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# Systematic implications of seed coat diversity in some representatives of the genus *Ipomoea* (Convolvulaceae)

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**Abstract:** Seed coat morphology of 15 species of *Ipomoea* L. was examined comparatively using scanning and light microscopy methods in order to evaluate their diagnostic value for systematic studies. Macro- and micromorphological characters, including seed shape, colour, size, seed surface, epidermal cell shape, anticlinal boundaries, and periclinal cell wall are presented. Descriptions of seed size, shape, colour, surface, and seed coat types are summarised for the genus. Taxonomic phylogenetic implications of the seed coat micromorphology are also discussed in comparison with the available gross morphological and molecular data. Results of the seed character analyses offer useful data for evaluating the taxonomy of *Ipomoea* both on subgeneric and sectional levels. Monophyly of both sections *Erpipomoea* Choisy and *Eriospermum* Hallier is not supported. A key for the identification of the investigated taxa based on seed characters is provided.

**Key words:** *Ipomoea*, cluster analysis, scanning electron microscopy, seed coat, subgeneric classification

## 1. Introduction

*Ipomoea* L. is a large, diverse genus of the Convolvulaceae comprising over 600 species of vines and shrubs that are widely distributed throughout the tropics and subtropics (Van Ooststroom, 1953; Austin, 1975; Austin and Huáman, 1996; Miller et al., 1999; Stefanovic et al., 2003).

The taxonomy and systematics of this critical group are highly controversial and dependent on different character marker systems.

Earlier treatments recognised subgenera and infrageneric taxa within *Ipomoea* (Choisy, 1845; Hallier, 1893; House, 1908). In a recent cladistic analysis of tribe *Ipomoeae* based on 45 morphological and palynological characters, Willkin (1999) suggested that *Ipomoeae* is a monophyletic tribe, but *Ipomoea* is a paraphyletic genus. Moreover, relationships among the Old World (Asian) *Ipomoea* species were further refined by Van Ooststroom (1953), who recognised 7 infrageneric taxa, whereas Verdcourt (1957, 1963) recognised 8 infrageneric taxa in his treatment of the African species.

Austin (1975, 1979, 1997) and Austin and Huáman (1996) divided *Ipomoea* into 3 subgenera, i.e. subgenus *Eriospermum* (Hallier f.) Verdcourt ex Austin, *Ipomoea*, and *Quamoclit* (Moench) Clarke.

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McDonald and Mabry (1992) carried out phylogenetic analysis of chloroplast DNA for 31 New World *Ipomoea* species, and this molecular study supported the monophyly of several traditionally recognised infrageneric taxa of *Ipomoea*. Das and Mukherjee (1997) studied seedling morphology and isozyme profiles of 12 species of *Ipomoea*, and they suggested 2 species groups. Miller et al. (1999) studied the phylogenetic relationships of 40 species representing the 3 subgenera and 9 sections within *Ipomoea* using sequence data from the ITS region and waxy sequences. They detected a close relationship between species of section *Pharbitis* (Choisy) Griseb. of subgenus *Ipomoea* and species of subgenus *Quamoclit*.

Manos et al. (2001) tested the phylogenetic relation of the genus *Ipomoea* with other genera from the tribe *Ipomoeae* based on morphology and concluded that *Ipomoea* is paraphyletic. Ogunwenmo (2003) investigated morphometric cotyledon characters of 18 *Ipomoea* taxa, and he suggested that cotyledon characters are of taxonomic significance in *Ipomoea*.

Miller et al. (2004) phylogenetically investigated 36 *Ipomoea* species by ITS sequence comparison. Results suggested that nuclear ITS studies generally agree with cpDNA studies in recognising 2 large clades of species. McDonald et al. (2011) studied 68 species and 2 varieties

of tropical and temperate North American *Ipomoea* using sequence data from ITS with parsimony, and Bayesian analyses revealed multiple origins of autogamy. They classified the species into 2 subgenera, subgenus *Eriospermum* and subgenus *Quamoclit*.

Abdel Khalik et al. (2012) studied 10 species of *Ipomoea* based on RAPD-PCR and SDS-PAGE analysis of seed proteins. They found a close relationship between *Ipomoea purpurea* (L.) Roth of section *Pharbitis* (Choisy) Griseb (subgenus *Ipomoea*) and species of the subgenus *Quamoclit*. Additional results derived from the RAPD molecular data indicated that *I. cairica* (L.) Sweet should be considered a well-separated section that may be related to section *Orthipomoea* (Choisy) Austin and section *Erpipomoea* Choisy is not a monophyletic group, whereas species of section *Orthipomoea* form a single monophyletic section.

Seed morphology provides a number of characters potentially useful for species identification, phylogenetic inference, and character-state evolution (Johnson et al., 2004; Attar et al., 2007; Moazzeni et al., 2007; Mostafavi et al., 2013). Observations in many plant groups have shown that seed morphology and anatomic features are rather conservative, which makes them taxonomically important (Esau, 1977; Barthlott, 1984; Werker, 1997; Abdel Khalik and Maesen, 2002; Akbari and Azizian, 2006; Abdel Khalik, 2010; Kaya et al., 2011; Abdel Khalik and Hassan, 2012; Bona, 2013). The species of *Ipomoea* equally exhibit diversity in fruit and seed morphology. However, affinities are sometimes shown among closely related taxa (Ugborogho and Ogunwenmo, 1995; Ogunwenmo, 1998). Data on the seed morphology of representatives of *Ipomoea* are rather limited and mostly confined to papers on Convolvulaceae systematics (Ogunwenmo, 2006; Abdel Khalik and Osman, 2007; Aitawade et al., 2009).

The aim of the present study is to estimate the importance of seed micromorphological characters for the infrageneric classification of *Ipomoea* by means of cluster analyses and to determine whether data on seed micromorphology can contribute additional knowledge about seed shape and seed coat in the studied taxa.

## 2. Materials and methods

### 2.1. Seed material

Representative species from the investigated subgenera *Eriospermum*, *Ipomoea*, and *Quamoclit* of *Ipomoea* were selected for seed micromorphological analysis. In total, the seed microsculpture of 15 taxa at the species level has been reanalysed. Only clearly visible, measurable characters were recognised.

Some of the investigated seeds were collected from mature capsules of living plants in Egypt, and others were taken from either herbarium specimens or from abroad as a loan. A list of voucher specimen localities is presented in Table 1. Only mature seeds were used for investigation. The dried seeds were first examined by dissecting scope (Olympus type BH-2), and 10–15 seeds for each taxa were chosen to cover the range of variation. For scanning electron microscopy (SEM), seeds were mounted on stubs with double adhesive tape. The stubs were sputter-coated with gold for 5 min in an E1100 (Polaron Equipment). After coating, the specimens were examined with a JEOL JSM 5300 scanning electron microscope using accelerating voltages at 20–25 kV. All photomicrographs were taken at the central laboratory of the Faculty of Science, Sohag University, Egypt. The terminology used here follows that of authors such as Barthlott (1981, 1984), Abdel Khalik and Maesen (2002), and Abdel Khalik and Osman (2007) for description of seed shape, cell shape, and seed coat ornamentation.

### 2.2. Characters selection coding

The principles for character selection were the independency of the characters and their stability within the taxa analysed (Stuessy, 1990; Davitashvili and Karrer, 2010). Seeds provide several qualitative and few quantitative characters. The focus is on qualitative characters of seed micromorphology that are easy to detect. One quantitative character (character 3) was measured for bigger samples, and means were grouped in magnitudes that could be treated statistically as qualitative characters. All characters were coded as in the Appendix.

### 2.3. Analysis of seed data

A total of 10 characters were measured in each species. UPGMA analysis was performed with NTSYS-pc 2.02k software (Applied Biostatistics Inc., Setauket, NY, USA). Cluster analysis was conducted by average taxonomic distance and UPGMA clustering (procedures SIMINT, SAHN, and TREE). The characters and character states scored and obtained from seed morphological characters are shown in the Appendix.

## 3. Results

The seed morphological characters of the studied taxa of the genus *Ipomoea* as shown by light microscopy and SEM are reviewed in Table 2 and Figures 1–5.

### 3.1. Seed colour

The colours of seeds are highly diagnostic and of systematic interest among taxa. The colour of seeds varies from yellow to brown in *Ipomoea sinensis* (Desr.) Choisy while it is dark brown in *I. triloba* L.; black in *I. eriocarpa* R.Br., *I. indica* (Burm.) Merr., and *I. stolonifera* (Cyr.) Gmel.; and black to brown in the rest of the species.

### 3.2. Seed shape

Seed shape in *Ipomoea* can be categorised as follows: elongate to pear shape in *I. quamocliti* L. (Figure 3); ovoid to subglobose in *I. cairica* (L.) Sweet, *I. carnea* Jacq., *I. cristulata* Hallier, and *I. eriocarpa* (Figure 1); broadly

**Table 1.** List of the studied species of *Ipomoea* sited according to traditional [Austin (1979, 1997) and Austin and Huáman (1996)] and more recent [McDonald (1991), Miller et al. (1999, 2004), and McDonald et al. (2011)] classifications.

| N | Taxa | Voucher | Austin and Huáman (1979, 1996, 1997) | McDonald and Miller (1991, 1999, 2004, 2011) | Present study |
|---|---|---|---|---|---|
| 1 | *Ipomoea cairica* | Egypt: Ismailia canal at Abu Zaabal, Hadidi et al. *s.n.* (CAI) | Subg. *Eriospermum* Sect. *Erpipomoea* | Subg. *Quamoclit* Sect. *Orthipomoea* | Group A |
| 2 | *I. carnea* | Egypt: Sohag, El Kola, canal bank, Abdel Khalik *s.n.* (SHG) | Subg. *Eriospermum* Sect. *Eriospermum* | Subg. *Eriospermum* Sect. *Eriospermum* | Group D |
| 3 | *I. cristulata* | Kew Garden, Millennium Seed Bank, serial number: 0347644 (). | Subg. *Quamoclit* Sec. *Mina* | Subg. *Quamoclit* Sect. *Mina* | Group A |
| 4 | *I. eriocarpa* | Egypt: Behiera, Abu Qir, Alaa Amer *s.n.* (CAI) | Subg. *Quamoclit* Sect. *Orthipomoea* | Subg. *Quamoclit* Sect. *Orthipomoea* | Group E |
| 5 | *I. heterotricha* | Kew Garden, Millennium Seed Bank, serial number: 0443926 (K) | Subg. *Eriospermum* Sect. *Eriospermum* | Subg. *Eriospermum* Sect. *Eriospermum* | Group B |
| 6 | *I. imperti* | Kew Garden, Millennium Seed Bank, serial number: 0396147 (K) | Subg. *Eriospermum* Sect. *Erpipomoea* | Subg. *Eriospermum* Sect. *Erpipomoea* | Group C |
| 7 | *I. indica* | PTBG 72c900, Kauai, HI, USA | Subg. *Ipomoea* Sect. *Pharbitis* | Subg. *Quamoclit* Sect. *Pharbitis* | Group B |
| 8 | *I. involucrata* | Kew Garden, Millennium Seed Bank, serial number: 0212975 (K) | Subg. *Ipomoea* Sect. *Involucratae* | Subg. *Quamoclit* Sect. *Involucratae* | Group A |
| 9 | *I. obscura* | Egypt: Gebel Elba, Wadi Yahameb, Abdel Khalik *s.n.* (SHG) | Subg. *Eriospermum* Sect. *Erpipomoea* | Subg. *Eriospermum* Sect. *Erpipomoea* | Group B |
| 10 | *I. pes-caprae* subsp. *brasiliensis* | Egypt: Giza, Tackholm, V. *s.n.* (CAI) | Subg. *Eriospermum* Sect. *Erpipomoea* | Subg. *Eriospermum* Sect. *Erpipomoea* | Group C |
| 11 | *I. purpurea* | Egypt: Idku, A. Amer 9720 (CAI) | Subg. *Ipomoea* Sect. *Pharbitis* | Subg. *Quamoclit* Sect. *Pharbitis* | Group C |
| 12 | *I. quamocliti* | Botanical Garden of Berlin, number: 920 (B) | Subg. *Quamoclit* sect. *Mina* | Subg. *Quamoclit* sect. *Mina* | Group A |
| 13 | *I. sinensis* subsp. *blepharosepala* | Egypt: Gebel Elba, Wadi Yahameib, Abdel Khalik et al. *s.n.* (SHG) | Subg. *Quamoclit* Sect. *Orthipomoea* | Subg. *Quamoclit* Sect. *Orthipomoea* | Group B |
| 14 | *I. stolonifera* | Egypt: N. Sinai, Rafah, Tackholm et al. *s.n.* (CAIM) | Subg. *Eriospermum* Sect. *Erpipomoea* | Subg. *Eriospermum* Sect. *Erpipomoea* | Group C |
| 15 | *I. triloba* | Kew Garden, Millennium Seed Bank, serial number: 0223353 (K) | Subg. *Eriospermum* Sect. *Eriospermum* | Subg. *Eriospermum* Sect. *Eriospermum* | Group B |

**Table 2.** Seed characteristics of studied *Ipomoea* taxa.

| N | Taxon | Seed colour | Seed shape | Seed surface | Seed size (length × width) mm | Epidermal cell shape | Anticlinal cell wall boundaries | Periclinal cell wall |
|---|---|---|---|---|---|---|---|---|
| 1 | *I. cairica* | Black to brown | Ovoid to subglobose | Pubescent with tufts of long silky hairs along the margins | 4–5 × 3–4 | Irregular, polygonal cells | Undulate, raised channelled; smooth to fine folds | Flat to concave; microreticulate |
| 2 | *I. carnea* | Black to brown | Ovoid to subglobose | Long woolly hairs all over | 7–9 × 5–6.5 | 4–5 gonal cells, elongate in 1 direction | Straight to slightly sinuous, slightly raised; folded | Flat to concave; reticulate |
| 3 | *I. cristulata* | Black to brown | Ovoid to subglobose | Pubescent | 2.5–4 × 1.5–2.5 | Irregular, polygonal cells | Undulate, raised-channelled; smooth to fine folds | Flat to concave; smooth |
| 4 | *I. eriocarpa* | Black | Ovoid to subglobose | Glabrous | 2.5–3 × 1.7–2 | Isodiametric, 5–6 gonal cells | Straight to slightly sinuous, raised; folded | Flat to convex; smooth to fine folds |
| 5 | *I. heterotricha* | Black to brown | Ovoid | Glabrous | 2.2–2.5 × 1.6–1.8 | Irregular, polygonal cells | Undulate, raised; smooth to fine folds | Flat to concave; smooth |
| 6 | *I. imperti* | Black to brown | Ovoid | Pubescent with tufts of long silky hairs along the margins | 7–9 × 5–7 | Irregular, polygonal cells | Straight to slightly sinuous, raised; smooth to fine folds | Flat to concave; smooth to fine folds |
| 7 | *I. indica* | Black | Ovoid | Glabrous | 4–5 × 3–4 | Irregular, polygonal cells | Undulate, raised; smooth to fine folds | Flat to concave; smooth |
| 8 | *I. involucrata* | Black to brown | Ovoid | Pubescent | 2–3.5 × 1.4–2.5 | Irregular, polygonal cells | Undulate, raised-channelled; smooth to fine folds | Flat to concave; folded |
| 9 | *I. obscura* | Black to brown | Ovoid | Dense pubescent | 4–5 × 3–3.5 | Irregular, polygonal cells | Undulate, raised; smooth to fine folds | Flat to concave; folded |
| 10 | *I. pes-caprae* subsp. *brasiliensis* | Black to brown | Broadly ovoid to subglobose | Pubescent | 7–10 × 6.5–8 | Irregular, polygonal cells | Straight to slightly sinuous, raised; smooth to fine folds | Flat to slightly concave; microreticulate |
| 11 | *I. purpurea* | Black to brown | Ovoid | Glabrous to pubescent | 4–5 × 2–3 | Irregular, polygonal cells | Straight to slightly sinuous, raised; smooth to fine folds | Flat to concave; folded |
| 12 | *I. quamocliti* | Black to brown | Elongate to pear shape | Glabrous to pubescent | 2.5–4 × 1.2 –2 | Irregular, polygonal cells | Straight to slightly sinuous, raised-channelled; smooth to fine folds | Flat to concave; smooth to fine folds |
| 13 | *I. sinensis* subsp. *blepharosepala* | Yellow to brown | Ovoid | Dense pubescent | 3–4 × 2–3 | Irregular, polygonal cells | Undulate, raised; smooth to fine folds | Flat to concave; smooth to fine folds |
| 14 | *I. stolonifera* | Black | Ovoid | Glabrous to pubescent | 2.5–4 × 1.5–2.5 | Irregular, polygonal cells | Straight to slightly sinuous, raised; fine folds | Flat to concave; fine folds |
| 15 | *I. triloba* | Dark brown | Ovoid | Glabrous | 1.6–2 × 1.1–1.5 | Irregular, polygonal cells | Undulate, raised; smooth to fine folds | Flat to concave; smooth to fine folds |

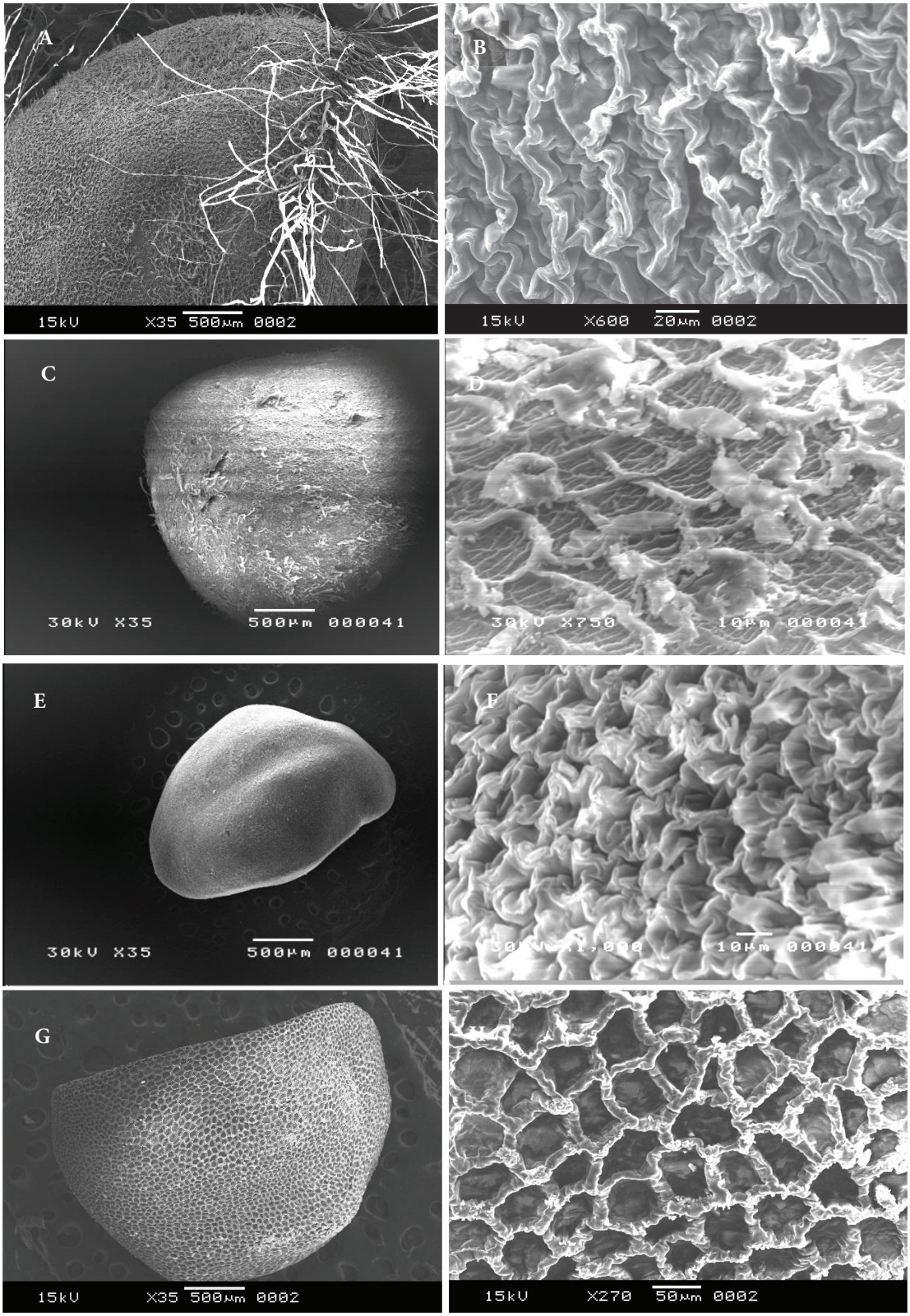


**Figure 1.** SEM photographs of seeds. A, B- *Ipomoea cairica*; C, D- *I. carnea*; E, F- *I. cristulata;* G, H- *I. eriocarpa*. A, C, E, G- entire seed; B, D, F, H- enlargement of seed coat.

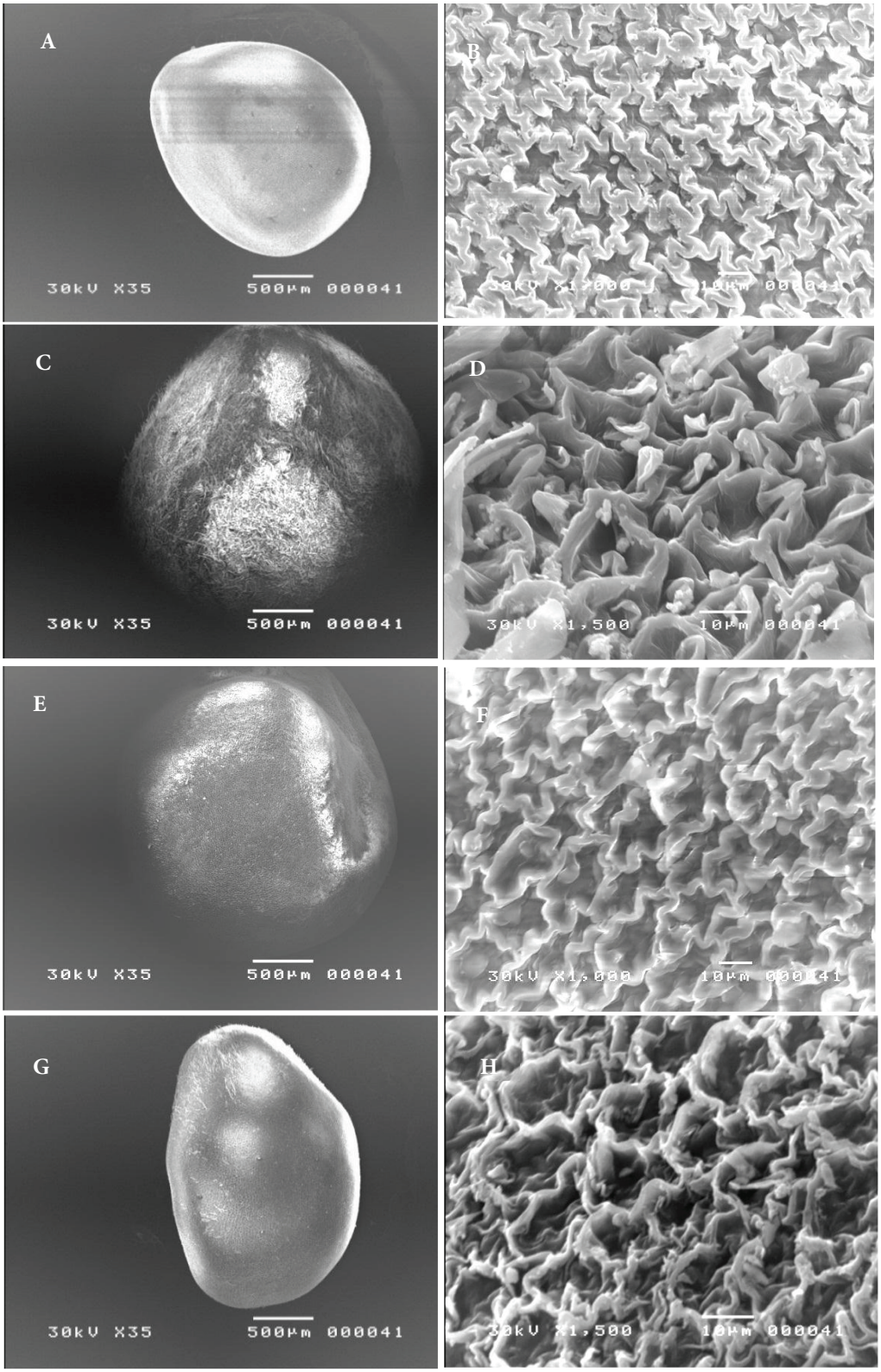


**Figure 2.** SEM photographs of seeds. A, B- *Ipomoea heterotricha*; C, D- *I. imperti*; E, F- *I. indica;* G, H- *I. involucrata*. A, C, E, G- entire seed; B, D, F, H- enlargement of seed coat.

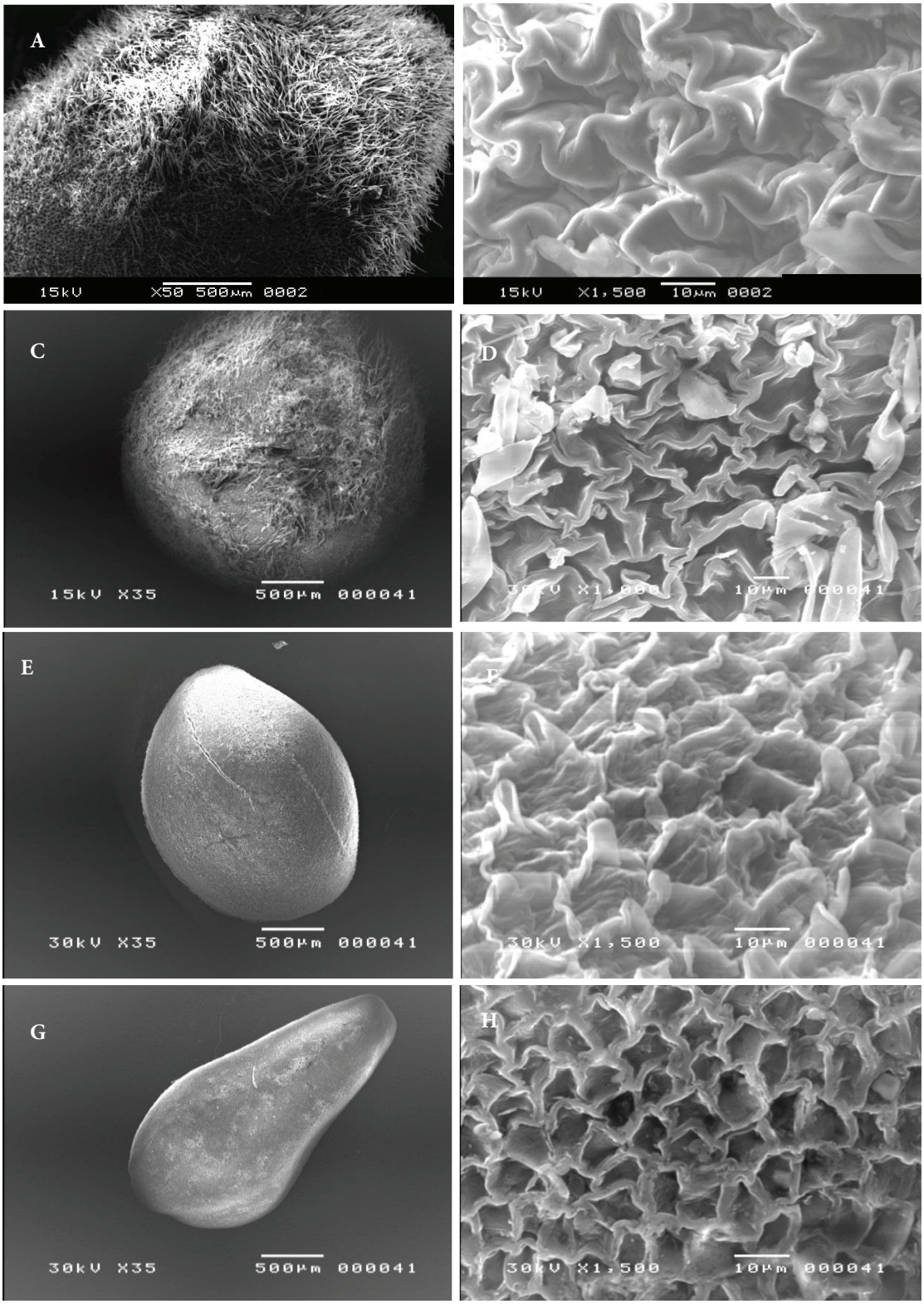


**Figure 3.** SEM photographs of seeds. A, B- *Ipomoea obscura*; C, D- *pes-caprae*; E, F- *I. purpurea;* G, H- *I. quamocliti*. A, C, E, G- entire seed; B, D, F, H- enlargement of seed coat.

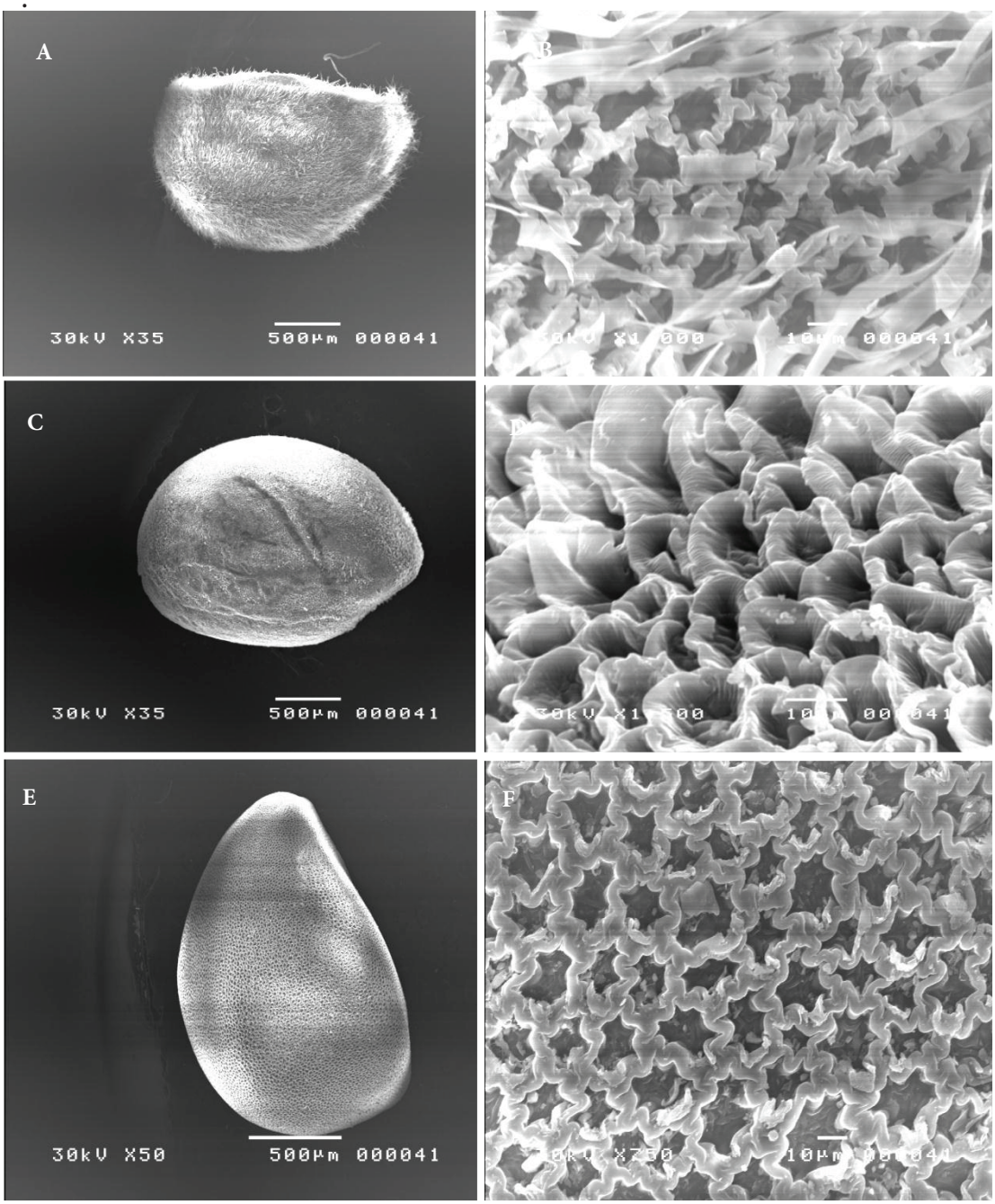


**Figure 4.** SEM photographs of seeds. A, B- *Ipomoea sinensis*; C, D- *stolonifera*; E, F- *I. triloba*. A, C, E- entire seed; B, D, F- enlargement of seed coat.

ovoid to subglobose in *I. pes-caprae* (L.) R.Br. (Figure 3); and ovoid in the rest of the *Ipomoea* species.

**3.3. Seed surface**

The seed surfaces of the studied taxa have great variation. They vary tremendously from glabrous in *Ipomoea eriocarpa*, *I. heterotricha* F.Didr., *I. indica*, and *I. triloba* (Figures 1–4); glabrous to pubescent in *I. purpurea* (L.) Roth., *I. quamocliti*, and *I. stolonifera* (Figures 3 and 4); pubescent in *I. cristulata*, *I. involucrata* P.Beauv., and *I. pes-caprae* (Figures 1–3); densely pubescent in *I. obscura* (L.) Ker Gawl. and *I. sinensis* (Figures 3 and 4); pubescent with tufts of long silky hairs along the margins in *I. cairica* and *I. imperti* (Vahl) Griseb. (Figures 1 and 2); and long woolly hairs all over in *I. carnea* (Figure 1).

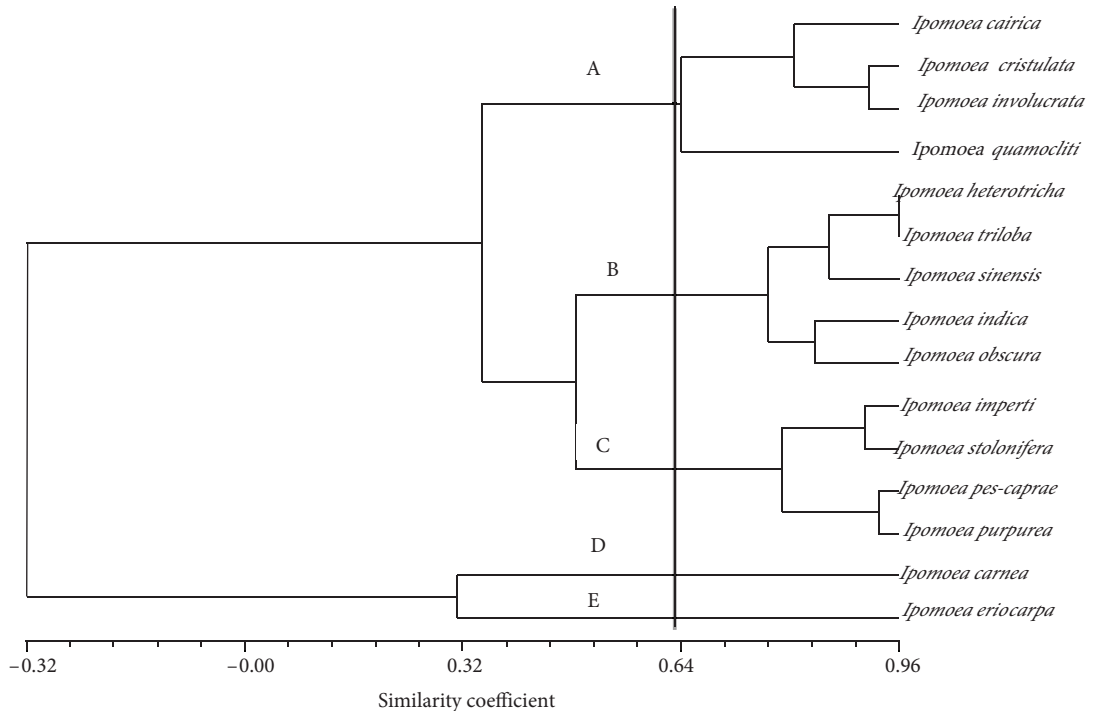


**Figure 5.** Dendrogram illustrating the relationships of the investigated species of *Ipomoea* based on seed characters.

### 3.4. Seed size

Seed dimensions vary significantly among the examined taxa. The biggest seeds were measured in *Ipomoea carnea*, *I. imperti*, and *I. pes-caprae*, at 7–10 × 5–8 mm; the smallest seeds were 1.6–4 × 1.1–3 mm in *I. cristulata*, *I. eriocarpa*, *I. heterotricha*, *I. involucrata*, *I. quamocliti*, *I. sinensis*, *I. stolonifera*, and *I. triloba*. The rest of the species have slightly bigger seeds measuring 4–5 × 3–4 mm (Table 2).

### 3.5. Epidermal cell shape

The cellular shapes can be of considerable diagnostic systematic value. The cells vary from 4–5 gonals to elongate in 1 direction in *Ipomoea carnea* (Figure 1), isodiametric to 5–6 gonals in *I. eriocarpa* (Figure 1), and irregular to polygonal cells in the rest of the taxa.

### 3.6. Anticlinal cell wall boundaries

These are mostly well developed. There are 2 types of cell wall boundaries: the first type is undulate in *Ipomoea cairica*, *I. cristulata*, *I. heterotricha*, *I. indica*, *I. involucrata*, *I. obscura*, *I. sinensis*, and *I. triloba* (Figures 1–4); the second type is straight to slightly sinuous in the rest of the taxa (Figures 1–4). Based on the relief of cell wall boundaries there are 3 types of boundaries: raised-channelled as in *I. cairica*, *I. cristulata*, *I. involucrata*, and *I. quamocliti* (Figures 1–3); slightly raised in *I. carnea* (Figure 1); and raised in the rest of the taxa (Figures 1–4).

### 3.7. Periclinal cell wall

The curvature of the outer wall can assist as a good diagnostic character. There are 2 different shapes for the outer periclinal cell wall: flat to convex in *Ipomoea eriocarpa* (Figure 1) and flat to concave in the rest of the taxa. The sculpture of the outer cell wall shows great variation among the studied taxa. There are 5 different shapes for the surface of the outer cell wall: smooth in *I. cristulata*, *I. heterotricha*, and *I. indica* (Figures 1 and 2); folded in *I. involucrata*, *I. obscura*, and *I. purpurea* (Figures 2 and 3); smooth to fine folds in *I. eriocarpa*, *I. imperti*, *I. quamocliti*, *I. sinensis*, and *I. stolonifera* (Figures 1–4); microreticulate in *I. cairica* and *I. pes-caprae* (Figures 1 and 3); and reticulate in *I. carnea* (Figure 1).

### 3.8. Cluster analysis

The results of the cluster analyses are presented in Figure 5. In the UPGMA dendrogram, 5 major group branches (A–E) with approximately 64% similarity are distinguished: 1) group A includes *Ipomoea cairica*, *I. cristulata*, *I. involucrata*, and *I. quamocliti*; 2) group B contains *I. heterotricha*, *I. triloba*, *I. sinensis*, *I. indica*, and *I. obscura*; 3) group C comprises *I. imperti*, *I. stolonifera*, *I. pes-caprae*, and *I. purpurea*; 4) branch D includes only *I. carnea*; and 5) branch E consists of *I. eriocarpa*. The subgenera and sections show intravariability among themselves. In general, UPGMA indicates that seed morphology follows the currently applied subgenera sectional classification of

*Ipomoea* by McDonald (1991), Miller et al. (1999, 2004), and MacDonald et al. (2011) but forms separate clusters with taxa of morphologically different sections.

**Key to the identification of *Ipomoea* based on seed characters**

1a. Seeds glabrous ...….....2
1b. Seeds glabrous to hairy .....……….....5
2a. Seed ovoid, 1.6–2 × 1.1–1.5 mm; dark brown ..... ..... *I. triloba*
2b. Seed ovoid to subglobose, 2.2–5 × 1.6–4 mm; black or black to brown . . . .....……... 3
3a. Seed size 2.2–2.5 × 1.6–1.8 mm; black to brown…… …….....…….....*I. heterotricha*
3b. Seed size 2.5–5 × 1.7–4 mm; black…..... 4
4a. Seed ovoid to subglobose; 2.5–3 × 1.7–2 mm; epidermal cell isodiametric, 5–6 gonal cel ls……..….....…. *I. eriocarpa*
4b. Seed ovoid; 4–5 × 3–4 mm; epidermal cell irregular, polygonal cells.....*I. indica*
5a. Seed glabrous to pubescent .....…6
5b. Seed haiy.....……..……...8
6a. Seed elongate to pear shape; anticlinal boundaries, raised channelled.....…*I. quamocliti*
6b. Seed ovoid; anticlinal boundaries, raised…...……... 7
7a. Seed size 2.5–4 × 1.5–2.5 mm; black……*I. stolonifera*
7b. Seed size 4–5 × 2–3 mm; black to brown..…….....……...*I. purpurea*
8a. Seed covered with long woolly hairs all over; sculpture of periclinal cell wall reticulate.....*I. carnea*
8b. Seed covered with pubescent or with tufts of long silky hairs along the margins; sculpture of periclinal cell wall smooth, fine folds to microreticulate…….....…..…..…9
9a. Seed covered with pubescence only.....…10
9b. Seed covered with pubescence with tufts of long silky hairs along the rgis……11
10a. Seed size 4–5 × 3–4 mm; anticlinal boundaries undulate, raised-channelled; sculpture of periclinal cell wall microreticulate..….....*I. cairica*
10b. Seed size 7–9 × 5–7 mm; anticlinal boundaries straight to slightly sinuous, raised; sculpture of periclinal cell wall smooth to fine folds. ……..…….*I. imperti*
11a. Seed covered with dense pubescent .....……12
11a. Seed covered with sparse ubescent….....……13
12a. Seed size 3–4 × 2–3; yellow to brown…..… *I. sinensis*
12b. Seed size 4–5 × 3–3.5; black to brown…..… *I. obscura*
13a. Seed size 7–10 × 6.5–8 mm; anticlinal boundaries straight to slightly sinuous, raid…….....*I. pes-caprae*
13b. Seed size 2–4 × 1.4–2.5 mm; anticlinal boundaries undulate, raised-channelled.....14
14a. Seed ovoid, 2–3.5 × 1.4–2.5 mm; sculpture of periclinal cell wall folded.....*I. involucrata*
14b. Seed ovoid to subglobose, 2.5–4 × 1.5–2.5 mm; sculpture of periclinal cell wall smoo……. *I. cristulat*

## 4. Discussion

Several authors have tried to provide an accepted system to split the genus *Ipomoea* into subgenera, sections, and series (Choisy, 1845; Hallier, 1893; House, 1908; Van Ooststroom, 1953; Verdcourt, 1957, 1963; Austin, 1975, 1979, 1997; Austin and Huáman 1996). These studies were based on 1 or 2 traits from these morphological characters such as life forms, leaves, sepals, petals, fruits, seeds, and pollen grains. In the present study a number of seed characters were used based on the details of seed coat structure. In general, the results show that different patterns of seed morphology are helpful in distinguishing various species (Table 2); they do not confirm the 3 subgenera and sectional classification of the genus *Ipomoea* proposed by Austin's classifications (Austin and Huáman, 1996; Austin, 1997) and somewhat confirm the subgenera and sectional classification of *Ipomoea* by McDonald (1991), Miller et al. (1999, 2004), and McDonald et al. (2011).

### 4.1. Subgeneric classification

#### 4.1.1. Subgenus *Quamoclit* (Moench) Clarke (groups A, E)

Within group A there is a close relationship with 0.64 similarity corresponding to *Ipomoea* subgenus *Quamoclit* including *Ipomoea cristulata*, *I. involucrata*, *I. quamocliti*, and *I. cairica*. Specialisations in seed morphology include black to brown seeds; irregular to polygonal cells epidermal cell shape; undulate, raised-channelled, smooth to fine folded anticlinal boundaries; and flat to concave periclinal cell wall.

Another branch of species represented by *Ipomoea eriocarpa* (branch E) shares the same seed shape and flat to concave periclinal cell wall, but differs in black and glabrous seeds; isodiametric, 5–6 gonal cells epidermal cell shape; straight to slightly sinuous, raised anticlinal cell wall (Figure 4).

In group A, *Ipomoea involucrata* corresponds to its previously recognised position within subgenus *Ipomoea* section *involucrate* (Van Ooststroom, 1953; Verdcourt, 1957, 1963; Austin, 1979, 1997; Austin and Huáman, 1996). Moreover, *I. cairica* has been treated previously as belonging to subgenus *Quamoclit* (Austin and Huáman, 1996), but Miller et al. (2004) treated it as an outgroup to the representative species from the subgenera *Quamoclit* and *Ipomoea*.

Miller et al. (2004) investigated 36 *Ipomoea* species from subgenera *Ipomoea* and *Quamoclit* using sequence data from the ITS region and did not establish support for these subgenera as distinct clades. Furthermore, species

from section *Pharbitis* (subgenus *Ipomoea*) were nested within species of subgenus *Quamoclit*. This result was shown previously by Miller et al. (1999) and with more samples from *Ipomoea* species for both ITS waxy sequence data. Wilkin (1999) also observed this same result based on morphological cladistics. McDonald and Mabry (1992) do not support these 2 subgenera as distinct clades. Furthermore, they identified 2 major clades within the *Quamoclit*. The first clade includes species of section *Mina* (Cerv.) Griseb. (*I. cristulata* and *I. quamocliti*) and species of section *Leptocallis* (G. Don) J.A.McDonald, and the second clade comprises species of section *Pharbitis* and others.

Abdel Khalik et al. (2012) found a close relationship between the *Ipomoea purpurea* of section *Pharbitis* and subgenus *Ipomoea* (Austin and Huáman, 1996) and species of the subgenus *Quamoclit*. Additional results derived from the molecular data of RAPD indicated that *I. cairica* should be considered a well-separated section that may be related to section *Orthipomoea* Choisy.

An interesting finding of this study is the close relationship of the *Ipomoea cairica*, which previously belonged to subgenus *Quamoclit* (Austin and Huáman, 1996) and was traditionally placed in different subgenera of *Quamoclit* and *Ipomoea* with other groups of species (*I. cristulata*, *I. involucrate*, and *I. quamocliti*). Seed morphology also supports the phylogenetic results of Abdel Khalik et al. (2012). Generally, these results agree with those of McDonald and Mabry (1992), Miller et al. (1999), and Abdel Khalik et al. (2012) regarding relationships among these species in an enlarged concept of subgenus *Quamoclit*.

#### 4.1.2. Subgenus *Eriospermum* (Hallier f.) Verdcourt ex Austin (groups B, C, and D)

Within the subgenus *Eriospermum*, 3 major clusters and branches with 0.82 similarities were identified. The first cluster (B) includes 2 species of section *Eriospermum* and *Erpipomoea*. The second clade includes section *Eriospermum* and one species from section *Erpipomoea*. These results mainly support the taxonomic system of the subgenus *Eriospermum* proposed by Verdcourt (1957, 1963), Austin (1979, 1997), and Austin and Huáman (1996) in their treatment of subgenus *Eriospermum*.

#### 4.1.3. Section *Eriospermum* Hallier f. (groups B and D)

Inside this cluster (group B), 2 species of section *Eriospermum* (*I. heterotricha* and *I. triloba*), 1 species of section *Erpipomoea* (*I. obscura*), and 2 species from subgenus *Quamoclit* (*I. sinensis* and *I. indica*) have been recognised with 0.82 morphological similarities. These species can be clearly defined on the basis of various features: ovoid seed, irregular to polygonal cells epidermal cell shape; undulate, raised, smooth to fine folded anticlinal boundaries; and flat to concave, smooth to fine folded periclinal cell wall.

An alternative branch of the species represented by *Ipomoea carnea* (branch D) shares the same seed shape and flat to concave periclinal cell wall, but differs in perennial woody tree habit; seed size (7–9 × 5–6.5 mm); long woolly hairs seed; 4–5 gonals, epidermal cell shape elongate in 1 direction; straight to slightly sinuous, raised anticlinal cell wall (Figure 2).

Within group B, *Ipomoea indica* corresponds to the previously recognised position within subgenus *Ipomoea* section *Pharbitis* (Van Ooststroom, 1953; Verdcourt, 1957, 1963; Austin, 1979, 1997; Austin and Huáman, 1996). However, McDonald (1991), Miller et al. (1999, 2004), and MacDonald et al. (2011) treated this species as subgenus *Quamoclit*, section *Pharbitis*.

McDonald and Mabry (1992) reclassified series *Batatas* (*I. triloba*) from subgenus *Quamoclit* to subgenus *Eriospermum* based on chloroplast DNA and RFLP.

Austin (1979, 1980) reported that sections comprising the woody and hairy-seeded species of subgenus *Eriospermum* hold together as a monophyletic group. However, McDonald and Mabry (1992) supposed that species of the same sections formed a polyphyletic group based on Dollo parsimony or a paraphyletic group on the basis of Wagner parsimony. Miller et al. (1999) suggested a close relationship of series *Batatas* (Choisy) D.F.Austin (*I. triloba*) and other species of section *Eriospermum*, and they classified the woody and hairy seeded species *I. carnea* and *I. arborescens* in a separate series [ser. *Jalapae* (House) D.F.Austin and *Arborescentes* Choisy]. They also suggested that the series *Jalapae* is not monophyletic. Abdel Khalik et al. (2012) showed that *Ipomoea carnea* and *I. heterotricha* are sister species of *I. triloba* and *I. stolonifera*, and they suggested that species of section *Eriospermum* form a monophyletic group and that there are close relationships between species of section *Eriospermum* and *I. stolonifera* (sect. *Erpipomoea* Choisy). Current observations in additional species confirmed the possibility that types or subtypes of seed coat can be diagnostic or indicative of phylogenetic relationships, and these results are in agreement with the phylogenetic results of McDonald and Mabry (1992), Miller et al. (1999), and MacDonald et al. (2011) and partially agree with Abdel Khalik et al. (2012), suggesting that species of section *Eriospermum* are not a monophyletic group.

#### 4.1.4. Section *Erpipomoea* Choisy (group C)

In this cluster (C), 3 species of section *Erpipomoea* (*I. imperti*, *I. pes-caprae*, and *I. stolonifera*) and 1 species (*I. purpurea*) of section *Pharbitis*, subgenus *Quamoclit* have been recognised with 0.85 morphological similarities. These species can be obviously defined on the basis of

various features: seed ovoid, black to brown, glabrous to short pubescence; irregular to polygonal cells epidermal cell shape; straight to slightly sinuous, raised, smooth to fine folded anticlinal boundaries; and flat to concave periclinal cell walls.

Within group C, *Ipomoea purpurea* corresponds to its previously recognised position within subgenus *Ipomoea* and section *Pharbitis* (Verdcourt, 1957, 1963; Austin, 1979, 1997; Austin and Huáman, 1996). However, McDonald (1991), Miller et al. (1999, 2004), and MacDonald et al. (2011) treated this species as subgenus *Quamoclit*, section *Pharbitis*.

Das and Mukherjee (1997) studied seedling morphology and the isozyme profile of 12 species of *Ipomoea*, and they revealed 2 broad clusters: the first group includes *I. obscura* and the second includes *I. pes-caprae*.

Moreover, Miller et al. (1999) found that section *Erpipomoea* is clearly not monophyletic, as they showed species from this section scattered within several well-supported clades from section *Eriospermum* and sections of subgenus *Quamoclit*. Additionally, Abdel Khalik et al. (2012) suggested that section *Erpipomoea* is not a monophyletic group.

Our results do not support the monophyly of section *Erpipomoea* of subgenus *Eriospermum*. This is due to the placement of *I. obscura*, *I. heterotricha*, *I. triloba*, *I. sinensis*, and *I. indica* within a separate subgroup with 0.82 genetic similarities and the rest of the species of section *Erpipomoea* with *I. purpurea* (subgenus *Quamoclit* sect. *Pharbitis*) within another cluster.

Our results are congruent with those of the above-mentioned authors' phylogenetic and isozyme study results (Das and Mukherjee, 1997; Miller et al., 1999; Abdel Khalik et al., 2012), which suggests that section *Erpipomoea* is not a monophyletic group.

## 5. Conclusions

The sculpture of seed coats offers a set of characters useful for the taxonomy of the genus. Earlier descriptions of *Ipomoea* seed types were mostly based on a single character, whereas in the present study several characters of seed microsculpture were used. The present study proves that seeds of *Ipomoea* display high diversity in shape, colour, size, surface, epidermal cell characters, anticlinal cell wall boundaries, and periclinal cell wall, and some species even have specialised structures. Seed coat morphology also provides some evidence for infrageneric classification and partly corresponds with the phylogenetic results of McDonald and Mabry (1992), Miller et al. (1999), McDonald et al. (2011), and Abdel Khalik et al. (2012). Current results do not support the monophyly of either section *Erpipomoea* or *Eriospermum*, as suggested by Austin (1979, 1980, 1997) and Austin and Huáman (1996).

Finally, seed coat analysis confirms that developmental variation in seed characters is taxonomically useful, not only because it gives us a better understanding of sculpture development but also because it allows us to formulate the taxonomy of *Ipomoea* on both the subgenera and sectional levels, and it is useful for construction of an identification key.

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## Appendix

List of characters and character states used in morphometric analysis of the genus *Ipomoea*.

**Seed characters**

**1.** Seed shape
   1. Elongate to pear-shaped
   2. Ovoid to subglobose
   3. Ovoid
   4. Broadly ovoid

**2.** Seed surface
   1. Glabrous
   2. Glabrous to pubescent
   3. Pubescent
   4. Densely pubescent
   5. Pubescent with tufts of long silky hairs along the margins
   6. Long woolly hairs

**3.** Seed size (mm) (length × width)
   1. 1.6–4 × 1.1–3
   2. 4–5 × 3–4
   3. 7–10 × 5–8

**4.** Seed colour
   1. Black

2. Black to brown
3. Brown
4. Yellow to brown

5. Epidermal cell patterns
   1. Isodiametric or 5–6 polygonal
   2. 4–5 gonal cells or elongate in 1 direction
   3. Irregular to polygonal cells
6. Anticlinal walls
   1. Straight to slightly sinuous
   2. Undulate
7. Relief of cell wall boundaries
   1. Slightly raised
   2. Raised
   3. Raised-channelled
8. Sculpture of anticlinal boundaries
   1. Smooth
   2. Smooth to finely folded
   3. Folded
9. Curvature of outer periclinal cell wall
   1. Flat to concave
   2. Flat to convex
10. Secondary cell wall sculpture
   1. Smooth
   2. Smooth to finely folded
   3. Folded
   4. Microreticulate
   5. Reticulate

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