (Nd) near the old station: N.D. Simpson, 15.2.1924*; Gunnar Tackholm, 7.1.1927. •
Delonix regia (Hook). Raf = . *Poinciana regia* Bojer. English name: Flame tree, Flamboyant, Royal poinciana, peacock flower. ①③ ⑥②	Early to mid summer (May, June, July) ① ⑤	Introduced late 19th century. A very common and popular street-tree in Egypt. (5) Herbarium specimens: - Ismailia: Loutfy Boulos, 22. 12. 1963. • - Orman Botanic Garden, Giza: Ezz Eldin, 10.6.1965 (Orman Herbarium); Ezz Eldin and Diwan, 1.7.1971 (Orman Herbarium). Agricultural Museum Garden, Giza: J. R. Shabetai, 1.6.1941.*

① Bailey (1949)
② Mabberley (1997)
③ Bailey & Bailey (1976)
④ Mondadori (1982)
⑤ El-Hadidi & Boulos (1988)
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Received 11 February 1999. Revision accepted 23 April 1999.

for electrophoresis or kept at -20 °C. For electrophoresis, 40 µl of the extract were mixed with an equal volume of a sample buffer (0.125 M Tris-HCl, pH 6.8, 2% SDS, 10% sucrose, 0.5% β-mercaptoethanol and 0.1% bromophenol blue as a tracking dye), denatured by boiling for 5 min in a water bath and cooled. Then, 20 µl of this mixture were loaded in 12.6% slab gel, which was prepared as described by Lammeli (1970). Electrophoresis was carried out in Tris-Glycine buffer (pH=8.3) at 4 °C and 125 volt for 2h using a Pharmacia low-molecular weight protein mixture as standard. Gel was then stained in 0.1 % Comassie Brilliant Blue R-250 for 1h, destained and photographed while wet and stored for subsequent examination. Total bands in the produced electropherogram were scored and their molecular weights were calculated using the standard protein marker (Table 2).

For the study of the seed coat surface using SEM technique, two seeds were mounted with colloidal silver on copper stubs, coated with a thin layer of gold in Polaron E 5000, the epidermal seed coat was photographed by a JEOL-T- Scanning Microscope at a magnification of 750, at the Electron Microscope Unit, Faculty of Science, Alexandria University. The terminology of Stearn (1966); Stant (1973); Barthlott (1981) and Boesewinkel & Bouman (1984) were used to describe the seed coat characteristics (Table 2). Macromorphological characters were obtained from relevant literature (Bailey, 1949; Bean, 1950; Bailey & Bailey, 1976; Wyman, 1977; Hillier, 1981; Mondadori, 1982; Mabberley, 1987; El Hadidi & Boulos, 1988; Ibrahim, 1996; Mabberley, 1997 and Shehata, 1997). (Table 3).

Received 11 February 1999. Revision accepted 23 April 1999.

Table 2: Summary of the SDS-PAGE of seed proteins and SEM of seed coat characters of the taxa studied and their codes used in the numerical analysis.

	Seed Characters			Taxa			
				(1) *C. gilliesii*	(2) C. pulcherrima	(3) *C. sepiaria*	(4) *D. regia*
	No	MW(KD)		SDS-PAGE Characters			
I	01	99.5		0	1	1	0
	02	96.5		1	1	1	1
	03	85.4		1	1	1	1
	04	67.0		1	0	0	1
	05	58.3		0	1	0	1
	06	52.4		1	1	1	0
	07	48.2		0	0	0	1
	08	44.0		1	0	0	1
	09	38.5		0	1	0	1
	10	36.0		0	1	1	0
	11	32.3		0	1	0	1
	12	29.2		0	0	0	1
	13	27.6		0	0	1	0
	14	25.2		1	1	0	1
	15	23.4		1	1	1	1
	16	21.3		1	1	0	1
	17	19.0		1	1	1	1
	18	18.6		0	1	0	1
	19	17.8		1	0	1	0
	20	16.5		1	1	0	1
	21	16.0		1	0	0	0
	22	15.4		0	1	1	1
		Epd. Cells		SEM- Characters			
II	23	**SH**	Ru.	0	-	-	-
			Rp.	-	1	-	-
			Rm.	-	-	2	2
	24	**UN**	Wa.	0	0	-	0
			Sw.	-	-	1	-
	25	**TH**	Vt.	0	-	-	0
			St.	-	1	-	-
			Th.	-	-	2	-
	26	**LV**	Ra.	1	1	1	1
	27	**ST**		1	0	0	0
III	28	**SH**	Il.	0	-	-	-
			Sc.	-	1	1	1
	29	**PA**		0	0	0	1

I = Protein bands, II = Anticlinal walls, III = Periclinal walls. Epd. Cells = Epidermal cells, Il. = Ildefined , KD = Kilo Dalton, LV = Leveling , MW = Molecular weight, PA= Papillae, Ra. = Raised, Rm. = Reticulate monomorphic, Rp. = Reticulate polymorphic, Ru. = Ruminate, Sc. = Slightly concave, SH = Shape, Sr. No = Serial number, ST = Steriation, St. = Slightly thick, Sw. = Slightly wavy, Th. = Thick, TH = Thickness, UN =Undulation, Vt. = Very thick , Wa. = Wavy.

Received 11 February 1999. Revision accepted 23 April 1999.

Table 3: Data matrix of the selected macromorphological Characters of the Studied taxa and their codes in the numerical analysis .

Characters		*C. gilliesii*	*C. pulcherrima*	*C. sepiaria*	*Delonix regia*
Main trunk colour	Grayish green		0	0	0
	Grayish brown	0	1	0	1
	Dark brown	1	0	0	0
Bark texture	Smooth glabrous		0	0	0
	Rough	0	1	0	1
Prickles on main trunk	Absent		0	0	1
	Few	0	1	0	0
	Dense	0	0	1	0
Prickles shape	Short, sharp-hooked		0	1	0
	Slightly hooked with a globose base.	0	1	0	0
Young twigs	Pubescent		0	0	0
	Glabrous	0	1	0	1
	Prickly	0	0	1	0
No. of pinnae	6-10		0	0	0
	4-8	0	1	0	0
	6-8	0	0	1	0
	10-20	0	0	0	1
Pinnae arrangement on rachis	Alternate		0	0	0
	Opposite	0	1	1	1
Pinnule size	Small (4x1.3 mm)		0	0	0
	Large (10x18 mm)	0	1	0	0
	Medium (5x15 mm)	0	0	1	1
Colour of leaves	Dark green	1	0	0	0
	Light green	0	1	1	0
	Green	0	0	0	1
Prickles on leaves	Absent	1	0	0	0
	On rachis	0	1	0	0
	On rachis &pinnae	0	0	1	0
Infloresence type	Racemose	1	1	1	0
	Corymb to Racemose	0	0	0	1
Pedicel length	2-2.5 cm	1	0	0	0
	2-4 cm	0	1	0	0
	1.5–2 cm	0	0	1	0
	4-7 cm	0	0	0	1
Pedicel texture	Galndular	1	0	0	0
	Pubescent	0	0	1	0
	Glabrous	0	1	0	1

Received 11 February 1999. Revision accepted 23 April 1999.

Table 3:Continued

Characters		*C. gilliesii*	*C. pulcherrima*	*C. sepiaria*	*Delonix regia*
Pedicel thickness	Stout	1	0	0	1
	Slender	0	1	1	0
Calyx texture	Glandular	1	0	0	0
	Glabrous	0	1	0	1
	Pubescent	0	0	1	0
Sepal shape	Imbricate unequal	1	1	1	0
	Valvate	0	0	0	1
Petal shape	Thin textured, equal	1	0	0	0
	Unequal imbricate	0	1	0	1
	Unequal sub-orbicular	0	0	1	0
Trichomes on floral parts	Present	1	0	0	0
	Absent	0	1	1	1
Idioblasts on floral parts	Present	0	1	0	0
	Absent	1	0	1	1
Stamens	Long (> 7 cm)	1	0	0	0
	Medium (< 4-6 cm)	0	1	1	1
Pod shape	Orbicular ovate (8x2 cm)	1	0	0	0
	Obovate 8x2 cm				
	Broad obovate 7x3 cm & peaked	0	1	0	0
	Large, narrow 40-50 x5 cm & oblong	0	0	1	0
		0	0	0	1
Pod colour	Light brown	1	0	0	0
	Brown	0	0	1	1
	Dark brown	0	1	0	0
Pod texture	Densely pubescent	1	0	0	0
	Glabrous	0	1	1	1
Pod dehiscence	Dehiscent	1	1	1	0
	Indehiscent	0	0	0	1
Seed shape	Orbicular ovate	1	0	0	0
	Obovate	0	1	0	0
	Globose to ovate	0	0	1	0
	Narrowly oblong	0	0	0	1
Seed colour	Brown with black mottling	1	0	0	0
	Brown	0	1	0	0
	Dark brown	0	0	1	0
	Yellowish with brown mottling	0	0	0	1
Seed germination	Hypogeal	0	0	1	0
	Epigeal	1	1	0	1

For data analysis, the recorded characters in each taxon i.e.SDS-PAGE protein bands, SEM features of seed surface, and the selected macromorphological characters were coded as in Tables 2&3 and used for creating the data matrix. Two phenograms, illustrating the relationships between the studied taxa were then constructed by calculating the average taxonomic distance (dissimilarity), using the NTSYS program package for IBM-pc as described by Rohlf (1993). One phenogram was based on the data of SDS-PAGE and SEM seed surface, and the second , on all features combined.

Received 11 February 1999. Revision accepted 23 April 1999.

Observations

The produced banding patterns of seed protein SDS-PAGE technique of the taxa studied are shown in fig. 1-A, and the micrographs of the SEM patterns of the seed coat surface in each of the taxa studied are given in fig. 1-B. Summary of SDS-PAGE and SEM characters and their codes are given in table 2. The phenograms illustrating the relationships between the taxa studied is presented in figs. 2 & 3. A total number of 22 protein bands with molecular weight (MW) ranging between 99.5 Kilodalton (KD) and 15.4 KD were recorded in the electropherograms of the four taxa (Fig. 1-A & Table 2). The highest number of bands (16) was recorded in *Delonix regia*, while the lowest band number (10) was observed in *Caesalpinia sepiaria*. Meanwhile, 12 and 15 protein bands were recorded in *C. gilliesii* and *C. pulcherrima* respectively.

Spermoderm of *Caesalpinia gilliesii* (Fig. 1-B & Table 2) was characterized by having striated irregularly ruminate, very thick, wavy, raised anticlinal walls, and ill-defined periclinal walls. *C. Pulcherrima* spermoderm differed from the above mentioned species in the following aspects:- polymorphic reticulate shape, slightly thick anticlinal walls and the smooth concave periclinal walls. *C. sepiaria* spermoderm is similar to that of the latter species except for the monomorphic reticulate shape and the thick slightly wavy anticlinal walls. *Delonix regia* spermoderm is similar to that of the above-mentioned species except for the very thick anticlinal walls and the papillate; slightly concave periclinal walls.

The phenogram constructed according to the analysis of the combined SDS-PAGE and SEM characters (Fig. 2) revealed the delimiting of the studied taxa into three major phenetic lines; the first one included only *C. gilliesii* and was clustered with the second line including *C. pulcherrima* and *Delonix regia* at the dissimilarity level of 1.42. The latter two taxa were clustered together at the dissimilarity level of 1.12. The third line included only *C. sepiaria* and that was delimited as a separate phenetic line.

One the other hand, the phenogram constructed according to the analysis of all characters (SEM,SDS-PAGE and selected macromorphological characters, clearly delimited *C. gilliesii* from the other three taxa. However, *C. sepiaria* was also to a large extent, differentiated from *D. regia* and *C. pulcherrima*) (Fig .3) .

Received 11 February 1999. Revision accepted 23 April 1999.

Received 11 February 1999. Revision accepted 23 April 1999.

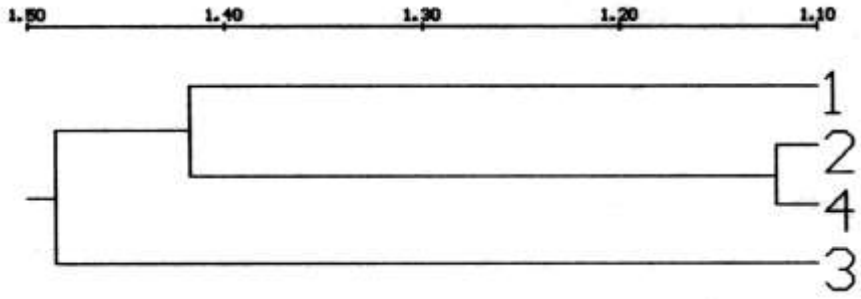

Fig. 2: The phenogram illustrating the relationships between the taxa studied (numbered as in Fig. 1) based on the variation in the combined SDS-PAGE of seed protein and SEM of seed coat surface characters.

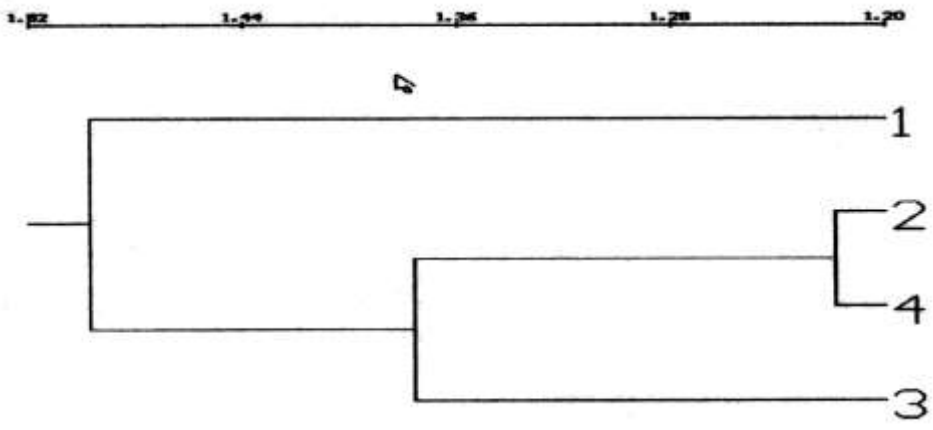

Fig. 3: The phenogram illustrating the relationships between the taxa studied (numbered as in Fig. 1) based on the variation in the combined SDS-PAGE of seed protein and SEM of seed coat surface and selected vegetative macromorphological characters.

Received 11 February 1999. Revision accepted 23 April 1999.

Received 11 February 1999. Revision accepted 23 April 1999.

Discussion

Bentham (1865), Taubert (1894) and Melchior (1964) have merged the related taxa *C. gilliesii*, *C. pulcherrima* and *Delonix regia* in the genus *Poinciana* as they are morphologically characterized by the tree or shrub habit, the unarmed or prickly bipinnate leaves, the terminal or axillary racemes of showy flowers, the androecium of 10 stamens with many staminodes, and the dorsifixed anthers. Pettigrew & Watson (1977) and Polhill & Vidal (1981) delimited these three taxa in the two related genera *Caesalpinia* and *Delonix* based mainly on the variation in habit and pod and seed characters.

The relationships based on average taxonomic distance between the studied taxa using SDS-PAGE & SEM criteria (Fig. 2) clustered *C. gilliesii*, *C. pulcherrima* and *D. regia* in one major group at the dissimilarity level of 1.42. Meanwhile, *C. gilliesii* was distinguished from the other two taxa that clustered together showing a closer relationship between *C. pulcherrima* and *D. regia*. Although *C. gilliesii* was closer to the cluster of *C. pulcherrima* and *D. regia* than *C. sepiaria*, yet, the degree of dissimilarity between *C. gilliesii* and the other two taxa clearly indicate a considerable difference between them. Consequently these results do not support the grouping of *C. gilliesii, C. pulcherrima* and *D. regia* in one genus as done by Bentham, (1865);Taubert, (1894) and Melchior, (1964). Moreover, the present results contradict the grouping of *C. gilliesii, C. pulcherrima* in one genus and the separation of *D. regia* in another genus as done by Pettigrew & Watson, (1977) and Polhill & Vidal, (1981).

C. gilliesii, C. pulcherrima and *D. regia* were grouped together due to the presence of the protein bands numbered 14, 16 & 20 with MW of 25.2, 21.3, & 16.5 KD, respectively, and the absence of band number 13 with a MW of 27.6 KD in addition to the undulation of anticlinal walls as revealed by SEM of seed coat surface data. *C. gilliesii* was distinguished from *C. pulcherrima* and *D. regia* due to absence of the protein bands numbered 5, 9, 11, 18 & 22 with MW of 58.3, 38.5, 32.3, 18.6 & 15.14 KD, respectively and the presence of the bands number 19 & 21 with MW of 17.8 & 16.0 KD respectively in addition to the ruminate shape, striated anticlinal walls and the ill-defined shape of periclinal walls .

SDS-PAGE data have revealed that *C. pulcherrima* and *D. regia* share 12 out of the 22 recorded protein bands. This relatively high number of common recorded bands was indicative of their common origin as observed between species of some other genera e.g. *Sesbania* (Saraswati *et al.* 1993; Badr *et al*., 1998) and *Lathyrus* (El-shanshoury 1997). SEM of seed coat data have revealed that both species are similar in the undulation, absence of striation , raised anticlinal walls, as well as the slightly concave shape of periclinal walls. Thus, the evidence obtained in the present study may indicate the possibility of merging *C. pulcherrima* with *D. regia* in the genus *Delonix.*

Nageshwar *et al.* (1984) have pointed out a close relationship between *C. sepiaria* & *C. pulcherrima* based on the similarity in steroids & phenol compounds. However, according to the present data it is clearly evident that these two species are quite different as they do not cluster together due to the absence of protein bands numbered 5, 9, 11, 14 & 20 with MW of 58.3, 38.5, 32.3, 25.2 & 16.5 KD respectively and the presence of bands numbered 13 & 19 with MW of 27.6 & 17.8 KD respectively in *C. sepiaria*. SEM data have also revealed that the latter species is different due to the reticulate polymorphic, undulated and slightly thick anticlinal walls.

Received 11 February 1999. Revision accepted 23 April 1999.

The phenogram constructed, utilizing all characters, Fig (3) furtherly supported the close similarity between *C. pulcherrima* and *D. regia* as the two taxa clustered at the dissimilarity level of 1.22. Yet according to the analyzed characters, *C. sepiaria* was more close to *C. pulcherrima* and *D. regia* than was *C. gilliessii*.

Prabha-choudhary & Choudhary (1987) also observed the close relationship between *C. pulcherrima* and *D. regia* based on phytochemical criteria including the similarity in phenol compounds extracted from fresh basal leaves. Shehata (1997) recorded a considerable number of embryological characters that are shared by *C. pulcherrima* & *D. regia* including that of anther, ovule and integument. She also pointed out the similarity in a number of morphological and anatomical features in these two taxa including those of androecium, gynoecium, trichomes, leaves and stem.

Clustering of all the taxa studied at considerably high degree of dissimilarity i.e. the level of 1.5; reflects an apparent variability between each of *C. gilliesii, C. sepiaria* and the group comprising *C. pulcherrima* & *D. regia*. SDS-PAGE data have indicated that all taxa shared only four out of the 22 recorded protein bands. These have the numbers of 2, 3, 15 & 17 with MW of 96.5, 85.4, 23.4 & 19.0 KD, respectively. SEM of seed coat surface data has revealed that they share only one character i.e. raised anticlinal walls. This provides further support to the possibility of merging *C. pulcherrima* with *D. regia* in the genus *Delonix* and retaining the other two taxa i.e. *C. gilliesii*, *C. sepiaria* as two different species of *Caesalpinia.*

C. gilliesii was shown to be standing apart from the other three taxa studied by some aspects:- ruminate spermoderm; smooth glabrous persistent grayish green bark; alternate pinnae arrangment on leaf rachis; dark green leaves; glandular pubescent floral parts. The geographical distrubution of this taxon is also different from the other three taxa. Its origin is in temperate regions (Argentina and Chile), while the others are centered in the tropics (Central America, India and Madagascar). In Egypt, it flowers in late winter and early spring, while the others flower in summer. Thus further research is still needed on this taxon in particular, to elucidate its relationships with the other taxa included in *Caesalpinia* , and other genera in the Caesalpineae .

Acknowledgement

The authors wish to express their deepest gratitude to Prof . Dr. Abdel Fattah Badr, professor of plant genetics & head of Botany Department , Faculty of Science , Tanta University for revising the manuscript , providing help in the computer analysis & his continuous encouragement .

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