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Ontogenetic Anatomy of *Streptocarpus grandis* (Gesneriaceae) with Implications for Evolution of Monophylly

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The monophylly of *Streptocarpus grandis* was examined ontogenetically and anatomically. When the seed is shed, the embryo is composed of a hypocotyl and two equal-sized cotyledons, lacking root and shoot apices. During germination, cell division and subsequent cell enlargement occur in the hypocotyl and cotyledons. The hypocotyl soon produces a primary root from its distal tip; this involves surface and subsurface cells at the point of attachment of the suspensor remnant. In the cotyledons, cell enlargement and differentiation occur basipetally, leaving small meristematic cells at the bases. These small cells give rise to the basal meristem in one of the two cotyledons, which contributes to an accrescent cotyledon. The groove meristem, which later differentiates into an inflorescence, arises in place of shoot apices when the cotyledons become visibly unequal in size. It later exhibits a *tunica-corpus* like configuration and differentiates directly into an inflorescence meristem. The evolution of this unique growth of one-leaved *Streptocarpus* is discussed with regard to morphogenetic data.

Key words: Anisocotyly, developmental anatomy, evolution, Gesneriaceae, one-leaf plant, ontogeny, *Streptocarpus grandis*.

INTRODUCTION

Most species of the Gesneriaceae have a conventional growth form, but species of the subfamily Cyrtandroideae show anisocotyly or unequal-sized cotyledons. In the Cyrtandroideae, some African *Streptocarpus* species and all members of the Malesian *Monophyllaea* have very unique monophylly, or a one-leaf type of growth form. They exhibit extreme anisocotyly; one cotyledon outgrows the other, no vegetative shoots are formed throughout the life history and, at maturity, the inflorescences arise from the accrescent cotyledon.

Since it was first reported by Caspary (1858; in Jong and Burtt, 1975) and independently by Crocker (1860), the one-leaf type of growth in the Gesneriaceae has received much attention (Hill, 1938; Schenk, 1942; Lawrence, 1958; Burtt, 1970; Bell, 1991; Cronk and Möller, 1997). This is because, contrary to the usual seedling, vegetative, and reproductive growth phases, it seems likely that one-leaf plants completely lack the vegetative growth phase. It is tempting to interpret the evolution of such a unique morphology in terms of heterochrony (Jong and Burtt, 1975). Recently, Tsukaya (1997) and Cronk and Möller (1997) noted an apparent resemblance between the *shoot meristemless* (*stm*) mutant of *Arabidopsis*, a model plant for molecular genetics, and the one-leaf plant.

The complete absence of a typical shoot apical meristem in Gesneriaceae seedlings has been reported repeatedly in some species of *Streptocarpus* and *Monophyllaea* (Jong and Burtt, 1975; Jong, 1978; Tsukaya, 1997). In contrast, the lack of a root meristem in the seedlings has received less attention.

Jong (1973, 1978) and Jong and Burtt (1975) proposed a new term, 'phyllomorph', to describe such a unique growth form, avoiding any attempt to describe the growth form using the root–shoot concept. Phyllomorphy means constructed as a leaf blade (lamina) with a proximal petiole-like stalk (petiolode). A phyllomorph is formed by the activity of three meristems: (1) the basal meristem, which is involved in lamina growth; (2) the petiolode meristem, which contributes to petiolode growth; and (3) the groove meristem, which is involved in inflorescence development (Jong and Burtt, 1975). According to the phyllomorph terminology, the one-leaf type of growth is interpreted as a cotyledonary phyllomorph, while the rosette form is an assemblage of phyllomorphs.

Streptocarpus may present an advantage over *Monophyllaea* in clarifying the evolution of one-leaf morphology. Since *Streptocarpus* includes both acaulescent (stemless) as well as caulescent (shooty) species (Hilliard and Burtt, 1971), phylogenetic and morphological comparisons can be made between acaulescent and caulescent congeneric species, while all species of *Monophyllaea* are acaulescent and monophyllous. A recent molecular phylogenetic analysis suggested that in *Streptocarpus* the unifoliate species represent a single clade, and this growth form has arisen only once from the caulescent growth form (Möller and Cronk, 1997).

Despite the efforts of previous researchers (Jong, 1973, 1978; Jong and Burtt, 1975), the ontogenetic establishment

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of one-leaf morphology is still poorly understood. The present study examines the anatomical ontogeny of the one-leaf morphology of *Streptocarpus grandis* N. E. Brown. This species is suitable for study because its developmental gross morphology has been fully described (Hilliard and Burtt, 1971); the cotyledons are equal in size until they are about 1 mm long, the petiolode (the portion between the small and large cotyledons) is short, the lamina of matured accrescent cotyledons becomes as large as 380 × 330 mm, and the inflorescences arise acropetally from the lamina base. This description of the morphogenesis follows Jong and Burtt's (1975) terminology.

MATERIALS AND METHODS

Seeds of *Streptocarpus grandis* were provided by the Hyogo Flower Center; they had been set by hand pollination. The seeds were grown in the Botanical Gardens of the University of Tokyo. Most seedlings were cultivated in a phytotron at 25°C with a 12 h photoperiod under plant-growth lamps at 800 lux at the Japan Women's University. A voucher specimen is housed in the herbarium of the Japan Women's University.

For anatomical study, seedlings and young to mature plants were fixed with FAA for more than 24 h. The fixed materials were dehydrated through an alcohol series, embedded in LKB Historesin (glycol methacrylate, Leica), and cut into 2 μm thick sections. These sections were stained with modified Sharman's stain (Jernstedt *et al*., 1992). The ordinary paraffin method was also used for large plants. In addition to sectioning, transmitted-light microscopy was employed on fixed materials to show the boundary between the mesophyll or ground-tissue cells and the meristematic cells. The mould-cast technique was used for SEM (Jernstedt *et al*., 1992). The moulds, which were made using dental paste, were filled with an epoxy adhesive and incubated at 60°C for 1 h to polymerize the adhesive. The casts formed were sputter-coated with platinum-palladium, and observed at 10 kV in Hitachi S-430 and S-800 electron microscopes.

RESULTS

Embryos and early seedlings

Seeds of *S. grandis* are very small (approx. 0·5 mm long) and exalbuminous. The seed embryo has only two organs: two cotyledons and an hypocotyl (Fig. 1). No young shoot apex (plumule) is observed between the bases of the two cotyledons. In longitudinal section, the cotyledons are eight or nine surface (protodermal) and inner-tissue (ground-meristem) cells long, and the hypocotyl is 11 or 12 cells. The distal tip of the hypocotyl is tapered and has no root meristem. The embryo contains numerous starch grains in each constituent cell. A procambium-like strand consisting of thin, elongate cells runs through the hypocotyl and both cotyledons, creating a Y-shape (Fig. 1, but the procambial strand in the cotyledons is not visible in this section). There are surface and subsurface cells distal to the procambium end at the hypocotyl apex, and the remnant of the suspensor is attached (Fig. 1).

In a germinating seed, the hypocotyl elongates first to emerge from the seed coat (Fig. 2). At emergence, the hypocotyl is 15 or 16 cells long, or nearly 1·4-times the length in the seed. There is no apparent increase in the number of cell layers in the hypocotyl between the surface layer and procambium for some time after germination (compare Figs 3 and 1). Consequently, hypocotyl elongation is due mainly to anticlinal cell division and subsequent cell enlargement (Fig. 2). The latter occurs in the distal portion first and proceeds upwards (Fig. 3). Cell-number increment and cell enlargement are also involved in cotyledon unfolding (Figs 4 and 5), although cell enlargement occurs later in the cotyledons than in the hypocotyl (Fig. 3).

Root development

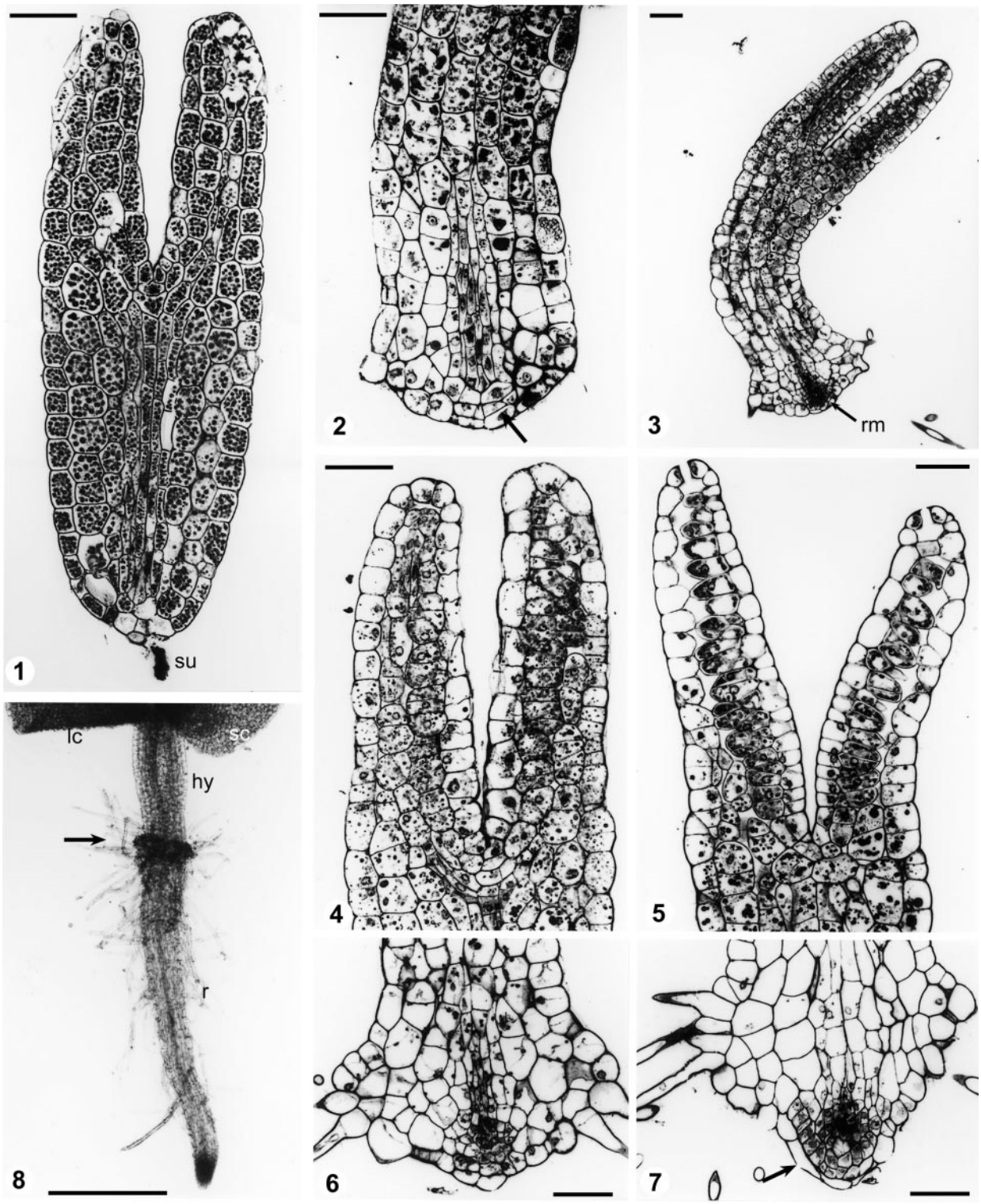
During germination, the hypocotylar tip becomes thick and round as a result of increased cell number in the surface layer as well as the inner tissue (Fig. 2). The surface cells at the attachment point of the suspensor remnant undergo periclinal divisions to form two tiers of thin cells (Fig. 2). Adjacent subsurface cells which are next to the procambial end then divide in various planes to give rise to a group of meristematic cells (Fig. 6). In the two tiers of thin cells, the proximal-tier cells divide mainly in the anticlinal plane, and the distal-tier cells elongate with no cell divisions (Fig. 7). The former cell layer may differentiate into the root protoderm, and the latter into a root-cap like structure. Thus the root meristem is derived from the surface and subsurface cells of the hypocotyl.

At the same time as root initiation begins, the end of the round hypocotylar tip becomes much enlarged transversely (Fig. 3), and produces root-hair-like trichomes on its surface (Fig. 6). As a result, a piliferous collar-like outgrowth is formed around the hypocotylar tip, which is contiguous with the downward growing root (Fig. 7). Even in well-grown seedlings with an elongated root, the collar-like outgrowth still remains at the point where the hypocotyl and root meet (Fig. 8).

After formation of the first root, the hypocotyl produces several adventitious roots (Fig. 19), which are endogenous in origin (data not shown). It is notable that the first root formed from the hypocotyl and those formed later are quite different in their mode of development.

Cotyledon unfolding and establishment of anisocotyly

A longitudinal section of the unfolding cotyledons shows basipetal tissue differentiation in the inner-tissue (Fig. 5). In the distal half, there are mature mesophyll cells accompanied by large intercellular spaces, but in the basal portion, there are smaller or just-divided cells with very few or no intercellular spaces. The SEM cast of the abaxial surface of newly-unfolded cotyledons also shows a gradual basipetal differentiation in surface-cell size and shape from the tip to the base of a lamina (Figs 9 and 10). There are large cells with undulating radial walls near the tip, somewhat smaller cells whose walls have little or no undulation in the middle, and small, rectangular cells at the very base (Figs 9 and 10). On the basis of longitudinal sections and



FIGS 1–8. Median longitudinal sections of embryo and seedlings (Figs 1–7), and transmitted-light micrograph of anisocotylous seedling (Fig. 8). Fig. 1. Embryo in seed, with seed coat removed. The embryo is composed of two cotyledons and a hypocotyl to which the suspensor remnant (su) is attached. Fig. 2. Elongated hypocotyl emerging from seed coat. Surface cells near the end of the procambial strand have been divided by periclinal walls (arrow). Fig. 3. Newly-germinated seedling. Root meristem (rm) is just initiated in hypocotylar tip. Fig. 4. Cotyledons in section near that of Fig. 3. Inner (ground-meristem) tissue as well as surface layers increase in cell number by anticlinal cell divisions. Fig. 5. Two or 3-d-old seedling with just-unfolding cotyledons. Note basipetal differentiation in the ground tissue. Fig. 6. Hypocotyl tip with root meristem at nearly the same stage as Fig. 3. Fig. 7. Root apical meristem covered with surface cell layer decaying like root-cap tissue (arrow). Fig. 8. Hypocotyl-root junction of an approx. 20-d-old seedling. A piliferous collar-like protrusion (arrow) demarcates the boundary between the hypocotyl (hy) and root (r). lc, Large cotyledon; sc, small cotyledon. Bars = 50 μm for Figs 1–7; 0·5 mm for Fig. 8.

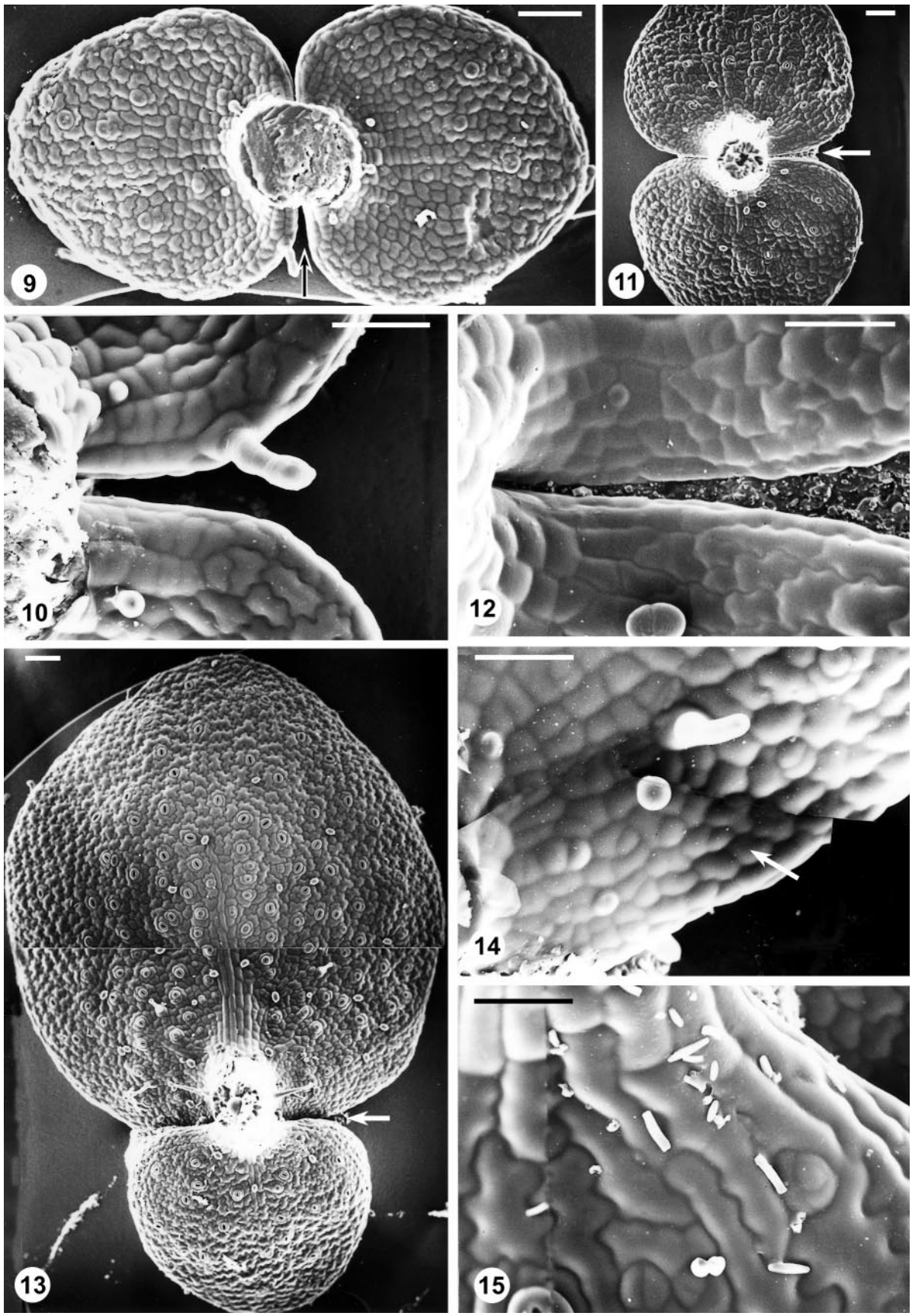


FIG. 9. For legend see facing page.

SEM views, it seems that, during lamina unfolding, cell division activity is lost gradually from the lamina tip to the base in the surface and the inner tissue, and basipetal cell enlargement occurs. The small cells in the extreme base still seem to be meristematic in both unfolding cotyledons of the seedling.

In SEM views of the abaxial surface of two unfolded cotyledons nearly 0·7 mm long, which do not yet show obvious anisocotyly, there is some difference between the two cotyledons in the surface cell organization of their lamina bases (Figs 11 and 12). In one lamina, the basal cells are small, whereas in the other lamina they are a little larger (Fig. 12). This difference becomes increasingly obvious during the development of anisocotyly (Fig. 13). The small basal cells in the large cotyledon increase in number to form the basal meristem (Fig. 14). In contrast, those in the small cotyledon become much more enlarged and finally differentiate into epidermal cells (Fig. 15). Thus the basal meristem is not formed in the small cotyledon.

A transmitted-light micrograph of a young large cotyledon shows that the basal meristem (dark area) is fan-shaped (Fig. 16). However, the boundary of the basal meristem is not obvious in either transmitted-light micrographs or SEM views. In the former, the basal meristem is surrounded by somewhat larger inner cells, which are distally contiguous to well-differentiated mesophyll cells (Fig. 16). In the latter, a group of small rectangular cells in the basal meristem is surrounded by somewhat larger cells with weakly undulating radial walls (Fig. 14).

Serial oblique longitudinal sections of a large cotyledon cut at nearly 40° to the midrib (Fig. 17) confirm that the basal meristem consists of actively dividing, small cells, and that the inner cells distally surrounding the basal meristem are somewhat larger with small intercellular spaces (Fig. 18). These inner cells may be non-meristematic. Similarly, somewhat larger surface cells with weakly undulating radial walls are also thought to be non-meristematic (Fig. 14). Therefore, the basal meristem is roughly demarcated by the somewhat larger inner cells (Fig. 18), or by surface cells with weakly undulating radial walls (Fig. 14).

In a transmitted-light micrograph (Fig. 17) the dark area of the large cotyledon appears to be interrupted by a narrow lighter area of the midrib base between both leaf-lamina bases. However, median (Fig. 21) and oblique (Fig. 18, bottom figure) longitudinal sections of the large cotyledon show that the subsurface and inner cells in the midrib base are actually meristematic but less stained, and contain far fewer chloroplasts. Such cell organization may make the midrib base appear lighter in the transmitted-light micrographs. In addition, the apparent restriction of the dark area to the leaf lamina may be caused by the 3D construction of the leaf base; the leaf-lamina bases on both sides of the midrib are directed upward a little and then outward (Figs 17 and 18, upper figure). In conclusion, the fan-shaped basal meristem of the seedlings is composed of meristematic cells in the lamina base, the basal meristem *sensu stricto*, as well as those in the midrib base.

The basal meristem becomes enlarged as the large cotyledon increases in size (Figs 16 and 17). In much older, larger cotyledons of young plants, the fan-shaped, dark area is much more enlarged, but becomes relatively elongate (Fig. 19).

Initiation of groove meristem and petiolode elongation

When the seedlings begin to show anisocotyly, several surface as well as subsurface cells between the two cotyledons undergo anticlinal divisions (Figs 20 and 21). In addition, the inner cells also divide in both the anticlinal and periclinal planes to give rise to a small meristem, called the groove meristem (Fig. 22). The meristem is not located exactly between the cotyledon bases, but instead at the base of the larger cotyledon. Associated with the formation of the groove meristem, the cells in the very base of the large cotyledon divide anticlinally (Fig. 22), and then elongate greatly. The groove meristem itself also contributes to the basal cells below it. As a result, a short petiole-like axis or petiolode is formed and intercalates below the lamina of the large cotyledon (Fig. 23).

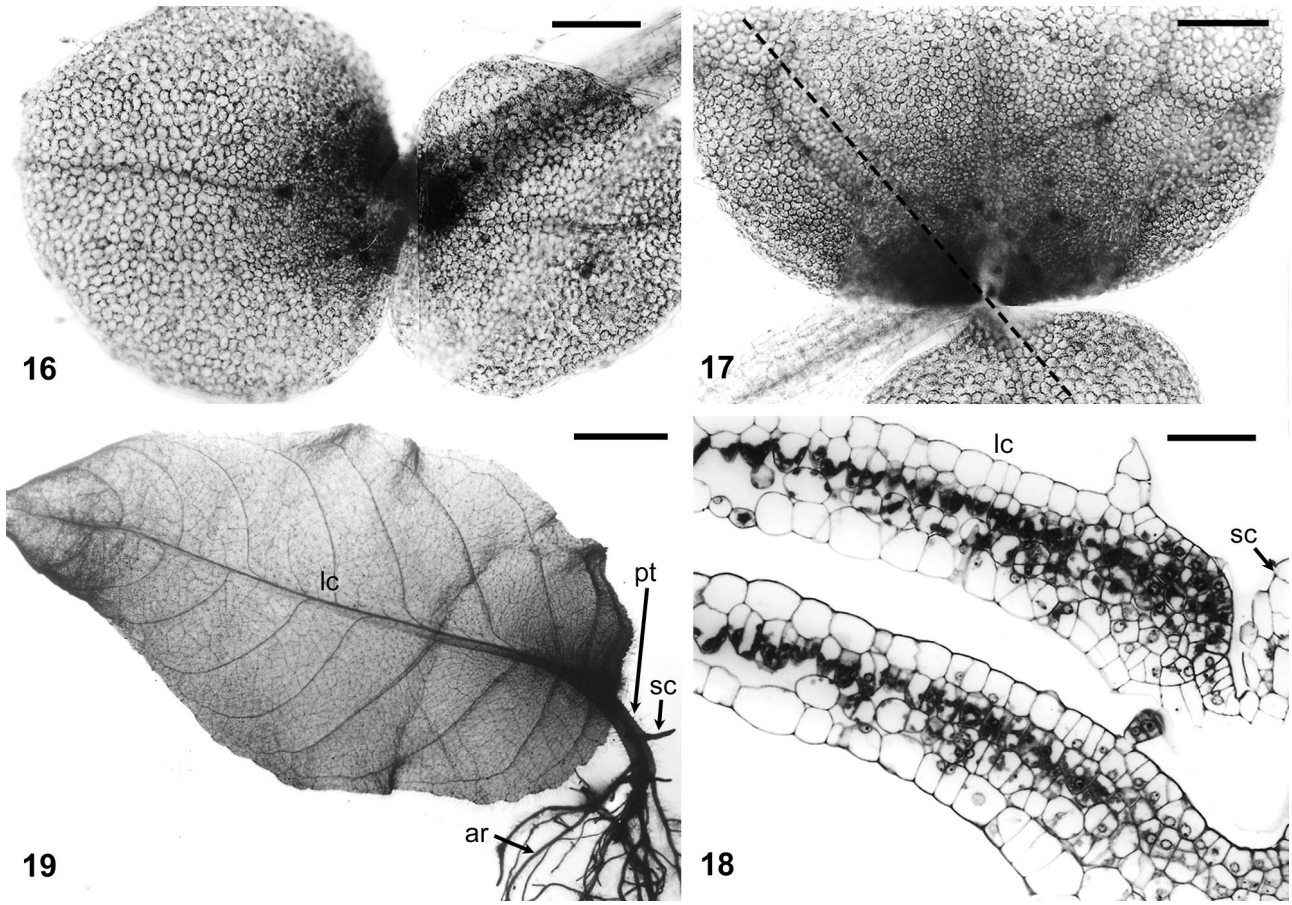
When the petiolode becomes more elongated, the petiolode meristem, an intercalary meristem consisting of several files of very thin cells, becomes distinct behind and at nearly the same level as the groove meristem (Fig. 24). After this developmental stage, the petiolode meristem enlarges distal and proximal to the groove meristem (Figs 25 and 28). The petiolode is about 5 mm long when the small cotyledon withers (Fig. 19). During further cotyledon growth, the petiolode meristem contributes more to elongation of the midrib than to the petiolode (Fig. 25). In mature inflorescence-bearing plants, the petiolode associated with the hypocotyl is 2 cm long.

The petiolode meristem is topographically distinguished from the basal meristem (Jong and Burtt, 1975). However, both meristems act together as an intercalary meristem. It is likely that the petiolode meristem is part of the basal meristem, not an independent *de novo* meristem.

Initiation of inflorescences

Accompanying the continued growth of the large cotyledon and petiolode, the groove meristem also increases in size (Fig. 26). Its surface and subsurface cells divide only in the anticlinal plane like a two-layered *tunica*, and the

FIGS 9–15. Cast SEM micrographs of the abaxial surface layer of cotyledons at various developmental stages. Arrows in Figs 9, 11 and 13 indicate magnified portions shown in Figs 10, 12 and 14 and 15, respectively. Fig. 9. Seedling, 14-d-old, with two newly-unfolded, equal-sized cotyledons. Fig. 10. Basal portions of two cotyledons with similarly sized small cells at the very base. Fig. 11. Seedling, 16-d-old, initiating anisocotyly. Fig. 12. Basal portions of two cotyledons. Basal cells are still small in the upper lamina of the figure, but a little larger in the lower lamina. Fig. 13. Seedling, 30-d-old, showing anisocotyly. Fig. 14. Base of larger cotyledon. The basal meristem consisting of small rectangular cells is surrounded by somewhat larger cells with weakly undulating radial walls (arrow). Fig. 15. Base of small cotyledon. Every surface cell becomes enlarged with undulating radial walls. Bars = 100 μm for Figs 9, 11, 13; 50 μm for Figs 10, 12, 14, 15.



FIGS 16–19. Transmitted-light micrographs of seedlings (Figs 16, 17, 19), and serial oblique longitudinal sections of large cotyledon (Fig. 18). Fig. 16. Anisocotylous young seedling. The basal meristem of the large cotyledon appears as a dark area. Fig. 17. More developed seedling with large cotyldeon 1·6 mm long. Dotted line indicates the orientation of serial oblique longitudinal sections cut through the basal meristem and groove meristem, as shown in Fig. 18. Fig. 18. Two serial oblique longitudinal sections of large cotyledon 1·2 mm long (lc), and small cotyledon (sc). The bottom figure is a section through the midrib near the groove meristem. The upper section is through a more proximal portion of the large cotyledon than the bottom section. The basal meristem consisting of densely stained small cells is contiguous with cells with small intercellular spaces. Fig. 19. Four-month-old plant with small cotyledon (sc) still persisting on the end of a short petiolode (pt) of the large cotyledon (lc). Due to its orientation, the small cotyledon is seen as a bar. The dark area becomes much more enlarged and relatively narrowed. There are many adventitious roots (ar) on the hypocotyl. Bars = 200 μm for Figs 16 and 17; 50 μm for Fig. 18; 5 mm for Fig. 19.

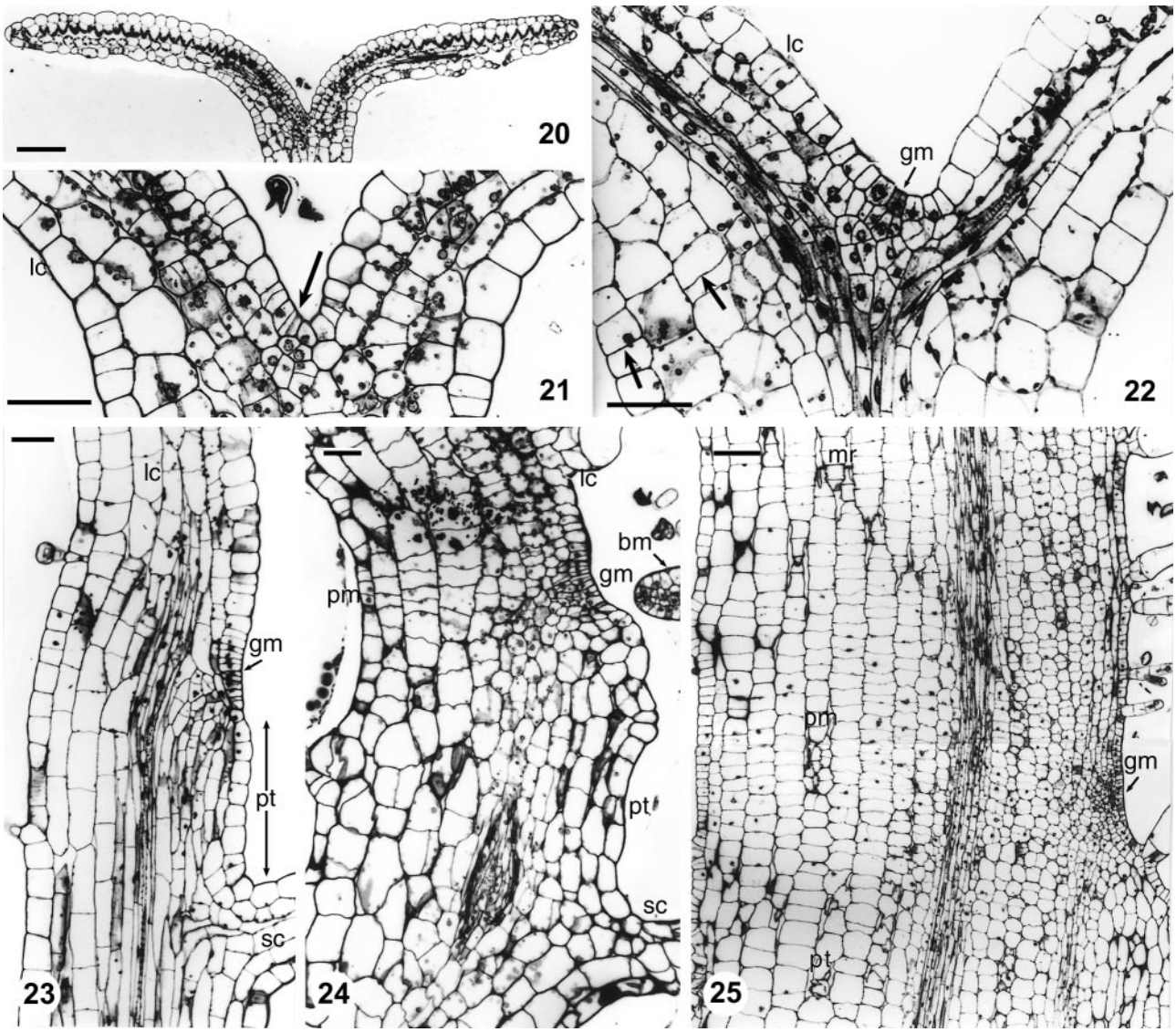
inner cells divide in various planes like a *corpus* (Fig. 27). Consequently, the groove meristem takes an apparent *tunica-corpus* configuration, as do many shoot apices of angiosperms. In a large cotyledon more than 10 cm wide, the groove meristem begins to initiate inflorescence meristems (Fig. 28). Several inflorescence primordia develop in an acropetal order and all are subtended by an involucral bract (Fig. 29).

DISCUSSION

Meristem- and organ-homology of unifoliate plants

The first root of seedlings of the subfamily Cyrtandroideae has been differently interpreted as primary or adventitious. In gross-morphological studies it is primary and the radicle (Weber, 1936, for *Streptocarpus wendlandii*, *Chirita sinensis*; Schenk, 1942, for *S. polyanthus*, *S. caulescens*, etc.; Tronchet *et al.*, 1960, for *S. grandis*). In anatomical studies, it is exogenous and primary (Oehlkers, 1923, for *Monophyllaea horsfieldii*), or endogenous and adventitious (Hielscher, 1883, for *S. polyanthus*; Weber, 1978, for *Rhynchoglossum gardneri*). Our results show that in *S. grandis* the apical meristem of the first root forms involving the surface and subsurface cells of the hypocotylar tip. Thus the first root appears to be of exogenous origin (Fig. 30).

The radicle or the root meristem in angiosperm embryos is normally initiated in the hypophyseal cell and/or its derivative cells, which are close to the suspensor and embryo proper (Esau, 1977; Raghavan, 1986; Fahn, 1990; Johri *et al.*, 1992; West and Harada, 1993; Howell, 1998). Embryogenetic studies on the subfamily Cyrtandroideae have been made on *Streptocarpus rexii* (Alimova and Yakovlev, 1982) and *Epithema carnosum* (Padmanabhan, 1967). In both species the embryos form the suspensor cells and the hypophyseal cell during embryogenesis. These mature embryos

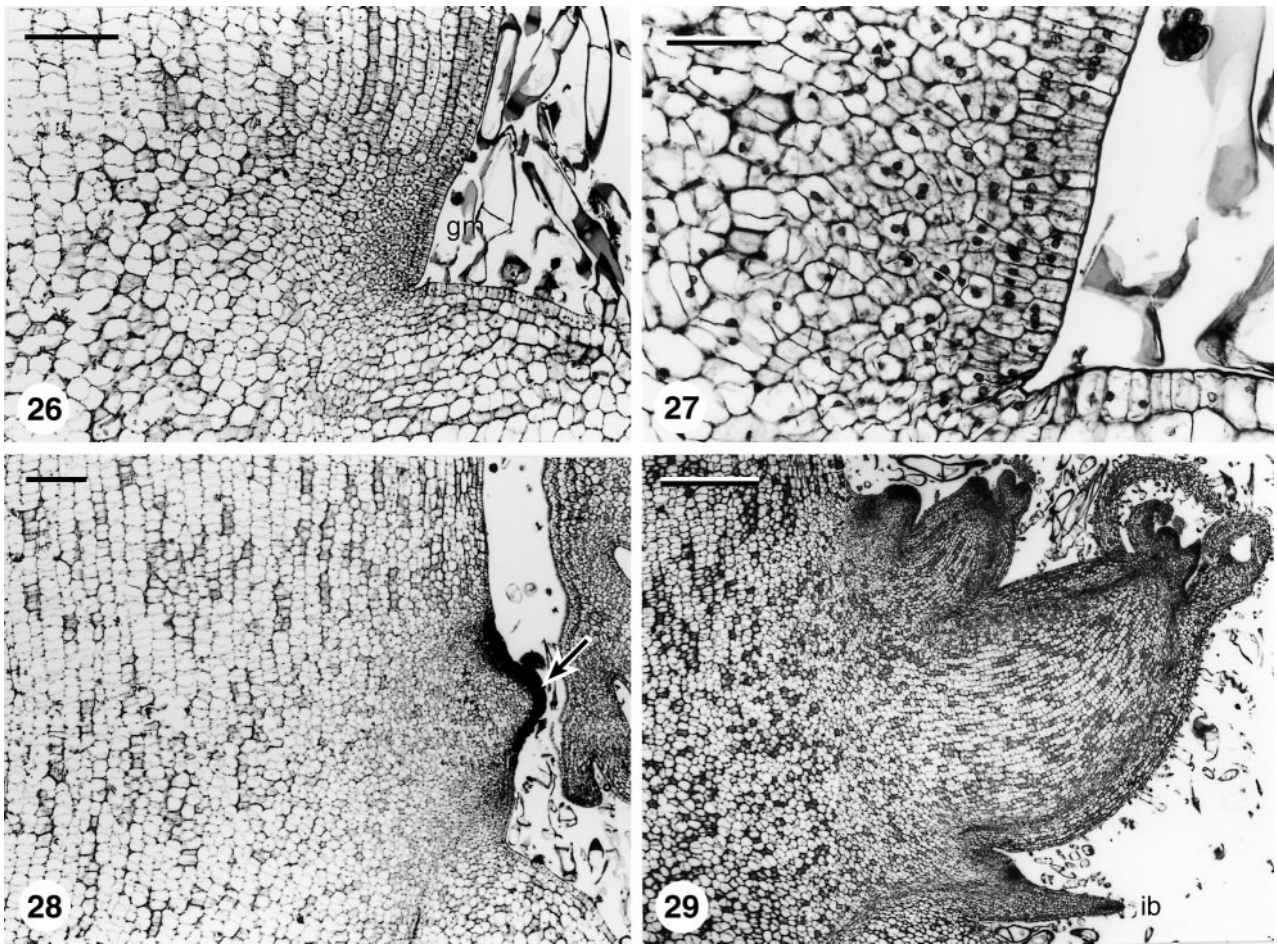


FIGS 20–25. Median longitudinal sections of seedlings and young plants. Fig. 20. Seedling just initiating anisocotyly. Fig. 21. Magnified figure of cotyledon bases in Fig. 20. Surface and subsurface cells between both cotyledons become meristematic (arrow). Note that the meristematic cells are slightly displaced towards the base of the large cotyledon (lc). Fig. 22. Anisocotylous seedling with newly-formed groove meristem (gm). Arrows indicate cell walls formed by anticlinal divisions which will contribute to early elongation of the petiolode of the large cotyledon (lc). Fig. 23. Large cotyledon 2 mm long. The groove meristem (gm) is transferred to the petiolode (pt) that intercalates between the large (lc) and small (sc) cotyledons. Fig. 24. Large cotyledon 7 mm long. The petiolode meristem (pm) is formed just behind the groove meristem (gm). The basal meristem (bm) of the large cotyledon (lc) is located at the same level as the groove meristem (gm). pt, Petiolode; sc, small cotyledon. Fig. 25. Large cotyledon 39 mm long. The petiolode meristem (pm) enlarges distal and proximal to the groove meristem (gm), and contributes more to elongation of the midrib (mr) than to the petiolode (pt). Bars = 100 μm for Figs 20 and 25; 50 μm for Figs 21–24.

are nearly identical in configuration to those of *S. grandis* examined here. Therefore, in *S. grandis* the root-initiating cells, that is, hypocotylar surface- and subsurface-cells between the rudimentary suspensor and the procambial end, may be hypophysis-derived cells.

At the beginning of root initiation in *S. grandis*, the hypocotylar surface cells divide periclinally to give rise to two tiers of thin cells, which later differentiate into the root protoderm and root-cap like structure. Similar periclinal divisions in hypophyseal derivatives have been reported in the embryonic root-cap formation of *Arabidopsis thaliana* (Scheres *et al.*, 1994). Therefore the first root of *S. grandis* may be embryologically equal to the embryonic root of other dicotyledons.

In some dicotyledon embryos, a collar-like thickening, called a 'collet' (Eames, 1961), is formed at the junction of the hypocotyl and root (radicle) (Bowman, 1994). One of the main characteristics of the epidermis at the junction in *Arabidopsis* is rapid formation of root hairs (Scheres *et al.*, 1994). A similar collar-like thickening with a piliferous epidermis appears at the conjunction between the hypocotyl and root in *S. grandis* (Tronchet *et al.*, 1960; this paper).



FIGS 26–29. Median longitudinal sections of well-grown plants showing inflorescence development. Fig. 26. Ten-month-old plant with large cotyledon 10 cm wide with distinct groove meristem (gm). Fig. 27. Close-up of the groove meristem shown in Fig. 26, showing organization of two-layered *tunica-corpus* formation. Fig. 28. Large cotyledon 16 cm wide of 13-month-old plant. The groove meristem has produced inflorescence meristems (arrow). Fig. 29. Larger cotyledon 21 cm wide of 23-month-old plant. Several inflorescence primordia are subtended by an involucral bract (ib). Bars = 200 μm.

On the basis of these two shared features, it is likely that the first root of *S. grandis* is primary and embryonic rather than adventitious (Fig. 30).

Cotyledon growth has been differently reported in various plants; it is attributed to cell enlargement rather than cell division (Tsukaya *et al.*, 1994), or to both cell division and cell enlargement (Avery, 1933). In the *S. grandis* embryo, cotyledon development begins with cell division through the cotyledons, and then cell enlargement occurs basipetally, like the tobacco cotyledon (Avery, 1933). Unlike ordinary cotyledon growth, in *S. grandis* cell division activity is retained in one cotyledon as the basal meristem for a long time, resulting in extreme anisocotyly. The large cotyledon can be divided into two parts: a tip, equivalent to the small cotyledon, and the promixal part formed later by the basal meristem (Fig. 30).

The present study also shows that the *S. grandis* embryo lacks the shoot apical meristem (SAM) at seed shedding. A small meristem called the groove meristem begins to differentiate between the bases of two cotyledons at a relatively late stage after germination. The groove meristem has been regarded as unique to some Gesneriaceae because it develops directly into an inflorescence meristem with no intervening vegetative growth phase (Jong and Burtt, 1975). However, the groove meristem has a *tunica-corpus* configuration just before inflorescence development (Jong and Burtt, 1975; Jong, 1978; this paper). Preliminary studies on the caulescent species, *S. pallidiflorus*, show that the SAM is produced in the same position and at nearly the same relative time as the groove meristem of *S. grandis*, and begins to produce foliage leaves soon after. Furthermore, recent molecular genetic studies confirmed that a SAM produces vegetative structures as well as reproductive structures, and production of both is just temporally segregated (e.g. Weigel, 1995; Evans and Barton, 1997). Therefore it is likely that the groove meristem is equal to the SAM, although its activity is much delayed compared with the ordinary SAM of other angiosperms.

In summary, the embryo of *S. grandis* remains in the early torpedo stage of embryogenesis. During or after germination the root apical meristem is formed, followed later by the SAM. The SAM grows very slowly, never forms foliage leaves, and at last differentiates directly into an inflorescence meristem.

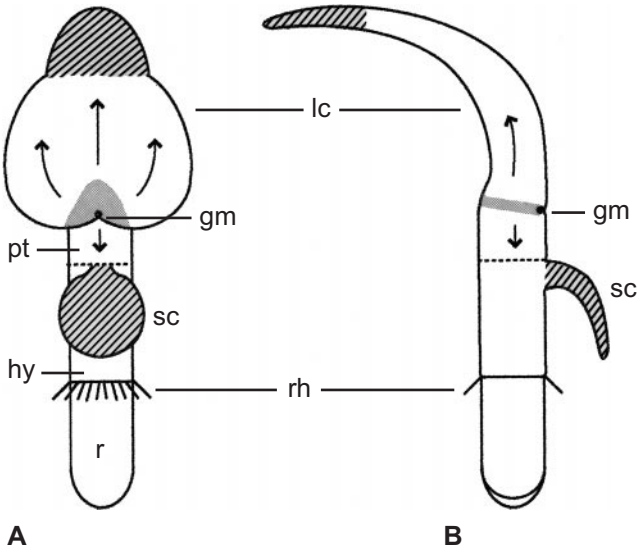


FIG. 30. Diagram illustrating anisocotylous development of seedling. Top (A) and longitudinal-sectional (B) views. The hatched areas indicate the small cotyledon and the tip portion of the large cotyledon that is formed at the isocotylous stage and is comparable to the small cotyledon. Arrows indicate the directions of cell production from the fan-shaped basal meristem (shaded areas in A). The petiolode meristem (shaded area), part of the basal meristem, is seen in B. gm, Groove meristem; hy, hypocotyl; lc, large cotyledon; pt, petiolode; r, root; rh, rhizoid; sc, small cotyledon.

Evolution of monophylly

The evolution of monophylly may be explained by meristematic modifications such as long-term retention of the basal meristem in one cotyledon, suppression of SAM activity without leaf formation, and differentiation of a reproductive meristem from the SAM. The mechanism underlying those changes is uncertain. However, useful information can be gained from relevant mutants and genes of *Arabidopsis* and other plants analysed by recent molecular genetic studies.

The basal meristem may be ontogenetically derived from the meristematic cells of the unfolding cotyledons. The evolution of the basal meristem might have involved a failure to switch off the cell-division phase at an appropriate time in seedling growth, or by novel acquisition of meristematic activity at the cotyledon (leaf) base. The latter resembles the evolution of dissected leaves which is assumed to have occurred by the imposition of SAM properties on the leaf-development program. Overexpression of the *knotted* (*kn1*) gene of maize or the *Arabidopsis kn1*-like gene, *KNAT1*, which are usually expressed in the shoot meristem, in leaves of transgenic tobacco plants or *Arabidopsis*, results in dissected leaves or meristems in leaves (Smith and Hake, 1994; Chuck *et al.*, 1996).

As noted earlier, delay of SAM activity may be one factor leading to the monophylly evolution. In their plant-hormone treatments on some acaulescent species of *Streptocarpus*, Rosenblum and Basile (1984) induced shoots from the groove meristem and otherwise isocotylous architecture. They argued that morphogenetic capacities, which have previously been thought to be lost or lacking in anisocotylous plants, are present but suppressed. Such suppression of SAM activity may be a factor in the monophylly evolution.

Tsukaya (1997) noted an apparent similarity between *Monophyllaea horsfieldii* seedlings and the *shoot meristemless* (*stm*) mutant of *Arabidopsis*. The *stm* mutant shows the same embryogenetic course as the normal embryo until the torpedo stage, but after that stage, SAM is never formed (Barton and Poethig, 1993). Cronk and Möller (1997) postulated that the unifoliate morphology devoid of a shoot apex might be interpreted as a loss-of-function mutant similar to the *stm* mutant. Delay of SAM activity might also occur by similar genetic mechanisms to a *paused* (*psd*) mutant of *Arabidopsis*, which fails to make the first leaf primordia for several days after germination (Conway and Poethig, 1997; Telfer *et al.*, 1997).

The change from a shoot apical meristem that fails to form foliage leaves to a reproductive meristem is also a major modification leading to monophylly. There is an apparent resemblance between the monophyllous plants and *embryonic flower* (*emf*) mutants of *Arabidopsis*. The *emf* mutant develops an inflorescence immediately after germination, bypassing the vegetative shoot stage (Sung *et al.*, 1992; Yang *et al.*, 1995).

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