MORPHOLOGICAL AND CHEMICAL STUDIES ON SOME SPECIES OF THE GENUS *BRACHYCHITON,L*. CULTIVATED IN EGYPT

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Abstract

Fruit and seed morphological characters, in addition to both nucleic acid and seed protein contents in five native species of *Brachychiton (B. discolor, B. acerifolius, B. australis, B. populeneus* and *B. rupestris)* have been studied. 56 selected characters of such studies are subjected to numerical analysis which resulted a dendrogram classifying the studied spp. into 2 groups. The 1st includes *B. discolor* which is similar to the 2nd (containing the 4 remainders) at a least similarity % level.

Introduction

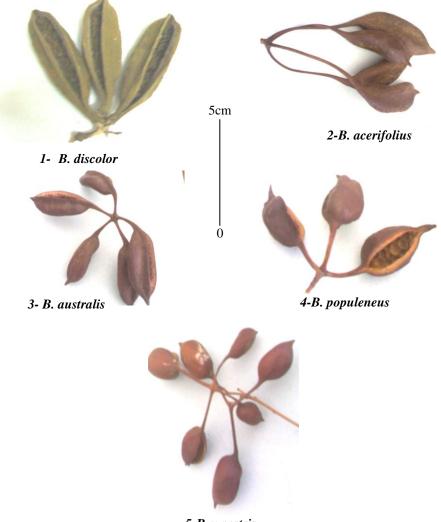
Sterculia, L. (Brachychiton, Schott & Lindl.) is the type genus of the Sterculiaceae, which is considered as a pantropic family; containing 67 genera and about 500 species (Alverson et al., 1999). The genus Brachychiton comprises about 100 spp. of which approximately 25 are said to occur in Africa (Chopra et al., 1956). They are either evergreen or partially deciduous trees, mostly native to Australia, and chiefly found in northern tropical and subtropical regions, with a few extending to arid regions (Mehrotra, 2000). Nucleic acids have provided a negligible amount at taxonomic information in plants so far. Several attempts have been made to utilize this type of data in the study of species relationships in various taxonomic groups. The comparison of DNA content between the different species could often provide useful information to the relationships between them (Greilhuber, 1977). Nucleic acids are initiated and extended from the shoot apex of seedlings. In Brachychiton spp., the concentration of nucleic acids increases, primarily in seeds (West and Gunckel 1968). Most useful methods for determination of the protein and isozymes are polyacrylamide and starch gel electrophoresis (Blakshear, 1984 and Zimniak et al., 1985). Seed protein electrophoretic profiles have been found to produce valid evidence for systematic treatment of several plant groups (Ladizinsiky and Hymotwitz., 1979; Jensen, 1984 and Badr, 1995). The use of seed profiles for taxonomic and evolutionary purposes are largely increased and widely used within many families and genera (Adrianse et al., 1969). On the other hand, Koyalamud, et al., (1989), stated that protein contents are different into B. discolor and B. acrefolius into seeds.

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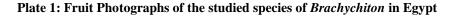
Materials and Methods

Morphological Studies:

Seeds of the studied spp. have been isolated from fruit specimens collected from different botanic gardens in Egypt (Plate 1) and are identified according to Bailey (1927). A list of the studied spp. and their localities is given in Table (1).







No.	Species	Locality
1 -	Brachychiton discolor, F. Muell	Orman Garden
2 -	B. acerifolius, (Cunn.) Muell	Agricultural Museum Garden
3 -	B. australis, L.	Qubba Palace Garden
4 -	B. populeneus, Dc.	Zoo, Giza
5-	B. rupestris,(Lindl.) Schum	Orman Garden

B- Chemical Studies

Nucleic Acid Extraction:

Nucleic acid was extracted from fresh root tips according to Mohamed and Capesius (1980).

-DNA & RNA Estimation:

i- DNA contents in the root tips are estimated according to Burton (1956).

ii- RNA contents are carried out according to Schneider method (1957).

Table 2: DNA and RNA contents (µg /gm dry wt) in root tips of the studied species

Characters	DNA Content	% of	RNA Content	% of
Studied Species	($\mu g/gm$. Dry wt.)	DNA	(μ g / gm. dry wt.)	RNA
B. discolor, F. Muell	37.30	0.0037	52.32	0.0052
B. acerifolius, (Cunn.) Muell	21.23	0.0021	31.24	0.0031
B. australis, L.	23.35	0.0023	29.83	0.0029
B. populeneus, Dc.	27.24	0.0027	39.31	0.0039
B. rupestris,(Lindl.) Schum	29.27	0.0029	43.27	0.0043

iii- Electrophoretic Investigations

-Seed Protein Estimation:

Characterization of seed protein fractions are carried out by using one dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Preparation and running of gel were carried out according to Laemmli (1970) and Stegeman *et al.*, (1981). The gel was stained with coomassie brilliant blue stain R- 250. The bands were determined and scanned by using Hoefer scanning densitometer GS 300. Gel of protein bands scanned and photographed in plate II.

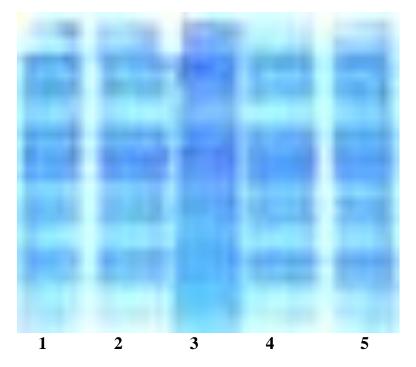


Plate II:SDS-Polyacrylamide gel electrophoresis illustrating storage seed protein of the studied species

This study is dependent upon the application of a total of 56 comparative morphological and chemical characters and their states at which, each is treated as a binary character (0 &1), on each of the 5 studied spp. of the genus *Brachychiton* in the data matrix (Table 3). The characters and states have been subjected to numerical analysis under a program using similarity and dissimilarity assessment percentage method (Khalifa, 1971). Calculation of similarity % is given in Table (4) from which the extracted dendrogram is shown in fig.(1).

Species						
Species		sn	s	snə		
State recognized	I-B. discolor	2-B. acerifolius	3- B. australis	4-B. populeneus	5-B. rupestris	
A- Morphological Characters:	1					
i- Fruit:						
1- Follicle: into three 0 / more 1	0	1	1	1	1	
2- Pedicel: long $0 / $ short 1	1	0	0	0	0	
3 -Size: large(12-20 cm long, 5-7						
breadth) 0 / medium or small 1	0	0	1	1	1	
4- Color: Pale brown 0 / brown 1	0	1	1	1	1	
5-Texture: hairy 0 / glabrous 1	0	1	1	1	1	
ii- Seeds:	0	1	0	0	0	
6 - Shape: rounded $0 / \text{ oval } 1$	0	1	0	0	0	
7- Color: black 0 / yellowish 1	0	1	1	1	1	
8- Texture: smooth 0 / curled 1	1	1	0	1	1	
B- Chemical Characters:						
i- DNA Content						
9- high conc.(37.50-29.0 μ g/gm) 0 / low (27.2-21.2	0	1	1	1	0	
$\mu g/gm)$ 1	0	1	1	1	0	
ii- RNA Content						
10 - high conc.(52.3-43.2 μ g/gm) 0 / low (39.3-31.2	0	1	1	1	0	
μg/gm) 1	0	1	1	1	0	
ii- Electrophoretic Characters:						
-Seed Protein Band:						
=Migration Distance(cm)	1		1			1
11 -0.12: Absent 0 / Present 1	1		1	1		1
12-0.20: Absent 0 / Present 1	0	-	1 0	0		0
13- 0.25: Absent 0 / Present 1	0	-		1		1
14- 0.29: Absent 0 / Present 1	1	-	1	1		1
15- 0.30: Absent 0 / Present 1 16- 0.34: Absent 0 / Present 1	0		1 0	0		0 1
10- 0.34: Absent 0 / Present 1 17- 0.43: Absent 0 / Present 1	0		0	1		0
17-0.45 : Absent 0 / Present 1 18-0.60 : Absent 0 / Present 1	1	1	0	0		0
19- 0.65: Absent 0 / Present 1	1		1	1		0
20- 0.86: Absent 0 / Present 1	0		0	0		0
21- 0.90: Absent 0 / Present 1 21- 0.90: Absent 0 / Present 1	1	-	1	1		1
21- 0.90. Absent 0 / Present 1 22- 1.00: Absent 0 / Present 1	0		1	0		0
23-1.30: Absent 0 / Present 1 23-1.30: Absent 0 / Present 1	0		1	0		1
23-1 .50: Absent 0 / Present 1 24-1 .65: Absent 0 / Present 1	1	-	0	0		0
24- 1.05: Absent 0 / Present 1 25- 1.87: Absent 0 / Present 1	0		1	0		1
26-1 .93: Absent 0 / Present 1 26-1 .93: Absent 0 / Present 1	0	-	1	1		0
27- 2.23: Absent 0 / Present 1	1	0	0	0		0
28-2 .50: Absent 0 / Present 1		1	0	1		1
29-3 .00: Absent 0 / Present 1 29-3 .00: Absent 0 / Present 1		0	1	1		0
30- 3.40; Absent 0 / Present 1			0	0		0
31- 3.60: Absent 0 / Present 1	1		0	1		1
32- 4.21: Absent 0 / Present 1			1	0		0
	0	1	1	0		U

Table 3: The resulted 56 binary characters of the studied spp.(Characters & states are symbolized for numerical analysis)

33- 4.22: Absent 0 / Present 1		0	1	1	1		1
34- 4.60: Absent 0 / Present 1		1	0	0	0		0
35- 5.91: Absent 0 / Present 1		1	0	1	1		1
36- 6.40: Absent 0 / Present 1		1	1	0	1		1
37-6 .60: Absent 0 / Present 1		0	0	0	1		1
38-6 .75: Absent 0 / Present 1	0	0	1		0	0	
39- 7.00: Absent 0 / Present 1	1	1	1		1	1	
40-7.12 : Absent 0 /Present 1	1	0	0	1	0	1	
41-7 .50: Absent 0 / Present 1	1	0	1		1	1	
42-7 .46: Absent 0 / Present 1	1	1	0	1	1	1	
43-7 .60: Absent 0 / Present 1	1	1	1		1	1	
44-7 .85: Absent 0 / Present 1	1	0	1		1	1	
45-8.00: Absent 0 / Present 1	0	1	0	1	1	0	
46- 8.17: Absent 0 / Present 1	1	1	0)	1	1	
47-8.20: Absent 0 / Present 1	1	1	1		1	1	
48 -8.43: Absent 0 / Present 1	1	0	0)	0	1	
49-8.63: Absent 0 / Present 1	0	1	1		1	1	
50 -8.80: Absent 0 / Present 1	1	0	0	1	0	0	
51-8.85: Absent 0 / Present 1	0	1	1		1	1	
52 -9.01: Absent 0 / Present 1	1	1	1		0	0	
53 -9.20: Absent 0 / Present 1	1	1	0)	1	1	
54-9.62: Absent 0 / Present 1	1	1	1		1	0	
55-9.84: Absent 0 / Present 1	0	0	1		0	0	
56-10.16: Absent 0/Present 1	1	1	1		1	1	

Table 4: Calculation of % ((similarity) among the studied species (56 morphological & chemical characters)

	ucal characters)			
Species	Related species	Characters	% Similarity	% Dissimilarity
1	2	25	44.64	55.36
1	3	21	37.50	62.50
1	4	27	48.21	51.78
1	5	32	57.14	42.85
1	2,4,5	17	30.35	69.65
2	3	30	53.57	46.43
2	4	39	69.64	30.35
2	5	38	67.85	32.14
2	4,5	32	57.14	42.86
2	3,4	24	28.50	72.50
3	4	35	62.50	37.50
3	5	31	55.35	44.64
3	4,5	27	48.21	51.79
3	2,4,5	19	33.93	66.07
4	5	44	78.57	21.43
1	2,3,4,5	5	8.92	91.08

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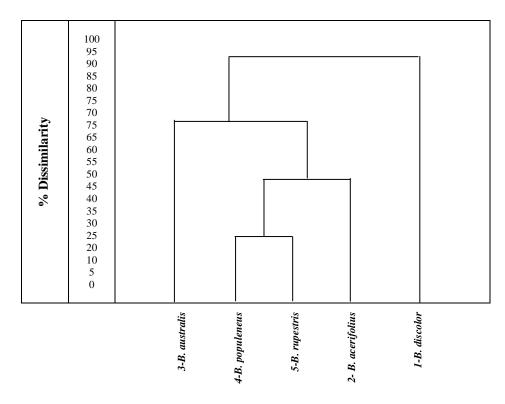


Fig. 1: A dendrogram of the 5 studied spp. in the genus Brachychiton, L. in Egypt

Results And Discussion

A- Morphological Characters

- **Fruit**: It is usually a follicle, grouped into clusters these are either into three ones (*B. discolor*) or in more than 3 (the remainder spp. studied). Pedicels are either, short (1-2 cm) in *B. discolor* or long (3-5 cm) in the remainders. Follicles may be; large-sized (12-18 cm long, 5-7 cm diameter) in *B. discolor* and *B. acerifolius*, medium-sized (5-9 cm length,2-3 cm breadth) in *B. australis, B. populeneus* while small-sized (2-3 cm length,1.5-2.5 breadth) in *B. rupestris*. Such follicles may be pale brown colored in *B. discolor* or smooth in the remainders.

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- Seeds: Oval-shaped in *B. acerifolius*, rounded in the remainders, black into *B. discolor*, yellowish in the remainders, smooth in *B. australis* and rough in the remainders.

B- Chemical Characters

i. DNA Content: Tables 2,3

Table (2) indicates that the highest amounts of 37.30 μ g/gm and 29.27 μ g/gm are recorded in *B. discolor* and *B. rupestris*, respectively (Table 3, charac. <u>no.</u> 9). Whereas the lowest amounts of 21.23 and 23.35 μ g/gm were recorded in *B. acerifolius* and *B. australis*. Finally, the value of 27.24 μ g/gm is recorded in *B. populeneus*.

ii. RNA Content:

Table (2) also shows that, *B. discolor* and *B. rupestris* reveal higher concentration values while *B. populeneus* produces medium value of $39.31\mu g/gm$, while *B. acerifolius* and *B. australis* are the lowest (Table 3, charac. <u>no.</u> 10).

iii- Electrophoretic Characters:

-Seed Protein Bands: Plate II, Table(3)

Seed protein within the 5 studied spp. are represented by 46 different bands ranged between 0.12 : 10.16 migration distance (Table 3, charac., <u>no</u>. 11-56). Only 6 bands of the migration distance of:0.12 ,0.29, 7.00, 7.21, 8.20 & 10.16 (Table 3, charac., <u>no</u>. 11, 14, 39, 43, 47&56) are widespread in all the studied spp. *B. discolor* is recorded with 26 bands (Table 3, charac. <u>no</u>. 11, 14, 16, 18, 19, 21, 24, 27, 30, (34-36), (39-44), (46-48), 50, (52-54) & 56). On the other hand, *B. acerifolius* recorded the presence of 25 bands (Table 3, charac. <u>no</u>. 11, 13, 14, 16, 18, 20, 23, 25, 28, (31-33), 36, 39, 42, 43, (45-47), 49, (51-54) & 56). Also 26 bands have been recorded in *B. australis* (Table 3, charac. <u>no</u>.11, 12, 14, 15, 19, (21-23), 25, 26, 29, 32, 33, 35, 38, 39, 41, 43, 44, 47, 49, 51, 52 & (54-56). *B. populeneus* is recorded with 28 bands (Table 3, charac. <u>no</u>.11, 13, 14, 16, 17, 19, 21, 26, 28, 29, 31, 33, (35-37), 39, (41-47), 49, 51, 53, 54, & 56). In addition there are 26 bands related to *B. rupestris* (Table 3, charac. <u>no</u>.11, 13, 14, 16, 21, 23, 25, 28, 31, 33, (35-37), (39-44), (46-49), 51, 53, 56.

Table (4), Fig.(1), isolate *B. discolor* from the remainder studied species at a higher level of dissimilarity % with a value of 91.08%. It is characterized by:

- Fruit follicles are: short pedicelled, pale brown, hairy, with black seeds are grouped into threes (Table 3, charac. <u>no</u>. 1,2,4,5&7).

- Higher nucleic acid contents (Table 3, charac. no. 9 &10).

- Presence of 8 own seed protein bands (Table 3, charac. <u>no</u>. 24, 27, 30, 33, 34, 49, 50, &51).

These are valid evidence for *B. discolor* systematic treatment as a separate group (Fig.1), that is in agreement with Greilhuber (1977), Ladizinsiky, and Hymotwitz (1979), Jensen (1984) and Badr (1995).

On the other hand, there is a higher similarity between *B. populeneus, B. rupestris* as they are similar into 44 characters (Table 3, charac. <u>no</u>.1, 2, (3-8), (11-16), (20-22), 24, 27, 28, (30-39), (41-44), 46, 47, (49-53), 55 & 56). They isolate at a higher level of similarity % with a value of 78.57%. *B. populeneus, B. rupestris* are more related to *B. acerifolius* by a similarity level of 57.14.The remainder *B.australis* is connected to the previous 3 species by a similarity level of 33.93%.

Table (3), indicates that they have \pm stable seed protein contents which can used as a taxonomic information for spp. relationships that encourage their categorization into the same group that connected to *B.discolor* group at a least(8.92%) level of similarity%.

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الملخص العربي

دراسات مورفولوجية وكيميائية علي بعض أنواع جنس براكيكيتون المنزرعة في مصر

وائل طه قاسم و محمود محمد منصور من قسم النبات والميكروبيولوجي- كلية العلوم (بنين)- جامعة الأزهر – مدينة نصر – القاهرة – مصر

تمت دراسة الصفات المورفولوجية للثمرة والبذرة وكذلك المحتوي النووي والبروتيني في عدد خمسة أنواع مصرية من جنس البراكيكيتون هم : . . B. acerifolius, B. نم استخلصت 56 صفة من نتائج الدراسة (australis, B. populeneus & B. rupestris) ثم استخلصت 56 صفة من نتائج الدراسة خضعت للتحليل العددي الذي أدي ألي رسم هيكلي Dendrogram صنف النباتات المدروسة إلي مجموعتين انفرد فيها discolor داخل مجموعة مستقلة تشابهت مع أفراد المجموعة الثانية عند أدني مستوي تشابة (22و 8 %).