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SEEDLINGS OF *LAXMANNIA GRACILIS* R.BR. (ANTHERICACEAE): FORMS AND EVOLUTIONARY VALUE

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ABSTRACT

Laxmannia gracilis *R.Br. germinates two types of seedlings, type A and type A1. The latter has permanently folded cotyledon, "a hair-pin-like structure", and a higher occurrence than the former. The vascular system of the said structure indicates an evolutionary trend of seedling form from type A to type A1 while those of the roots are not specificantly different.*

INTRODUCTION

Criteria other than floral and gross morphological characters are increasingly being employed in the evaluation of the taxonomy and phylogeny of plant taxa.[1-3] The morphological and anatomical study of seedlings forms a part of this evaluation.

Laxmannia gracilis R.Br. traditionally belongs in the family Liliaceae[4] but has been moved into the family Anthericaceae by Dahlgren and Clifford[5] and Dahlgren *et al.*[6] This species is restricted to eastern Australia while the other twelve members of the genus are endemic to central and south-western Australia.[7] It is commonly found in eucalypt open forest on poor sandy soils and clay loam. As a herb *Laxmannia* is unique in that it relies on its seeds to regenerate the population after a fire.[7] Seedlings of *L. gracilis* can be found easily in the wild but no morphological and anatomical work has been reported on either this species or others in the genus. This investigation is concerned with the gross morphology and anatomy of the seedlings of *L. gracilis.*

MATERIALS AND METHODS

Seeds of *Laxmannia gracilis* were collected from CSIRO Scientific Area No1, Beerwah (26° 51′ 30″ S, 152° 59′ 30″ E), Queensland. The seeds were soaked in deionized water for two hours then sown in pots with vermiculite. Seedlings were raised in a controlled temperature room (22°C) with a 12 hours light/12 hours darkness cycle. The gross morphology and germination patterns were noted.

Seedlings one to three weeks old were fixed in 70% FAA (Formalin-Acetic acid-Alcohol) for one week before being dehydrated in a tertiary butanol series and embedded in paraffin wax m.p. 58°C. Longitudinal and transverse 10 μm sections were stained with Safranin 0 and Fast Green.[8]

RESULTS

Morphology

Laxmannia gracilis seeds are 15-18 mm long, proximo-distal compressed, black, without strophiole (Fig.1A), and bear regular lobes on the testa (Fig.1B). Germination occurred 15-20 days or more after soaking. This species has two types of seedlings, both of which have epigeal phanerocotylar germination (Fig.2). The first is seedling type A[5] which has a green cylindrical cotyledon with the plumular leaves emerging through a pore in the sheathing base. The base of the cotyledon remains at soil level but the testa is raised above the soil when the cotyledon elongates (Fig.2A). At about two weeks of age, the cotyledon elongates until it is approximately 7 mm long and then provision is made for the emergence of the first leaf by a cotyledon slit or pore (Fig.2D).

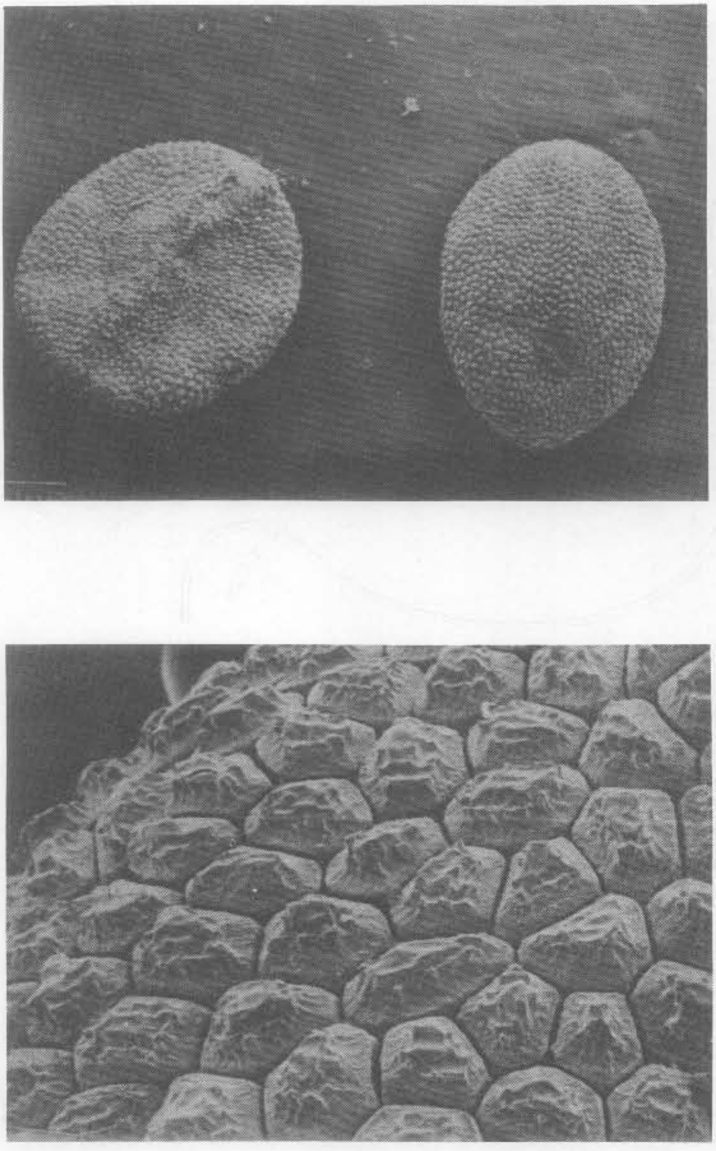
The second and new type of seedling, which is here called type A1 (Fig.2B-3), is similar to type A but has a cotyledon with a hair-pin-like structure which was wrongly described as a part of the root of *Dianella* seedling.[9] This structure develops until it is ca. 5-10 mm long at two weeks, then the first plumular leaf emerges in the same way as in type A. The tip of the cotyledon still connects to the testa and absorbs reserved food from it while the base of the cotyledon forms a short tube covering the first plumular leaf and the plumular bud. That tube is different from the ligule in ligulate seedlings because it does not have an apical pore.

The first leaves of both seedling types are similar, i.e. almost cylindrical with a ligulate base. The primary root sometimes develops a hair tuft at its proximal end but this is an uncertain character for distinguishing the types of seedlings. About ninety percent of seedlings are of the new type with the hair-pin-like structure (Fig.2D-E), whilst only five percent or less are type A (Fig.2A). The remainder are intermediate between these two forms (Fig.2B-C).

Anatomy

The photosynthetic organs of both seedling types are the cotyledon and the leaf. Transverse sections of these organs show epidermal cells that are almost isodiametric or square in shape, with a lightly cutinized outer wall. The mesophyll is slightly differentiated into palisade and spongy forms.

The type A seedlings are similar to some other species of Anthericaceae in having a double vascular bundle which consists of two collateral vascular bundles lying close together in an area bounded by bundle-sheath-like cortical parenchyma (Fig.3A). This double vascular bundle extends from the tip of the cotyledon to its base. Here it fuses to the plumular trace which is still not well differentiated in the two-week-old seedling, so the xylem of the cotyledon appears to be embedded in plumular meristematic tissue. The root stele is diarch (Fig.3B-C).



A

B

Fig. 1 SEM micrographs of *Laxmannia gracilis* seed. A: Whole seed (× 24), B: Higher magnification of the testa surface in A. (× 300).

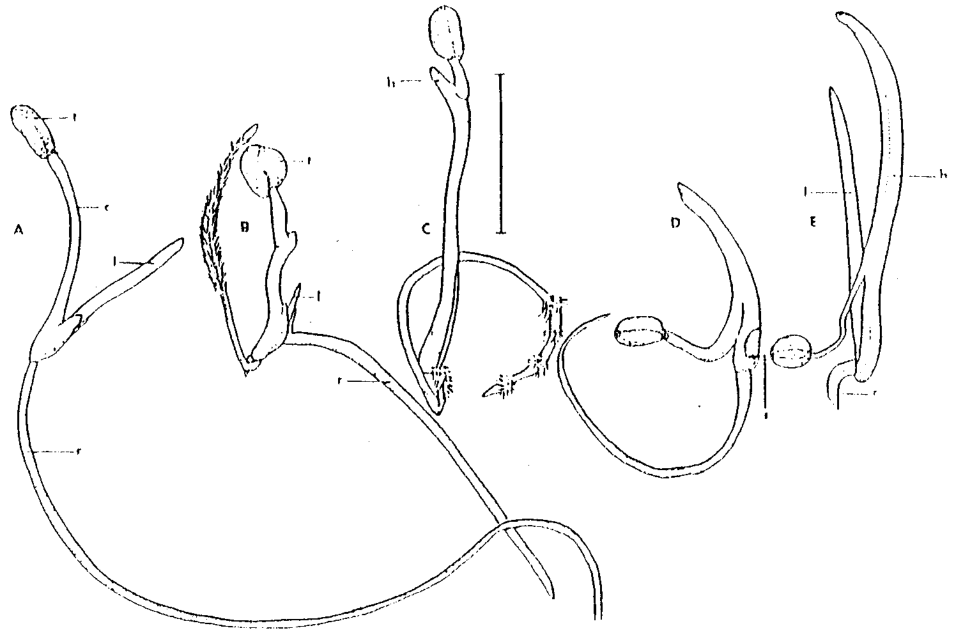


Fig. 2 *Laxmannia gracilis* seedlings. A: seedling type A; B-E: seedlings type A1. (all drawn from living materials) (c = cotyledon, h = hair-pin-like structure, l = the first leaf, r = primary root, s = slit, t = testa; scale = 5 mm).

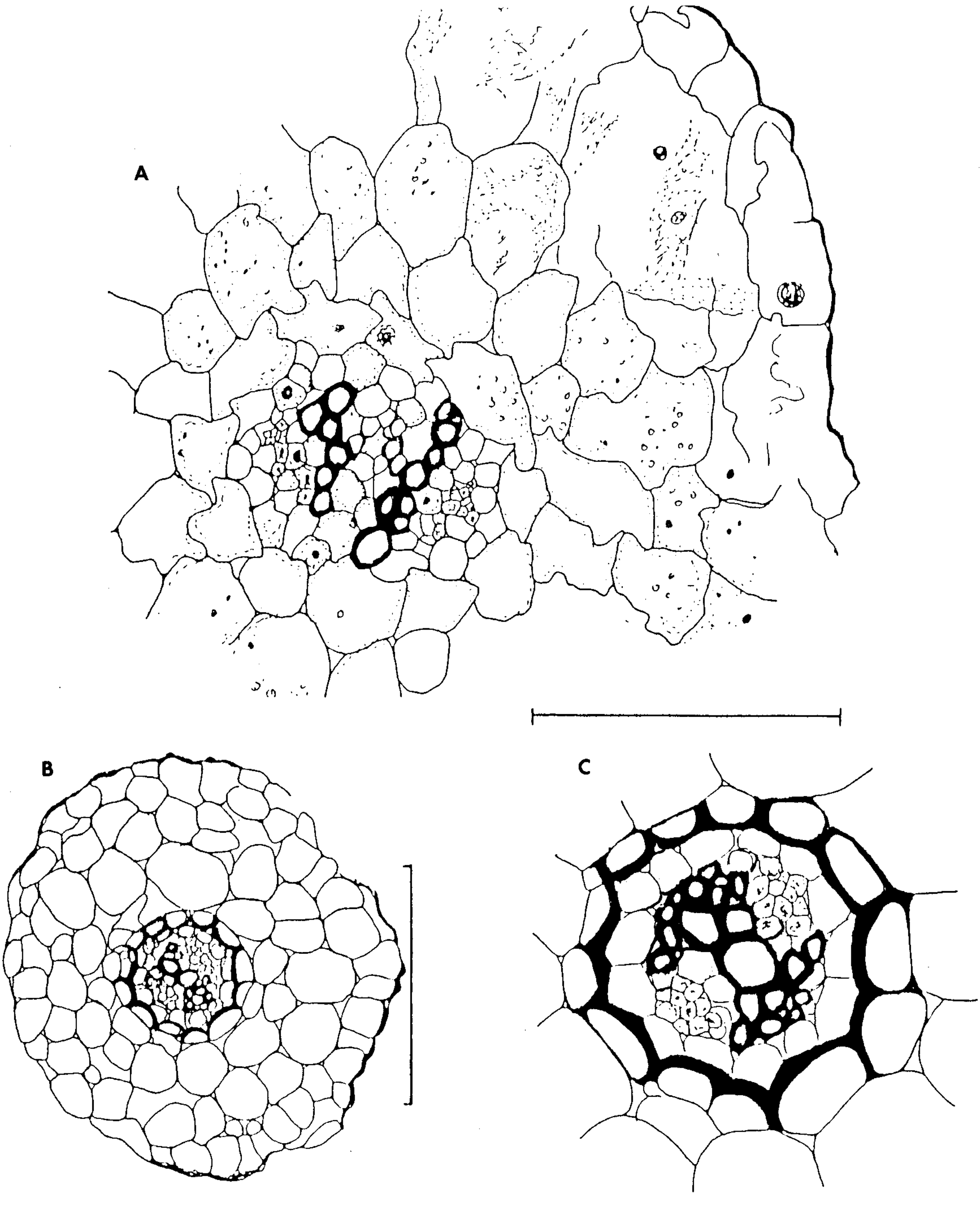


Fig. 3 Transverse sections of *Laxmannia gracilis* seedling type A. A: cotyledon showing a double vascular bundle; B: root whole section; C: enlargement of section B showing diarch stele. (scale A and C = 0.1 mm; B = 0.3 mm)

The position of the vascular tissue in the seedling type A1 at two weeks old is shown in Fig.4. Sections from the levels marked show the following features. The root (Fig.5) has a diarch stele that is without a definite pericycle or endodermis, and is surrounded by large, isodiametric cortical cells and an epidermis. The transition from primary root to stem occurs over about 150 μm. Figures 6A-C are of sections through the base of the cotyledon, the shoot apex and the base of the first foliage leaf. At this stage, the vascular tissue of the first leaf consists of meristematic (provascular) tissue, and that of the shoot apex and cotyledon of meristematic tissue and a few vessel elements respectively. The first leaf is within the tubular cotyledon and consists of meristematic tissue throughout its whole length (Fig.6D-E). The vascular system of the cotyledon is a double vascular bundle proceeding through its entire length, but when the cotyledon forms the hair-pin-like structure, its vascular bundles coalesce ((Fig.7A-C), and form a single strand made of four bundles surrounded by an indefinite boundary of xylem patches (Fig.7D). Towards the tip of the hair-pin-like structure, those xylem patches fuse and form a crescent (Fig.7E). The numbers of xylem and phloem elements are gradually reduced to the tip of the hair-pin-like structure and are finally absent leaving only cortical cells and epidermis in that area (Fig.8).

DISCUSSION

Richard[10] distinguished two morphological types of germination of monocotyledons, namely remotive and admotive. In the remotive type, elongation of the cotyledon carries the plumular bud some distance from the seed, while in the admotive type the two are in proximity since the cotyledon does not elongate after germination and carry the bud away from the seed. Martius[11] included an ocrea or ligule in the admotive type to describe seedling possessing an ascending sheath around the young bud. Klebs[12] classified germination under the criteria of morphology, physiology, and the response of the young plant to the environment but did not include any anatomical work. Arber[13] initiated some anatomical data to his morphological study on monocotyledonous seedlings. Later Boyd[14] proposed a seedling classification and suggested a terminology and description for three types of seedlings based on morphology and anatomy. Boyd's classification has been followed in the work of Dahlgren and Clifford.[5] Of the three types of seedlings they surveyed in the monocotyledons, none appears similar to the new type described here for *L. gracilis* with the long hair-pin-like structure (Fig.2D-E). Seedlings of *Tricoryne elatior* R.Br. (Anthericaceae), *Dianella caerulea* (Phormiaceae), *Scilla* spp. (Hyacinthaceae) and *Dasylirion* sp. (Dracaenaceae) show a transitory trend towards the development of the hair-pin-like structure.[9,13,15] Seedlings, probably of a species of *Allium*, as reported by Goebel,[16] may be very similar to the seedling type A1 of *L. gracilis*. This hypogeal phanerocotylar seedling possesses a cotyledon the upper part of which,[17] next to the tip, functions as a suctorial organ, and remains thin and thread-like and eventually dies. The lower portion of the cotyledon[17] becomes an almost cylindrical foliage leaf. Here the persistent part of cotyledon has grown out slightly beyond the thread-like transitional portion from the point where they are joined in a knee-like bend.

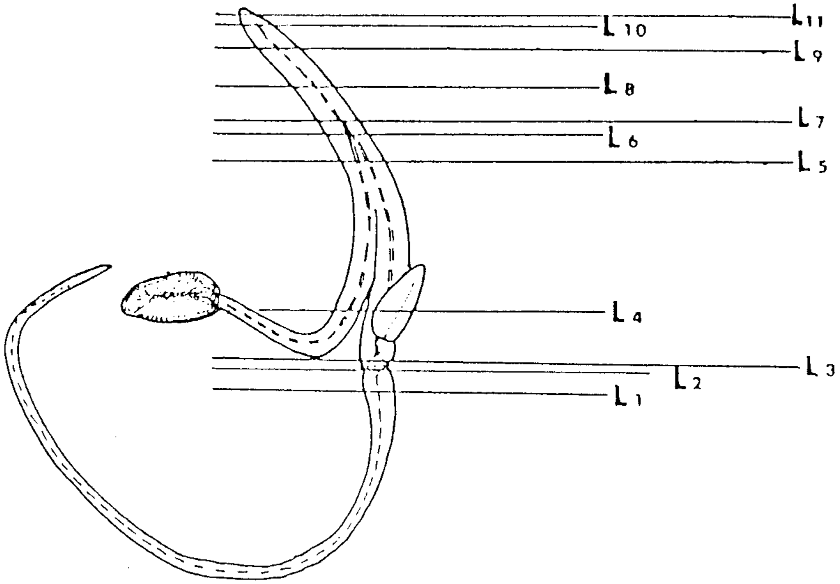


Fig. 4 *Laxmannia gracilis* seedling type A1 showing positions of sections. (L = level)

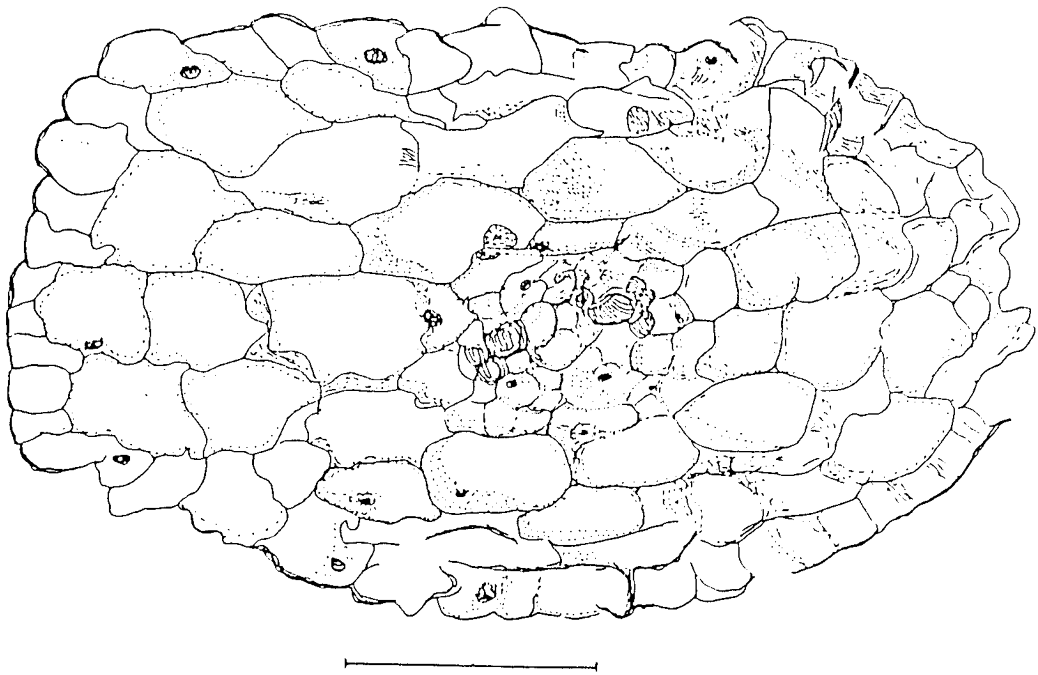


Fig. 5 Transverse section of root of *Laxmannia gracilis* seedling at L1 in fig.4. (scale = 0.1 mm)

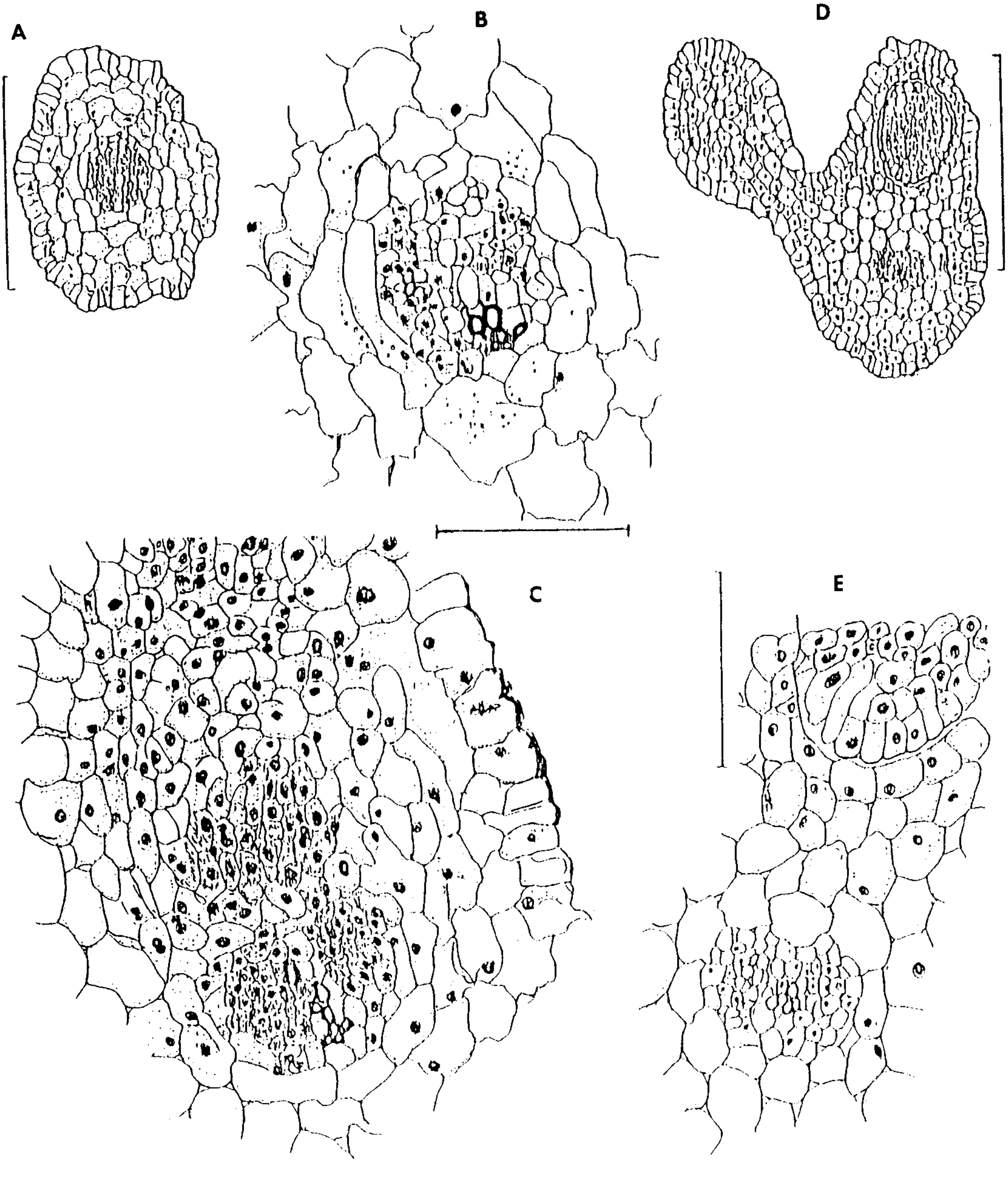


Fig. 6 Transverse sections of cotyledon of *Laxmannia gracilis* seedling type A1 at different levels shown in fig.4: A: section at L2; B: enlargement of vascular bundle of A; C: section as L3 showing stelic part; D: section of hair-pin-like structure at L4 showing the first leaf embedded in tubular structure of cotyledon; E: enlargement of vascular bundle of D. (scale A and D = 0.3 mm; B,C and E = 0.1 mm)

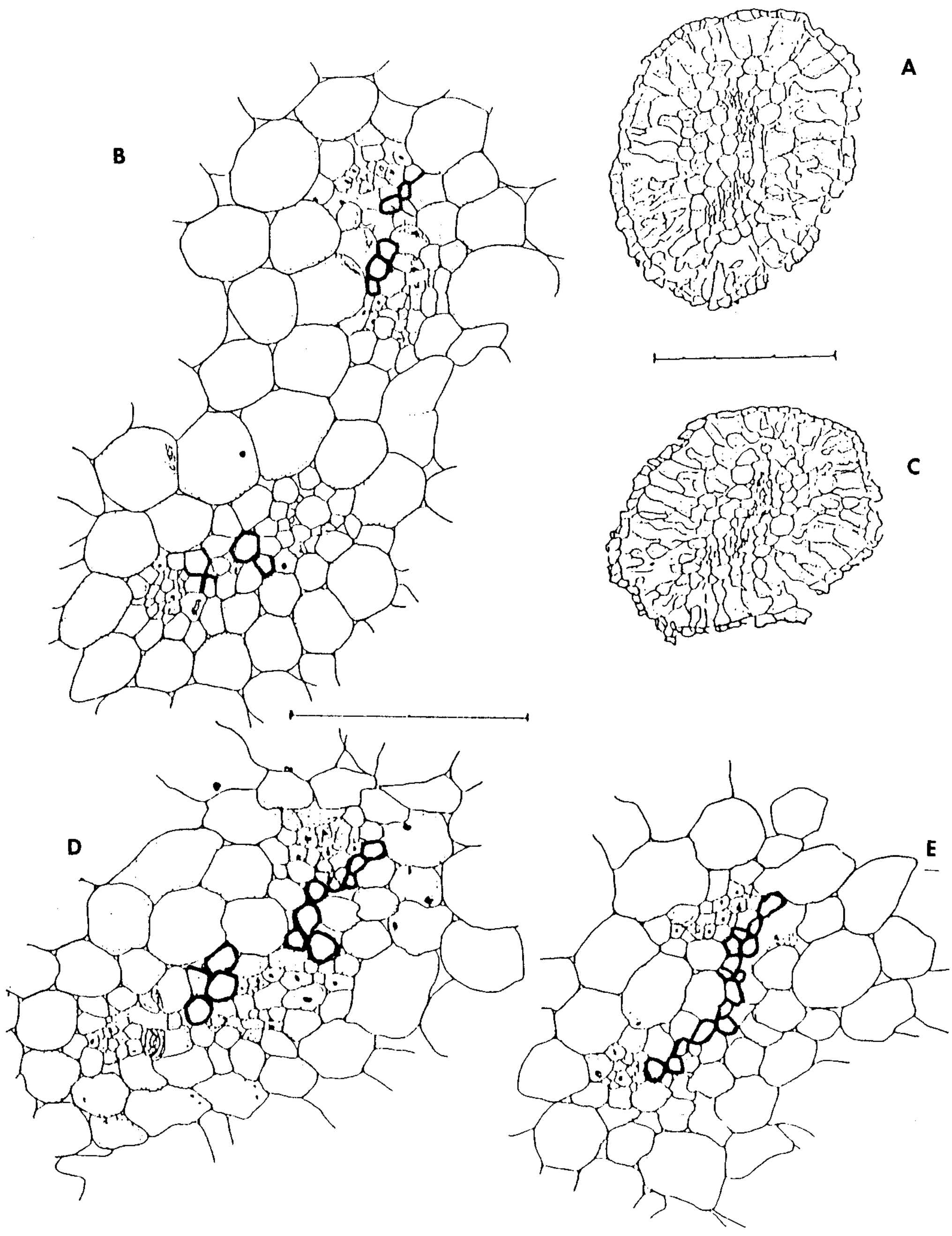


Fig. 7 Transverse sections of hair-pin-like structure of *Laxmannia gracilis* seedling type A1 at different levels shown in fig.4: A: section at L5; B: enlargement of vascular bundles of A; C: section at L6; D: vascular bundles of section at L7; E: section at L8 showing crescent of xylem. (scale A and C = 0.3 mm; B,D-E = 0.1 mm)

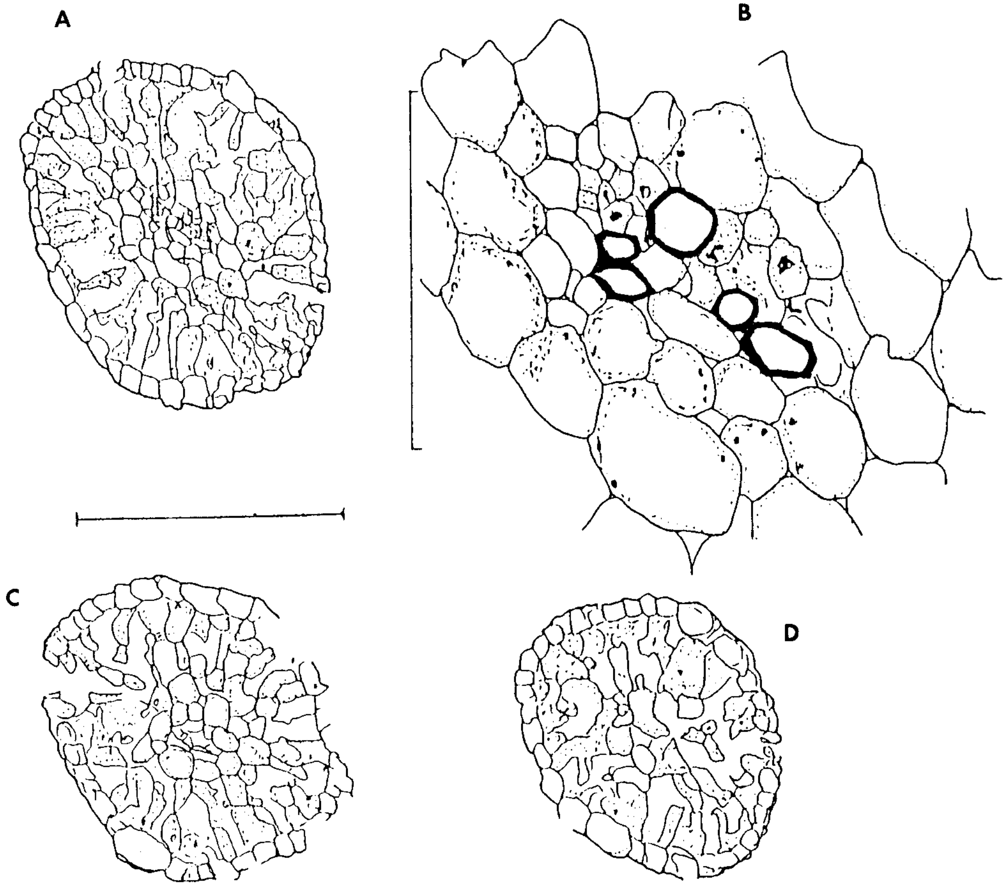


Fig. 8 Transverse sections of hair-pin-like structure of *Laxmannia gracilis* seedling type A1 at different levels as shown in fig.4: A: section at L9; B: enlargement of vascular bundle of A; C: section at L10; D: section at L11; showing absence of vascular system. (scale. A, C and D = 0.3 mm; B = 0.1 mm)

L. gracilis seedlings with the hair-pin-like structure can be derived from the normal type A seedlings. The hair-pin-like structure probably functions as a boring organ during germination.[16] The upper portion adjacent to the tip of the cotyledon may function as a suctorial as well as a photosynthetic organ because it is attached to the testa and possesses chlorophyll. During germination, both the plumule and the first leaf need to be protected so that a protective organ in the form of a complete or incomplete tubular cotyledon is advantageous. In most epigeal phanerocotylar monocotyledonous seedlings, the cotyledon protects the plumular bud by means of a low open sheath which arises around the cotyledonary slit. The function of the cotyledonary slit is similar to that of the ligule in the ligular seedling type, and clearly demonstrates the lateral origin of the ligule.[14] This seedling type A1 shows the connection between the cotyledonary sucker and the ligule through the connection of the vascular system to the plumule and the short tube covering it.

Boyd[14] suggested that the vascular system of the cotyledonary sucker is related to the physiological demand of the entire seedling and that the length of the cotyledonary stalk, but not its vascular anatomy, is affected by the depth of sowing or circumstances of germination. The epigeal phanerocotylar cotyledon has the additional function of a photosynthetic organ and often it is the only photosynthetic part of the seedling in the juvenile period. It also conducts the products of photosynthesis to the plumule and roots. In addition, the cylindrical epigeal phanerocotylar cotyledon provides a smaller surface to the air than a flat cotyledonary blade and so reduces water loss. The long green cylindrical cotyledon of *L. gracilis* seedlings, plus the extra growth of the hair-pin-like structure offers a greater surface area for manufacturing food through photosynthesis than in seedling type A with a cylindrical cotyledon. The vascular strands in the cotyledon and the hair-pin-like structure function not only as a pathway to transport assimilated photosynthetic products but also help provide the strength needed for the cotyledon to grow through the soil during germination. It may be argued that a hair-pin-like structure is not a special organ but merely the folded cotyledon which fails to detach itself and that this failure may be the result of an unequal rate of all division of the tissue on either side of the cotyledon, then the compact tissue, the double vascular bundle, fuse to give a crescent of xylem. This is followed by reduction to a single bundle at the tip of the structure. If the hair-pin-like structure achieves in detachment, it may become a seedling type A. Otherwise the hair-pin-like structure may be similar to that found in *Dasylirion* sp. and *Elettaria* sp. in which the single cotyledonary stalk grows vertically upwards from the seed but its basal part (usually in sheath form) bends downwards, carrying with it the plumular bud so that the stalk and the basal part of the cotyledon form a sharp angle with one another.[13] The first plumular leaf, however emerges from the apical end of the tubular ligule in both *Dasylirion* and *Elettaria* seedlings, which therefore differs from the lateral emergence found in *L. gracilis* seedlings. The cotyledonary stalk with abbreviated tissue (e.g. seedlings of *Billbergia zebrina* in Bromeliaceae) or the ligule without vascular tissue (e.g. seedlings of grasses) however, are considered more efficient in giving a more direct connection between the endosperm, the plumule and the root stele of seedlings.[14] The development of a photosynthetic organ and transport system to manufacture and transport food to every part of the plant are equally important and compensate for the limited source of endosperm in most small size seeds including those of *L. gracilis.* Xerophytic plants usually produce phanerocotylar seedlings with a cylindrical cotyledon, an adaptation to dry conditions.[14] The seedlings of *L. gracilis* seem to have this adaptation, the larger percentage of seedlings being of type A1 with the hair-pin-like structure. The presence of seedlings intermediate between this and type A suggests a trend of adaptation and modification of a plant organ and may be a significant feature for phylogenetic study within and between taxonomic units.

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บทคัดย่อ

Laxmannia gracilis R.Br. สร้างต้นกล้า 2 ชนิด คือ A และ A1 ซึ่งต้นกล้าแบบ A1 นี้แสดงการงอตัวอย่างถาวรของใบเลี้ยงซึ่งมีลักษณะคล้ายคลิปหนีบผม ต้นกล้าในลักษณะนี้เป็นแบบที่พบมากในธรรมชาติ และจากกายวิภาคของระบบท่อลำเลียงภายในโครงสร้างที่คล้ายคลิปหนีบผมนี้แสดงให้เห็นแนวโน้มของวิวัฒนาการในการเปลี่ยนแปลงจากต้นกล้าแบบ A ไปสู่แบบ A1 แต่จากกายวิภาคของราก ระบบนี้ไม่แสดงให้เห็นความแตกต่างอย่างมีนัยสำคัญ