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A Scanning Electron Microscopy Study of the Seed and Post-seminal Development in *Angelonia salicariifolia* Bonpl. (Scrophulariaceae)

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The external morphology of seeds and post-germination developmental stages of *Angelonia salicariifolia* Bonpl. (Scrophulariaceae) were investigated using scanning electron microscopy. Some structural features of the seed exotesta and seedling in *Angelonia* are presented for the first time and are of potential taxonomic value for this neotropical genus. The seeds are very small (0·9–1·7 mm long and 0·5–0·9 mm wide), ovate, with a reticulate-crested exotesta, reticules arranged uniformly in longitudinal rows, with a high density of microcilia-like projections on the cell wall of the reticule base and on the edge of the crests. The hilum is located beside the micropyle at the narrow end of the seed. Germination is epigeal. During germination the radicle develops, followed by elongation of the hypocotyl and primary root. At this stage dense root hairs develop on the lower part of the hypocotyl. The apical bud—located between the cotyledons—begins to develop after the cotyledons have unfolded. The cotyledons are equal in size, sessile and ovate. The seedlings have two types of trichomes, one characteristic of the cotyledons and first pair of leaves (glandular, sessile, four-celled head with quadrangular shape) and the other characteristic of the hypocotyl and epicotyl (stalked, erect, elongate and three-celled with dome-shaped unicellular head).

Key words: Seed morphology, exotesta-ornamentation, micromorphology, post-seminal stages, seedling, trichomes, SEM, *Angelonia salicariifolia* Bonpl., Scrophulariaceae.

INTRODUCTION

In Brazil, many genera in the family Scrophulariaceae show great ornamental potential. Of these, *Angelonia salicariifolia* Bonpl. stands out for its ornamental characteristics and potentialities (Souza, 1996). Corrêa (1926) commented on the ornamental and medicinal uses of this species.

Angelonia Bonpl. is a neotropical genus, comprising about 27 species (D'Arcy, 1979). Barringer (1983) classified *Angelonia* as belonging to the tribe *Angelonieae* Pennell along with *Monopera* Barringer and, later, also with *Basistemon* Turcz (Barringer, 1985). The Scrophulariaceae are represented by 32 genera and 143 species in Brazil (Souza, 1996). Of these genera, *Angelonia* has the second highest number of species in Brazil (18), occurring in open areas of 'caatinga', 'cerrado' or 'campo-rupestre' habitats. Caatinga is characterized as an open xerophyte vegetation, adapted to hot semi-arid climates, with an annual mean temperature of 24–29 °C and an annual mean rainfall between 280 and 750 mm. The cerrado can be described as a shrubby and wooded savanna, with a tropical humid climate and two pluviometric seasons, with a total annual mean rainfall between 750 and 2000 mm, while campo rupestre is an open savanna-like vegetation found on stony or sub-stony areas, common at the top of quartzitic mountains where the soil is shallow (Watanabe, 1997).

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A. salicariifolia, popularly known as 'malva-de-lagoa' or 'angelonia', shows a geographically disjunct distribution in the neotropical region, with three recognized centres of occurrence: the Antilles, Colombia, Venezuela and the Guianas; the Brazilian northeast Caatinga, extending along part of Bahia to the Rio de Janeiro coastland; and the South of Mato Grosso, Paraguay and Argentina (Souza, 1996). The species is herbaceous, 25–70 cm tall, erect to suberect, simple or branched, with opposite, simple, sessile, lanceolate and serrate leaves with an acute apex. The inflorescence is a terminal raceme, 10–30 cm long. The corolla is magenta, violet, blue, rose or lilac, rarely white; the fruit is a globose capsule with rounded apex, and is 0·4–0·7 cm in diameter (Souza, 1996).

Scanning electron microscopy (SEM) has been particularly useful in studying seed coat microsculpturing in the Scrophulariaceae due to the wide variation in seed coat structure and the small size of the seeds (Chuang and Heckard, 1972; Canne, 1979, 1980; Vijučić *et al.*, 1993; Juan *et al.*, 1994). The wide variety of seed shape, size and testal pattern of the Scrophulariaceae offers much systematic and evolutionary information (Elisens and Tomb, 1983). Thus studies of seed morphology are of taxonomic value, providing information that facilitates identification of species and genera.

Morphological studies of post-germination developmental stages and seedlings also allow species identification at different stages of development under field conditions, facilitating differentiation between very similar taxonomic

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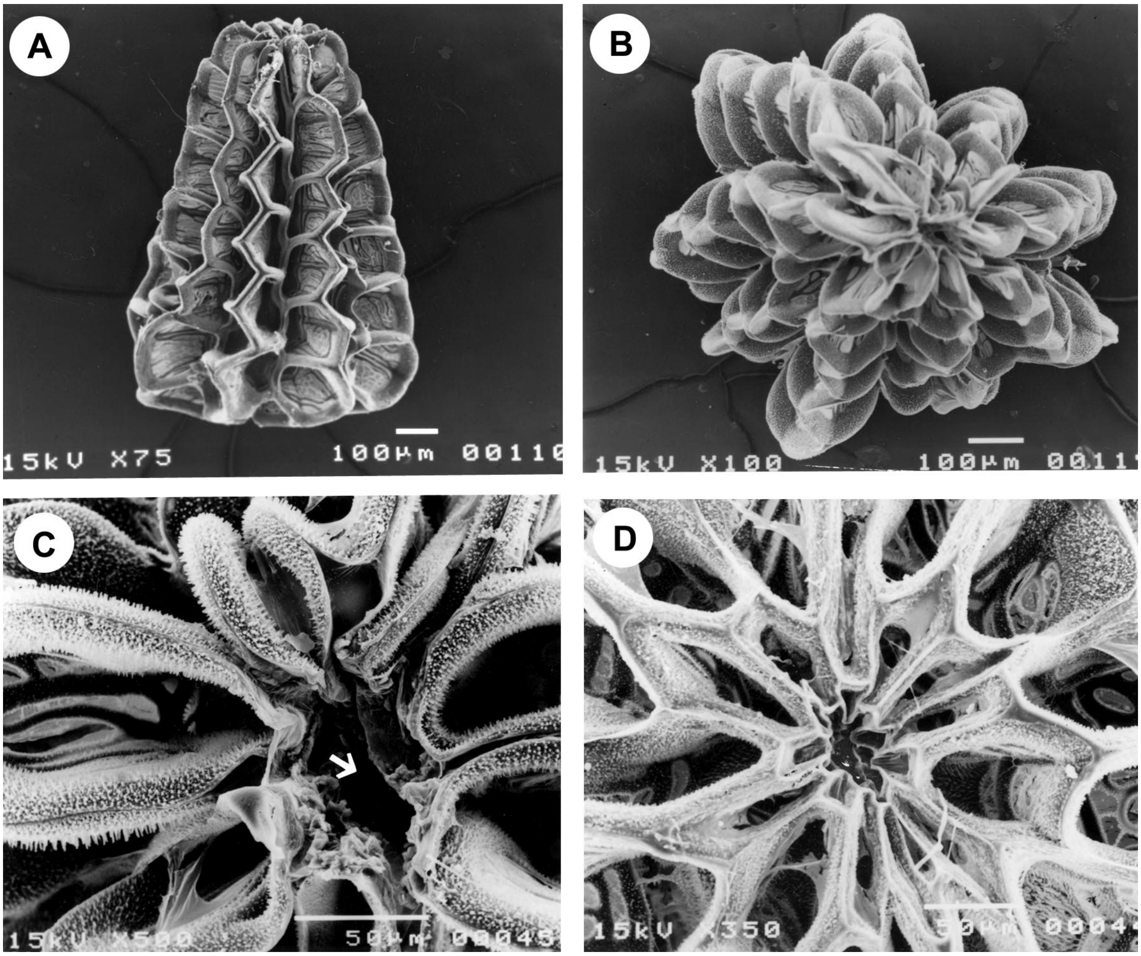

FIG. 1. Scanning electron micrographs of the whole seed of *Angelonia salicariifolia*. A, Longitudinal view (×75); B, view of the micropylar region (×100); C, detail of the micropyle (arrow) and dense microcilia in the cell wall of the reticule (×500); D, detail of the seed base (×350), bar = 50 µm.

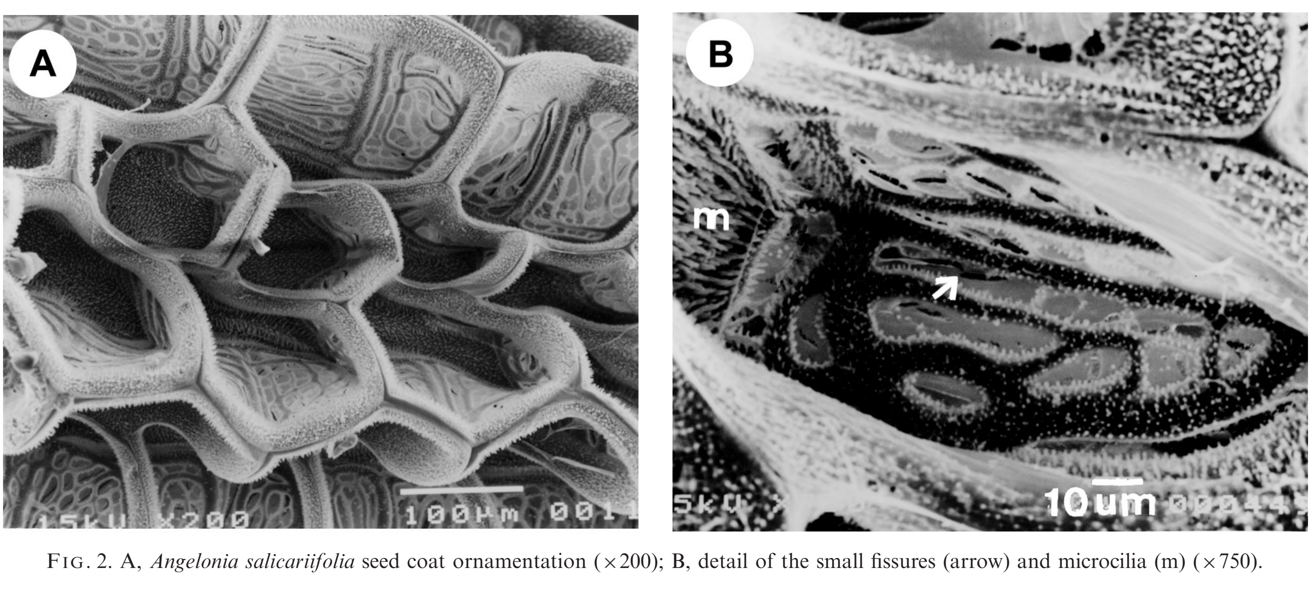

FIG. 2. A, *Angelonia salicariifolia* seed coat ornamentation (×200); B, detail of the small fissures (arrow) and microcilia (m) (×750).

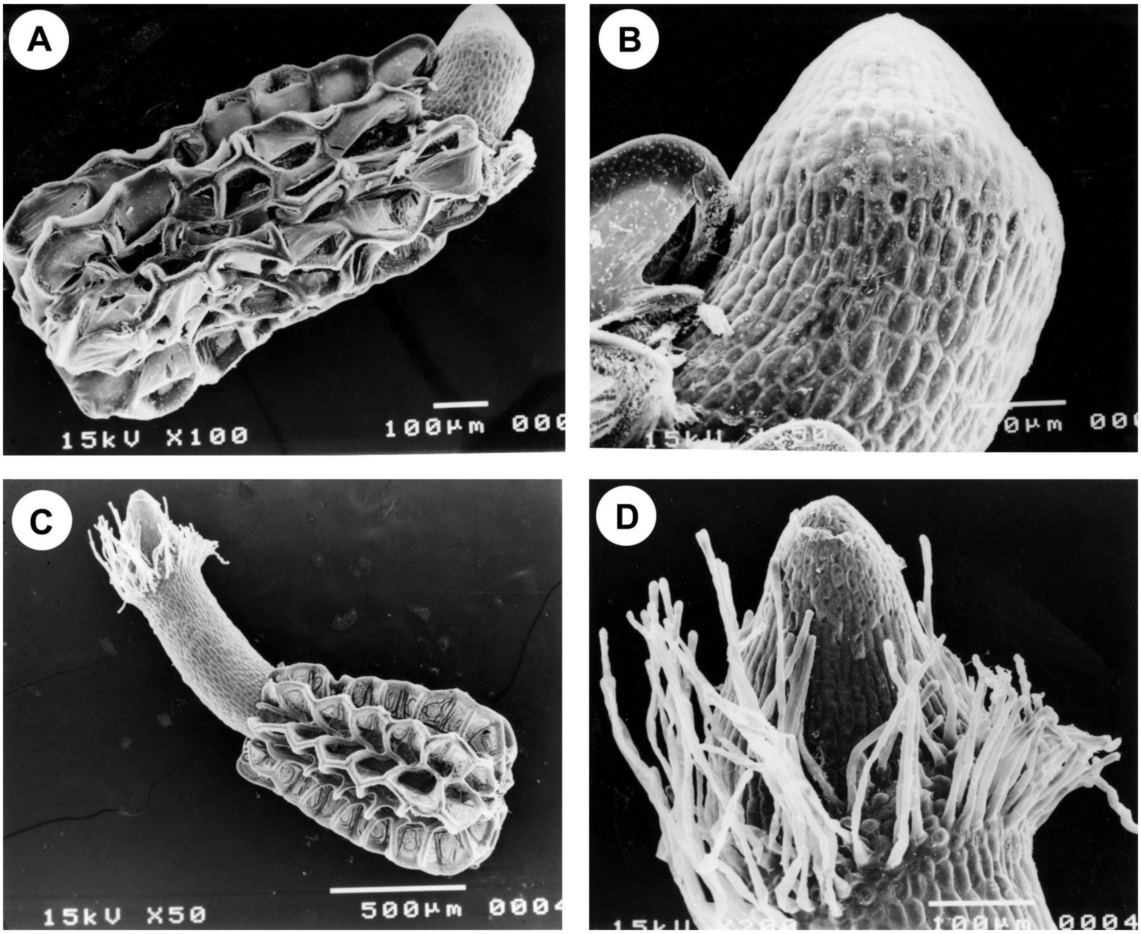

FIG. 3. Post-germination developmental stages of *Angelonia salicariifolia*. A, Radicle emergence through the micropyle (third to fourth day after seed imbibition) (×100); B, detail of the radicle and basal hypocotyl (×350), bar = 50 μm; C, hypocotyl elongation and initial development of root hairs (fifth day after seed imbibition) (×50); D, detail of the basal disc with root hairs (×200), bar = 100 μm.

groups. Several recent taxonomic studies in other families have shown that seedling morphology can be used to delimit genera, species and even varieties (Canne, 1983). In addition, morphological studies of seed and post-germination developmental stages aid the development of adequate cultivation techniques, complement taxonomic and ecological studies and also provide information about dispersal methods, thus increasing knowledge of the reproductive biology of the species.

To date, SEM techniques have not been used to investigate seed and seedling morphology in *Angelonia*. Thus, this work was undertaken to study the external morphology of mature seeds and post-germination developmental stages.

MATERIALS AND METHODS

Seeds were collected in September 1999 from dehiscent fruits of plants of *A. salicariifolia* cultivated in the municipality of Jaboticabal (21°15′22″ S, 48°15′18″ W, altitude of 610 m), São Paulo State, Brazil. The local climate is Cwa, following Köppen classification, that is subtropical humid, having a distinct dry winter season and a rainy summer, with the temperature of the hottest month above 22°C (Volpe *et al*., 1989).

Biometrical data of mature seeds were obtained using a stereoscope with a micrometre graticule. Valves of mean seed length and width at the widest point were based on the measurement of 110 seeds.

To obtain different post-germination developmental stages, recently collected seeds were germinated in four plastic boxes (11 × 11 cm) on a layer of filter paper moistened with 7 ml distilled water. Boxes were kept in a germination cabinet with continuous light at a constant temperature of 25 °C; these conditions have previously been found to promote adequate seed germination (Pinto *et al*., 1999, 2000). Twenty-five seeds were placed in each box and observed daily to obtain the different post-germination developmental stages. As each different stage was reached, the material was sampled and placed in a glass flask with 3 % glutaraldehyde in 0·05 M potassium phosphate buffer solution, closed immediately, wrapped in plastic film and stored at 10 °C.

The observed seed and post-germination developmental stages were pretreated in one of two ways. Dried seeds were

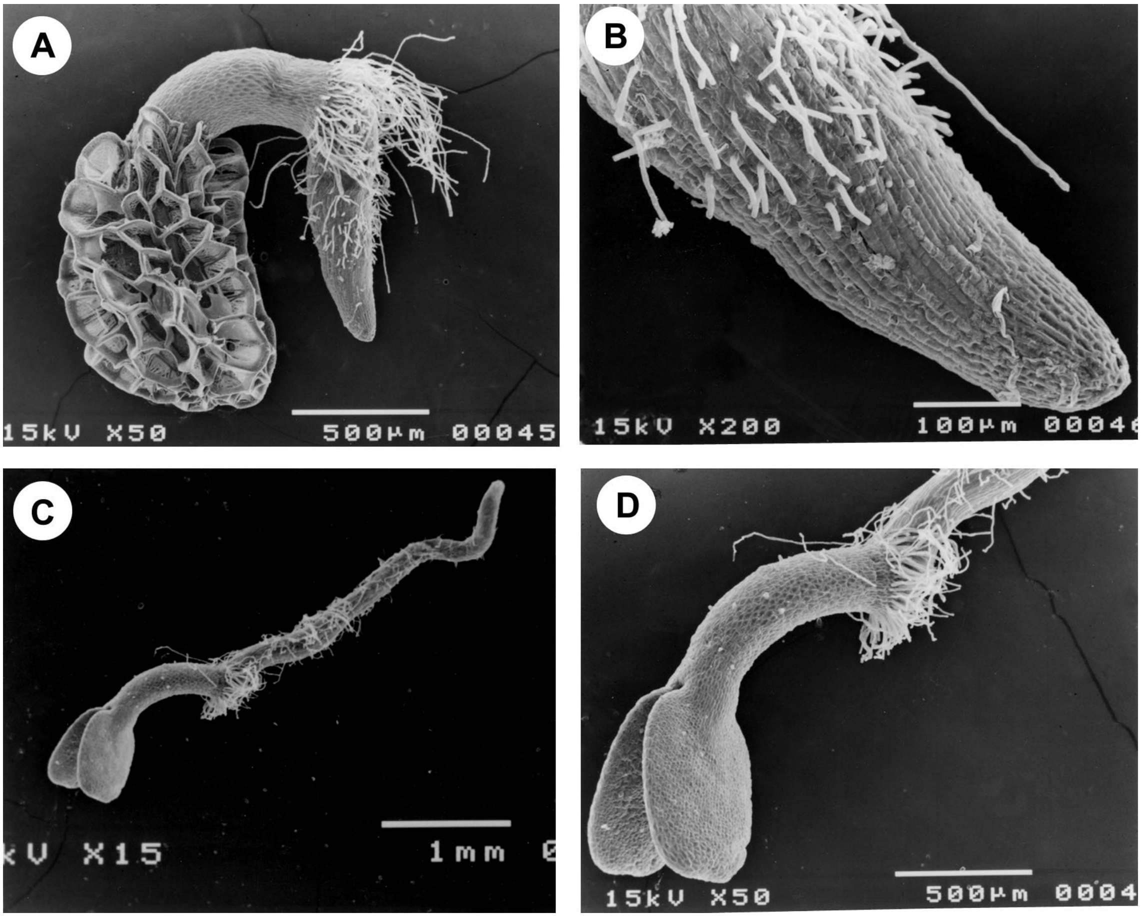

FIG. 4. Post-germination developmental stages of *Angelonia salicariifolia*. A, Elongation of the primary root and the development of the root hairs (×50); B, detail of the root apex (×200); C, seedlings with cotyledons starting to unfold at the sixth day after imbibition (×15); D, detail of the cotyledons starting to unfold (×50).

mounted directly on double-sided adhesive tape affixed to SEM stubs. Post-germination stages were fixed in 3 % glutaraldehyde in 0·05 M potassium phosphate buffer, washed in the same buffer, then post-fixed in 2 % osmium textroxide in the same buffer for 26 h. After rinsing in buffer, they were dehydrated in a graded ethanol series, critical point dried in CO_2 (Balzers EMS-850), and mounted on sticky tape affixed to aluminium stubs.

Mounted specimens were coated with gold in a sputter coater (Denton Vacuum Desk II) and examined using a scanning electron microscope (Jeol JMS-5410) operating at 15 kV.

Seed germination type was established by germinating 100 seeds in four boxes filled with sand and kept in a germination cabinet under the same conditions. Terminology for seed and post-seminal developmental stages follows Corner (1976) and Damião (1993).

Representative voucher specimens (seeds and seedling stages) are held in the Herbarium of the Departamento Biologia Aplicada, FACV/UNESP, Jaboticabal, São Paulo, Brazil.

RESULTS

Morphometric data and SEM micrographs showed that mature dry seeds of *A. salicariifolia* are small and range in length from 0·9–1·7 mm and in width from 0·5–0·9 mm. As seen under the stereomicroscope, seeds have a bright sandy colour when dry and are hyaline when soaked in water, making the embryo partially visible through the testa. When the seed is dry the testa has a firm consistency, but after soaking in water for approx. 15 s it acquires a smooth consistency. The seed shape is ovoid (length/width = 1·44) and the embryo is positioned longitudinally (Fig. 1A, B). The seed is exalbuminous and the hilum is located beside the micropyle at the narrow end of the seed. At each end of the seed there is a hole where the exotesta sculpturing meets (Fig. 1C, D); this leads to the micropyle and hilum at the narrow end of the seed (Fig. 1C).

Electro micrographs revealed details of a reticulate-crested type of testa ornamentation not reported previously for the genus *Angelonia* (Fig. 2A and B). The reticulate aspect is formed by hyaline-walled cells arranged uniformly

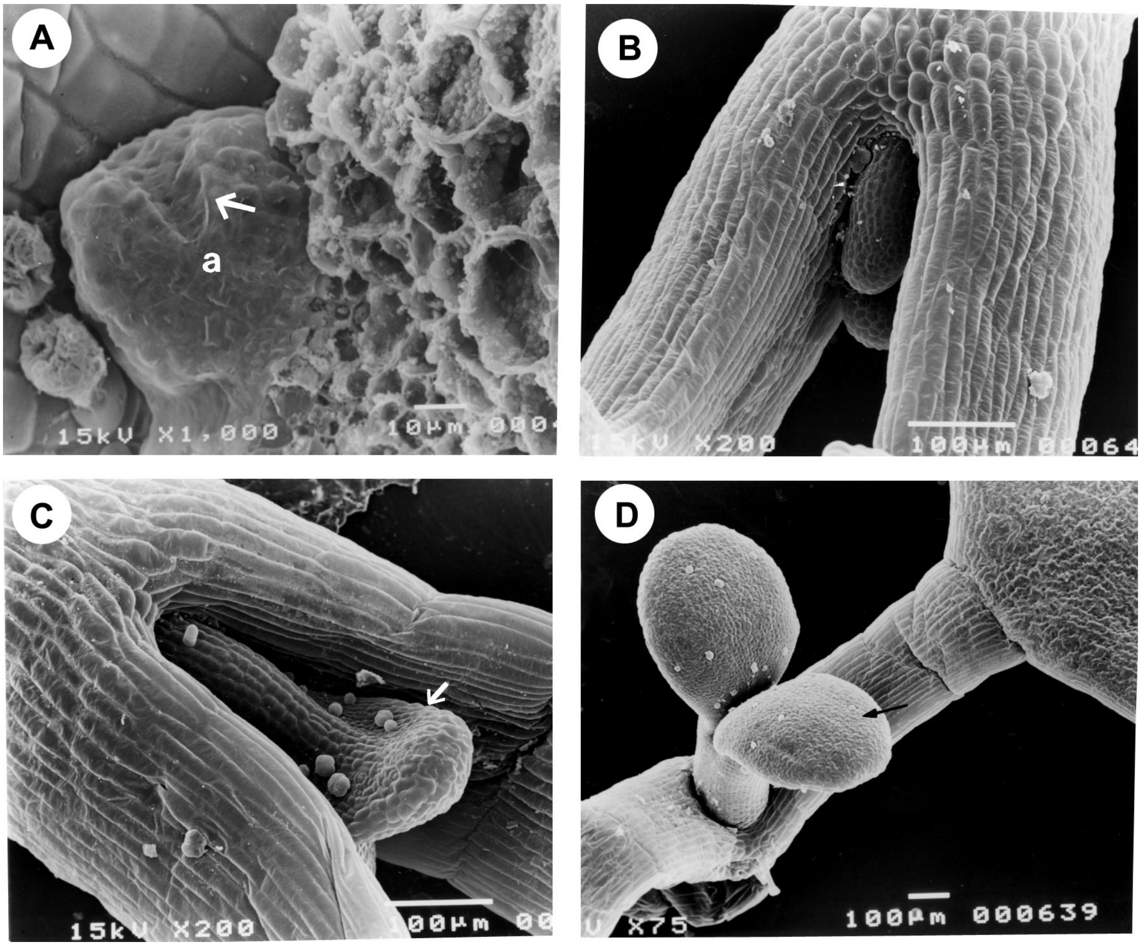

FIG. 5. Post germination developmental stages of *Angelonia salicariifolia* on the seventh day after inbibition. A, Apical bud (a) and leaf primordia (arrow) between the cotyledons (×1000); B, bud elongation (×200); C, epicotyl and the first pair of true leaves (arrow) (×200); D, first pair of true leaves (arrow) (×75).

in longitudinal rows. The hyaline walls of the cells have small fissures (Fig. 2B). The cell walls of the reticule base and of the edge of the crests have small projections which are relatively short and numerous. These projections are probably hairs and are referred to as microcilia (Fig. 2B).

The sequence of post-germination developmental stages, from emergence of the hypocotyl and radicle to the first pair of true leaves, is shown in Figs 3–5. Radicle emergence through the micropyle begins between the third and the fourth day after seed imbibition (Fig. 3A and 3B). On the fourth and fifth days, the hypocotyl elongates and a basal disc is formed in the region between the hypocotyl and radicle; root hairs develop on it (Fig. 3C and D). The hypocotyl keeps growing and the primary root elongates; dense root hairs form on the lower part of the hypocotyl (Fig. 4A and B). Between days 5 and 6, the cotyledons break out of the integument and start to unfold (Fig. 4C and D). The cotyledons open completely and, on the seventh day, the apical bud develops between the cotyledonary leaves (Fig. 5A and B). This bud elongates and originates the epicotyl and the first pair of true leaves (Fig. 5C and D).

During seed germination, the hypocotyl grows quickly and the cotyledons become elevated above the level of the substrate, thus germination is epigeal. The two cotyledons are sessile and ovate, being approximately 405 µm wide and 667 µm long; they are of equal size. Stomata and only one type of trichome are present on the abaxial and adaxial surfaces of the cotyledonary leaves (Fig. 6A). The stomata are dispersed randomly over the whole cotyledon and are situated at the same level in relation to the other epidermal cells (Fig. 6A–C). They are of the paracytic type (Metcalf and Chalk, 1950) and the guard cells and subsidiary cells are kidney shaped (Fig. 6B and C). Trichomes present on the cotyledons are glandular and sessile, with a four-celled head which is quadrangular in shape (Fig. 6D).

The hypocotyl is approximately 550 µm long and cells on its surface are arranged in a regular reticulate pattern (Fig. 7A). Stomata and trichomes occur on the seedling hypocotyl (Fig. 7A and B). Again, the trichomes are of only one type, although in this case they are stalked, erect, elongate and three-celled with a dome-shaped unicellular head (Fig. 7B and C).

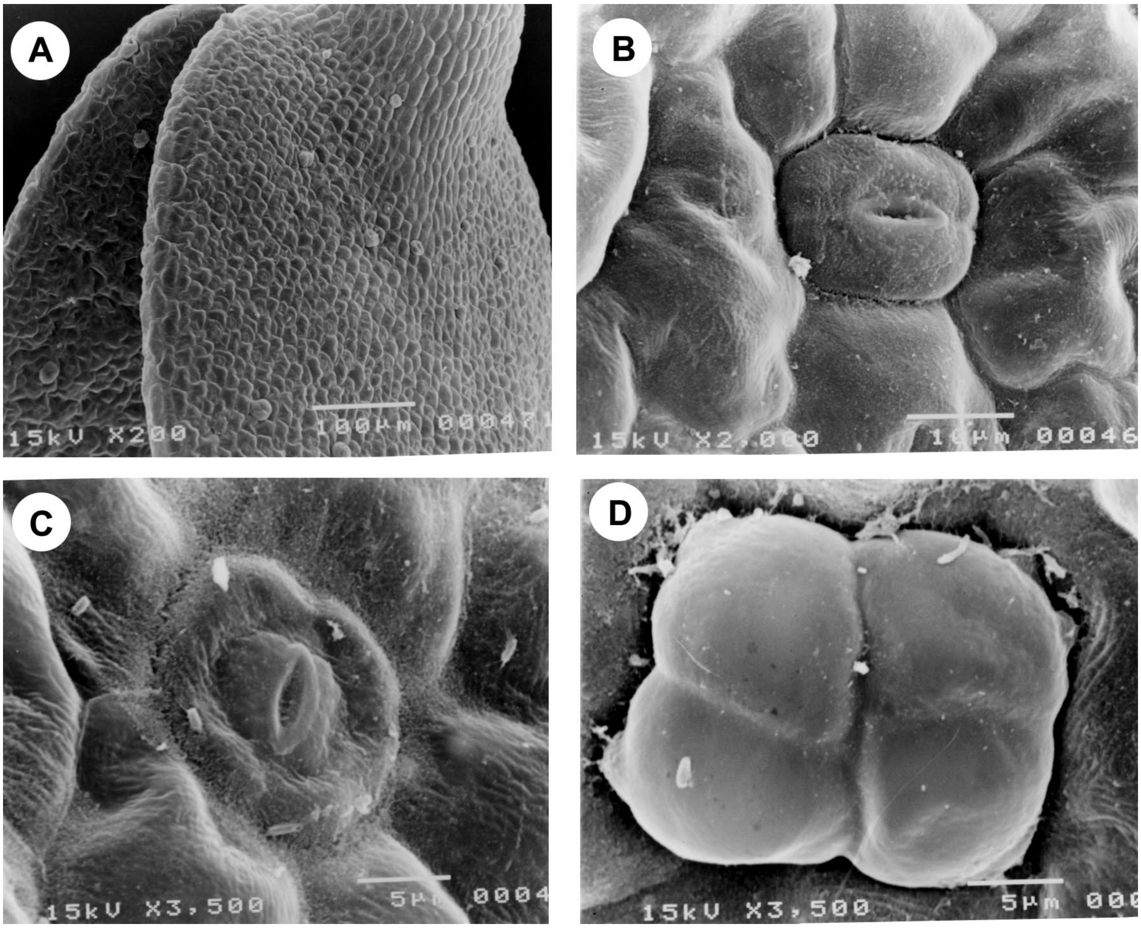

FIG. 6. A, Trichomes and stomata on the abaxial and adaxial surfaces of the cotyledons of *Angelonia salicariifolia* (×200); B, stoma on the cotyledon adaxial surface (×2000); C, stoma on cotyledon abaxial surface (×3500); D, cotyledonary trichome with four-celled sessile head (×3500).

The epicotyl also has trichomes of the type seen on the hypocotyl (Fig. 7D), whilst the first pair of true leaves has trichomes like those seen on the cotyledonary leaves (Fig. 7D).

DISCUSSION

According to Ichaso (1978), seeds are a good taxonomic feature by which to distinguish between different genera—and some species—of Scrophulariaceae. This author established and described 17 seed types, based on seed shape and testa structure. Later, Ichaso (1980) reported that in the Scrophulariaceae, seed characteristics are a taxonomically robust character and can be used to separate the Scrophulariaceae from the Solanaceae and Acanthaceae. According to Ichaso (1980), *Angelonia* is distinguished from other genera of Brazilian Scrophulariaceae by its relatively large seeds of the reticulate-crested type. The seed size observed in this study was in the range found by Ichaso (1980) for *Angelonia* (over 1·0 mm); this species is grouped among the genera that have large seeds (*Alectra* Thunb., *Gerardia* L., *Escobedia* Ruiz et Pav., *Physocalyx* Pohl, *Maurandya* Ort., *Antirrhinum* L. and *Nothochilus* Radlk.).

Seed colour is another useful character helping to differentiate between *Angelonia* and the genus *Esterhazya* Mikan. Both these genera have seeds of the reticulate-crested type, but in *Esterhazya* the seeds are totally black and opaque, making it impossible to see the embryo. In *Angelonia*, however, the seed reticulum is formed by hyaline cell walls allowing a partial view of the embryo as shown here and by Ichaso (1980).

Seed shape varies sufficiently in the genus *Angelonia* to allow a distinction to be made between species (Ichaso, 1980). Seeds of *A. salicariifolia* differ in shape and exotesta structure from those of the *Angelonia* species studied by Ichaso (1980). Thus, there are species with a pyramidal inverted shape and well developed crests such as *A. goyazenzis* and *A. campestris* with perpendicular and oblique crests. *A. pubescens* has an obovoid shape and walls leaning to the base, whilst in *A. cornigera* the crests stoop to the seed apex and base. The morphological similarities observed between seeds of *A. gardneri* (Ichaso, 1980) and *A. salicariiforlia* (present study) could be explained by the fact that *A. gardneri* is included in the synonymy of *A. salicariifolia* by Souza (1996). *A. gardneri* belongs to the group of *Angelonia* species that have the largest seeds,

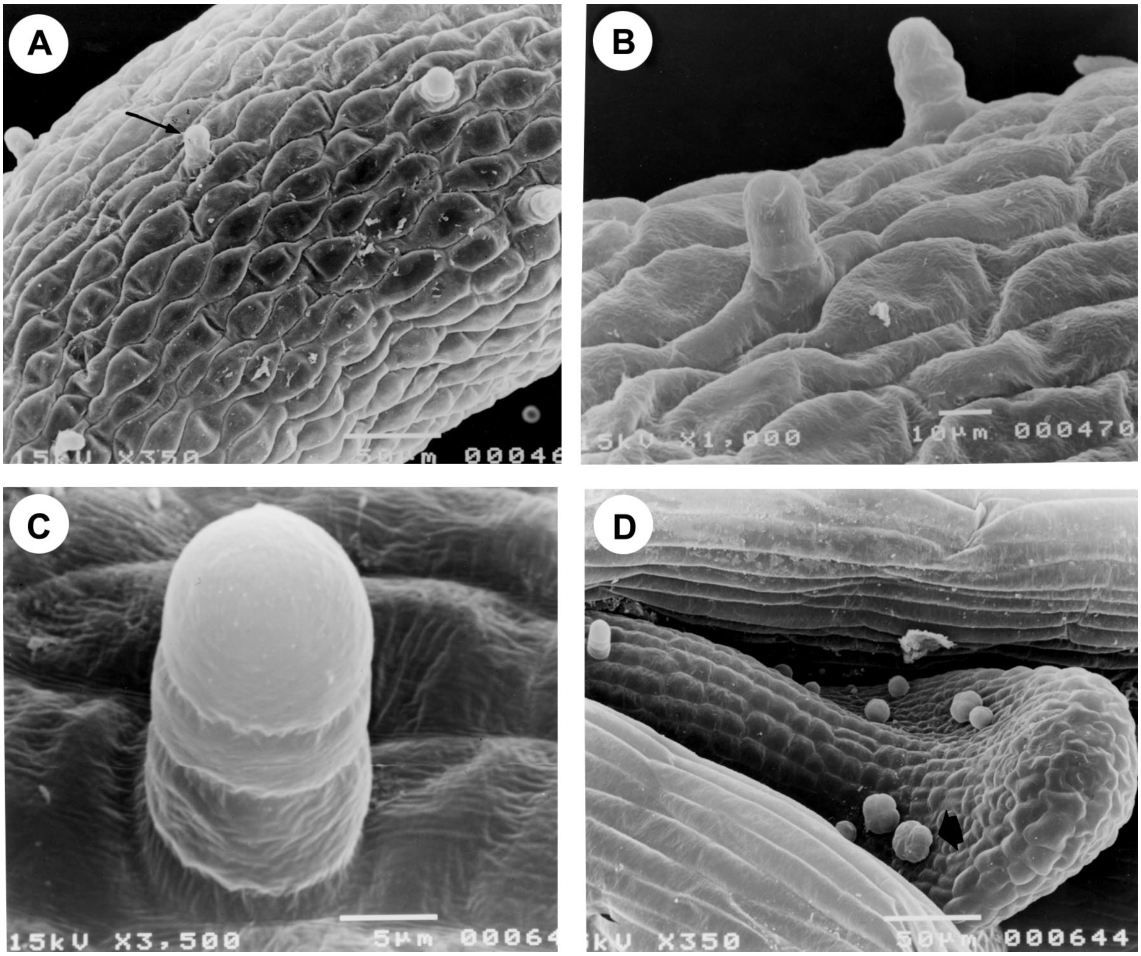

FIG. 7. Trichomes of hypocotyl, epicotyl and primary leaves of *Angelonia salicariifolia*. A, Trichomes on hypocotyl surface (arrow) (×350), bar = 50 µm; B, detail of hypocotyl three-celled, dome-shaped trichomes with unicellular head (×1000); C, epicotyl three-celled, dome-shaped trichome with unicellular head (×3500); D, primary leaf sessile trichome with four-celled head (arrow) (×350).

exceeding 2·0 mm (Ichaso, 1980). In the present study, seed size was less than 2·0 mm; this may be due to the environmental conditions during seed formation or because seed size in Scrophulariaceae is influenced by their packing in the capsule (Elisens and Tomb, 1983).

The seed exotesta ornamentation found in *A. salicariifolia* confirms previous descriptions for *Angelonia* (Barroso, 1952; Thieret, 1954; Ichaso, 1978, 1980; Barroso *et al*., 1999). However, the projections on the cell walls of the seed exotesta reticulum, referred to as microcilia, are a newly discovered character that could be of potential taxonomic value following further SEM studies of other species of *Angelonia* and other genera of the Scrophulariaceae.

The small fissures observed in the hyaline walls of the cell that give the reticulate aspect may help to ease the passage of air streams and thus assist in seed dispersal. Many seeds of the Scrophulariaceae are adapted to anemochory (Ichaso, 1980; Elisens and Tomb, 1983).

The absence of endosperm in seeds of *A. salicariifolia* is a tribal character that unites *Angelonia*, *Basistemon* Turcz. and *Monopera* Barringer in the tribe *Angeloniea* (Barringer, 1985).

SEM observations of seedlings of *A. salicariifolia* revealed the presence of different types of trichomes on the cotyledons, hypocotyl and epicotyl. The trichomes may have a taxonomic significance, which could be confirmed by future studies on the seedlings of more genera and species of Scrophulariaceae. Studies of seedling morphology of 18 species of *Agalinis* Raf. (Scrophulariaceae) (Canne, 1983) revealed that three major trichome types occur repeatedly among numerous species, while other hair types are restricted to one or a few taxa. Canne (1983) verified that the presence or absence of hairs on the hypoctyl and types of trichomes on the adaxial surface of the cotyledons as well as variation in the shape and size of cotyledons, is often distinctive at the species level. The four-celled dome-shaped sessile trichomes observed on the cotyledons of *A. salicariifolia* are similar to the quadrangular trichomes observed on the cotyledons of all species of *Agalinis* investigated by Canne (1983). It is interesting to find similar trichomes in two rather distantly related genera of Scrophulariaceae.

It is important that this study is expanded to more than one population of *A. salicariifolia*, taken from different parts of its disjunct distribution, and also to other species of *Angelonia* to establish the amount of intra- and inter-specific variation in seed and seedling characters and to increase understanding of these characters.

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