INVITED SPECIAL PAPER

Organization of the root apical meristem in angiosperms[1]

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Although flowers, leaves, and stems of the angiosperms have understandably received more attention than roots, the growing root tips, or root apical meristems (RAMs), are organs that could provide insight into angiosperm evolution. We studied RAM organization across a broad spectrum of angiosperms (45 orders and 132 families of basal angiosperms, monocots, and eudicots) to characterize angiosperm RAMs and cortex development related to RAMs. Types of RAM organization in root tips of flowering plants include open RAMs without boundaries between some tissues in the growing tip and closed RAMs with distinct boundaries between apical regions. Epidermis origin is associated with the cortex in some basal angiosperms and monocots and with the lateral rootcap in eudicots and other basal angiosperms. In most angiosperm RAMs, initials for the central region of the rootcap, or columella, are distinct from the lateral rootcap and its initials. Slightly more angiosperm families have exclusively closed RAMs than exclusively open RAMs, but many families have representatives with both open and closed RAMs. Root tips with open RAMs are generally found in angiosperm families considered sister to other families; certain open RAMs may be ancestral in angiosperms.

Key words: angiosperms; basal angiosperms; eudicots; meristem organization; monocots; phylogeny; root apical meristems.

The origin and characteristics of the flowering plants have fascinated botanists for a long time (see Soltis et al., 2005), but these characteristics have generally been presented in terms of evolution of flowers, fruits, leaves, and stems. Their roots have received far less attention, mostly because they are unseen underground or in water or because they are not nearly as showy as the aboveground plant parts. They are often looked upon as being the anchoring parts of the plant with a rootcap covering the growing tip of the root and, thus, not as interesting as the aboveground portion with its leaves, flowers, and fruits attached to stems. Yet, roots might provide a set of characters valuable to our understanding of plant evolution and phylogeny. The angiosperms were once subdivided into monocots and dicots, but now into basal angiosperms (ANAs [*Amborella*, Nymphaeales, Austrobaileyales] and magnoliids), monocots, and eudicots.

During the nineteenth and early twentieth centuries researchers attempted, to various degrees, to characterize vascular plants by the organization of their growing tips or root apical meristems (hereafter, RAM); some of the most important of these were Hanstein (1870), Van Tieghem (1870), Janczewski (1874a–c), De Bary (1884), Sachs (1872), and Kroll (1912; see also Haberlandt, 1914). These were analyzed and summarized in depth by Vecchierello (1928). The organization or pattern of cells within the RAM continued to be of great interest to botanists during the middle of the twentieth century with the reports of Guttenberg (1940, 1960, 1968, and related works), Popham (1966), Pillai et al. (1961a, 1965, and related papers), Voronin (1964), and Voronkina (1975) (see review of early works by Byrne, 1974).

While Guttenberg (1960, 1968) argued against trying to produce phylogenetic considerations of the root apical meristem, Pillai (e.g., Pillai and Pillai, 1961a–d; Pillai et al., 1961a, 1965; Pillai, 1963) argued that open patterns of apical organization (that lack defined boundaries between cortical, epidermal, and rootcap RAM regions) were generally ancestral to closed patterns (that have defined cell boundaries between cortical, epidermal, and rootcap RAM regions). Pillai et al. (1965) had evidence in the form of drawings that common initials (indistinct zone of cells for all tissue regions within root tips) were present in magnoliids and possibly in the eudicots. Recently, as molecular information has provided new tools for analyzing phylogenetic relationships, there has been renewed interest in trying to relate organization of the root apical meristem to phylogeny by Barlow (1995, 2002), Clowes (2000), and the Rost group (Groot and Rost, 2001; Groot et al., 2004).

Concomitantly during the past two decades, using mutants primarily of *Arabidopsis thaliana*, other groups of researchers have emphasized areas such as cell and tissue patterns, gene expression patterns, and cell differentiation (e.g., Dolan et al., 1993; Rost and Bryant, 1996; Benfey and Scheres, 2000; Cnops et al., 2000; Kidner et al., 2000; Sabatini et al., 2003; Ueda et al., 2005). To try to understand the basis of root development and structure, these researchers chose *Arabidopsis* as the model organism because of its simplicity (Benfey and Scheres, 2000). The closed RAM, a root apex with separate tiers or cell layers

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of initials for the stele, cortex, and rootcap epidermis, became the type meristem of flowering plants, at least for the eudicots. Closed RAMs of young roots in *Arabidopsis* and other Brassicaceae have been well known for a long time (e.g., Kadej, 1970; Pillai and Pillai, 1977; Baum and Rost, 1996; Wenzel and Rost, 2001). However, despite all of the work on root development and structure in *Arabidopsis*, it was not until the thorough analysis by Baum et al. (2002) that the developmental reorganization in its RAM structure became clear.

As the study of plant anatomy has given way to the study of plant development, recent research and texts generally give the impression that a closed, three-tiered RAM in the eudicot, *Arabidopsis*, or in the monocot, *Zea*, is the norm for angiosperm RAM structure and development (e.g., Raghavan, 2000), although earlier texts (e.g., Esau, 1953, 1977; Popham, 1966) and the findings of the Pillai group (1961–1975) had left no doubt about the wide range of RAM types among angiosperms (see also Evert, 2006). It appears that the characteristic organization of a plant's RAM may appear in the first days after germination or emergence from the parent structure (cf. Clowes, 1961, 2000, and Guttenberg, 1968).

There has been analysis of the RAMs of some basal angiosperms. The early descriptions of *Nymphaea* by Conard (1905) and later Pillai and Kavathekar (1975) characterized the nymphaeaceous RAM as an open transversal RAM (plate of cells across the apex) like that in cucurbits or legumes, but it appears to have been Guttenberg and Müller-Schröder (1958), Voronin (1964), and Voronkina (1974) who first clearly reported the nymphaeaceous RAM to be monocot-like with the epidermis associated with the cortex and not with the rootcap (sensu Esau, 1953, 1977). Clowes (1994, 2000), and Seago et al. (2000b, 2005) have described the RAM of various members of Nymphaeales, and the association between epidermis and cortex in their RAM is now well established.

Considerable attention has also been paid to the open transversal RAMs of the legumes, in which the epidermis is associated with lateral rootcap, by such researchers as Torrey (1955), Popham (1955), Hayat (1963), Pillai and Sukumaran (1969), Pillai et al. (1974), Pillai and Raju (1975), Chiang and Tsou (1974), and Wenzel et al. (2001). Patel et al. (1975) gave a comprehensive schematic representation of these RAMs.

Other studies described ontogenetic changes from closed to open meristems in RAMs during elongation of the primary root (e.g., Guttenberg, 1968; Seago and Heimsch, 1969; Byrne and Heimsch, 1970; Seago, 1971; Armstrong and Heimsch, 1976). Clowes (1981) expanded earlier concepts of Guttenberg (1940, 1968) and Armstrong and Heimsch (1976) to demonstrate the developmental change from closed to open meristems in the roots of flowering plants. Then, following the notion of Eames and MacDaniels (1947) that epidermal origin in monocots was either associated with cortex origin or had a separate origin in aquatic monocots, Clowes (1990, 2000) related epidermal origin to RAM organization in aquatic monocots and later across a fairly broad spectrum of the flowering plants. More recently, Groot and Rost (2001) and Groot et al. (2004) reported open vs. closed patterns in many eudicots. Hamamoto et al. (2006) related production and release of rootcap cells to meristem type in dicots.

The study of Clowes (2000) is particularly important because it included many members of the Nymphaeales and magnoliids, distinguished between open and closed meristems, and also distinguished between the site of origin of the epider-

mis and its developmental relationship to the cortex or rootcap. Groot and Rost (2001) and Groot et al. (2004) examined some general characteristics of RAM organization in eudicots. Other studies have examined basal angiosperms or magnoliids (Pillai et al., 1961a; Voronkina, 1974; Pillai and Kavathekar, 1975; Seago et al., 2000b). In fact, most recent papers have basically ignored the many researches that came from the Pillai group (1961–1975), except for one citation by Clowes (2000). Pillai et al. (1961–1975) characterized many different angiosperm groups and even some gymnosperms and showed differences and similarities in apices between angiosperms and gymnosperms. Within the angiosperms, they even postulated RAM phylogenetic trends, challenging Haberlandt's view (1914) that closed meristems were ancestral and expanding upon Guttenberg's view (1960) that open meristems were ancestral.

Clowes (2000) considered the RAMs of different ranks of roots, while Groot and Rost (2001) and Groot et al. (2004) dealt with primary roots, i.e., the root derived from the radicle of the embryo after germination, but they partially relied upon information that came from other authors who analyzed adventitious roots in some species. Dicotyledonous flowering plants (Heywood, 1985) usually produce primary roots from the radicle of the embryo, and the products form their root systems, but basal angiosperms are often dominated by adventitious roots. Monocots, while often producing primary roots, have root systems that are characteristically adventitious and that are often associated with a clonal habit of growth (see Arber, 1920; Weaver, 1926; Heywood, 1985). Adventitious roots usually arise from shoots and replace or supplement the earlier roots (see Arber, 1920; Les and Schneider, 1995; Feild et al., 2004).

Because molecular methods have helped plant systematists and morphologists to produce a revised phylogenetic scheme for the flowering plants, we interpret RAM organization and cortical structure within the context of one of these phylogenetic schemes (APG, 2003; P. F. Stevens, University of Missouri at St. Louis, personal communication). The groups referred to here, as modified from APG (1998, 2003) and Soltis et al. (2005), are basal angiosperms (i.e., ANA grade [*Amborella*, Nymphaeales, and Austrobaileyales] and magnoliids); monocots including commelinids; and eudicots, including basal eudicots, nonasterid eudicots (basal core eudicots, basal rosids, eurosids I, eurosids II), and asterids, including euasterids I and euasterids II with their included orders and families (the ANAs, magnoliids, and eudicot groups made up the dicots in the past [for background, see APG, 1998, 2003; Soltis et al., 2005]).

Before these current phylogenies were identified, one of us (C.H.) initiated this study in the mid 1950s to look for any obvious phylogenetic trends within angiosperm RAM organization, with an emphasis on dicotyledonous flowering plants, sensu Heywood (1985). The work was expanded to include monocots and other ANA angiosperms. We also wanted to determine whether the RAM organizational pattern is related to cellular patterns within the root cortex because the kind of association between the cortex and its initials seemed just as important in determining cell and tissue patterns produced in the root cortex as the location of the origin of the epidermis (Clowes, 2000). Heimsch's (1960) study of expanding root cortex in tomato, following his analysis of the barley root tip related to vascular differentiation (Heimsch, 1951), was foundational to this approach.

MATERIALS AND METHODS

In analyzing RAMs in the roots of angiosperms, it is very difficult to deal with just one kind of root. Many traditional dicot species have no roots or primary roots are not produced,others have primary roots that are too ephemeral to produce any characteristic primary root and primary root system, or the root system is dominated by adventitious roots, plants being primarily clonal in reproduction (e.g., *Ceratophyllum*, Arber, 1920; *Nelumbo lutea, Trapa natans*, J. L. Seago, personal observation; Arber, 1920; *Nymphaea* spp., Conard, 1905; *Podophyllum peltatum*, J. L. Seago, personal observation; ANA basal angiosperms, and most monocots, respectively; see also Feild et al., 2004; Soltis et al., 2005). Accordingly, like Clowes (2000), we found it necessary to compare different ranks of roots, such as primary roots and adventitious roots, to obtain a sampling of roots from a wide variety of flowering plant groups. For magnoliids and eudicots, the overwhelming numbers of specimens are of primary roots, but for the ANA basal angiosperms and monocots, most roots examined are adventitious, which is basically consistent with their respective growth habits. However, adventitious and primary root tips are similar, e.g., for *Lycopersicon*, cf. our results, Heimsch (1960), and Byrne et al. (1975); for *Typha*, cf. Seago and Marsh (1989, 1990) and this study, which included primary roots; and for *Zea*, cf. Seago and Wolniak (1976) and Varney and McCully (1991), among others. When we were unsure that we could obtain satisfactory main roots (primary or adventitious), we examined lateral roots of two species, which turned out to be important for the basal angiosperms.

We did not examine some of the unusual root apices associated with unusual habitats such as running water over waterfalls (e.g., Podostemaceae: Imaichi et al., 1999; Ota et al., 2001; Koi and Kato, 2003; Koi et al., 2006) and parasitism (e.g., Orobanchaceae: Okonkwo, 1970).

Cell and tissue patterns can be shown by the analyses of Körper-Kappe (⊥ or ⊤ shaped) cell files that began with Schüepp (1917, 1926; see also Kadej, 1963; Kadej, 1966) and that were used in various ways by many researchers (e.g., Clowes, 1950, 1961; Tomlinson, 1969; Patel et al., 1975; Seago and Marsh, 1989). We do not show Körper-Kappe patterns here, except minimally in drawings, although we used them microscopically and photographically, especially in analyzing roots that were problematic.

We examined six orders and 10 families of basal angiosperms, 10 orders and 28 families of monocots, and 29 orders and 94 families of eudicots; the species numbered 427 (Appendix S1, see Supplemental Data with online version of this article).

Seeds, bulbs, corms, etc., were obtained from many sources. Seeds, especially of magnoliids and eudicots, were generally germinated on moist filter paper in a refrigerator or cool room (4–8°C). Soon after germination, the early stages of seedlings were placed between moist sheets of filter paper in beakers, and tips of primary roots, mostly 1–5 cm long, were harvested during the first week. Other seeds were germinated and grown at room temperatures. Some seeds did not produce primary roots upon germination; after young leaves emerged, rhizomes elongated and produced more leaves, and adventitious roots originated and grew from the rhizomes, as in *Nelumbo lutea*.

For almost all monocots and for several species of basal angiosperms (such as *Amborella trichopoda*, *Nymphaea cordata*, *Cabomba caroliana*, *Brasenia schreberi*, *Schisandra chinensis*, and *Illicium floridanum*) and eudicots (such as *Podophyllum peltatum*, *Pachysandra terminalis*, and *Ilex* sp.) for which we were unable to obtain primary roots, we used young adventitious roots, sprouted from bulbs, corms, rhizomes, or stem bases that were typically only a week or two old, 1–5 cm long, and grown in a vermiculite-soil mixture, in open water if aquatic plants, or in some cases, taken directly from field-grown plants. For *Illicium floridanum*, after we discovered that adventitious roots had a seemingly unusual organization, we also included lateral roots off adventitious roots from cuttings. We did not harvest root tips from seedlings or plants in which the tips had browned or had any sign of damage, degradation, or death. We did examine primary roots of a few selected monocots and determined that they were identical structurally to the adventitious roots. We have included specimens of species from our own studies in which roots were grown similarly to and to the same lengths as the roots of species itemized here, and we included species in the thesis work of a graduate student of C.H., L. Libous (1982) who grew the plants under similar conditions.

Specimen preparations of most root tips began by fixation in CRAF solution, FAA, or FPA (Sass, 1958). The tips were processed through a graded alcohol series into normal or tertiary butanol and embedded in paraffin wax or the synthetic Paraplast Plus (Sherwood Medical Industries, St. Louis, Missouri, USA). Handling of small, delicate specimens was facilitated by use of pipettes and moving specimens to small tubes closed at one end with fritted glass that allowed drainage of solutions. Tissue was sectioned on a rotary microtome at 8 or 10 μm and mounted on slides, stained variously (often with hematoxylin and safranin or safranin and fast green); coverslips were affixed with balsam or synthetic resins. Specimens were viewed with various microscopes, but photomicrographs for this paper were taken with a digital camera on a Zeiss Axiophot light microscope at the same magnification. Kodak 35 mm print and slide films were used to record and study images.

There can be great difficulties in ascertaining the median longitudinal section of RAMs in flowering plants (Clowes, 2000). To determine that sections were median, we counted the number of sections thru a series of root longisections with the stele into the tip of the RAM and then selected the median section. That is easiest to do for roots with closed, tiered meristems; open meristems of different types were much more difficult to assess because the patterns of cells were often neither symmetrical nor regular across the median section. And roots with slightly open RAMs could appear closed (tiered) near the median section, especially if slightly biased, because they had a partial boundary between outer cortical initials and the most apical lateral rootcap-epidermal initials of the RAM. However, slightly biased sections were not necessarily eliminated because the tiers of cortical initials overlying the tip of the stelar initials and the columellar initials overlying the cortical initials in closed RAMs could be determined even in biased sections by the complete, thickened border between the cortical and columellar initials in all sections. Certain open meristems were easily distinguishable in biased sections because the curving border between cortical and rootcap-epidermal regions at the periphery of the initials zone or cortical-epidermal initials and rootcap was clearly maintained and observed near the cell wall boundaries and cell files or packets (see principles illustrated by Barlow, 1987). In Fig. 1, the patterns of cell files and packets within RAMs and adjacent tissues are partially shown by Körper (e.g., Fig. 1D) and Kappe (e.g., Fig. 1M) line patterns (see Schüepp, 1926; Kadej, 1963; Seago and Wolniak, 1976), and tissues are colored to enhance visualization of the RAMs and derivative tissues.

RESULTS WITH COMMENTARY

The patterns of RAM organization are presented in Table 1 by angiosperm group, as modified from APG (1998, 2003) and Soltis et al. (2005) and are depicted schematically in Fig. 1. We treated basal angiosperms (ANAs and magnoliids; Figs. 1, 2–12), monocots (Figs. 1, 13–22), and eudicots (Figs. 1, 23–35) separately. Roots with RAM common initials are shown in Figs. 1A, 2, 4–5, 10–12, with RAM epidermal origin separated by a cleft from lateral rootcap in Figs. 1B–C, 6–9, with RAM epidermis origin separated from lateral rootcap without a cleft in Figs. 1D–I, 13–22, and with RAM epidermal origin associated with lateral rootcap origin in Figs. 1J–O, 3, 23–35.

Basal angiosperms—ANA-grade basal angiosperms have much more varied RAMs than magnoliids.

Common initials (CI)—RAMs in some roots of *Amborella* (Fig. 2), some roots of the Austrobaileyales (*Illicium*, Fig. 10), and in the magnoliids examined, including the Magnoliales (Fig. 11), Laurales (Fig. 12), and Piperales, have a zone of *CI* (Fig. 1A; Table 1) for the columella, lateral rootcap, epidermis, and cortex, but the stele may be separate. Boundaries between the initials of tissues are usually indistinct and vary within a specimen and between specimens (Figs. 2, 10, 11). In individual root tips, the epidermis may be aligned with the rootcap on one side of a median section, to the cortex on the other side, to the epidermis with Kappe patterns proximally and to cortex with Körper patterns distally, or to neither rootcap nor cortex. In adjacent sections, differing patterns of epidermal associations can be observed. The tip of the stele may manifest curved files into the tip wherein the pericycle is the boundary, but often the outer layer of stele is not distinct from the inner layer of cortex. Although there is a columella and it appears to abut the stele tip and the cortical initials, its origin as a set of initials is not clear.

TABLE 1. Types of root apical meristems (RAMs) by angiosperm group, order, and family; classification modified from APG (2003) and P. F. Stevens (University of Missouri at St. Louis, personal communication). Mostly adventitious roots in monocot groups and primary roots in eudicot groups; primary roots, except where noted.

Angiosperm group	Family	RAM type(s)
Basal angiosperms		
ANA		
Amborellales	Amborellaceae[al]	CI, OCD, OD
Nymphaeales	Cabombaceae[a]	TB
	Nymphaeaceae[a]	OTvB
Austrobaileyales	Schisandraceae[l]	TM, ETM
	Illiciaceae[al]	CI, ETM
Magnoliids		
Magnoliales	Magnoliaceae	CI
	Annonaceae	CI
Laurales	Calycanthaceae	CI
	Lauraceae	CI
Piperales	Aristolochiaceae	CI
Monocots[a]		
Acorales	Acoraceae	OEM
Alismatales	Araceae	OEM
	Hydrocharitaceae	TM, ETM
	Aponogetonaceae	TM, ETM
	Butomaceae	TM, ETM
	Alismataceae	TM
Pandanales	Pandanaceae	CM
Dioscoreales	Dioscoreaceae	OEM
Liliales	Melanthiaceae	OM
	Liliaceae	OM, OEM
Asparagales	Orchidaceae	TM, ETM
	Iridaceae	TM, ETM, OEM
	Alliaceae	OM
	Amaryllidaceae	OTvM, OEM
	Hyacinthaceae	OEM
	Agavaceae	OEM, OM
	Anthericaceae	CM
Commelinids[a]		
Arecales	Arecaceae	CM
Zingiberales	Musaceae	TM, ETM, OEM
	Strelitziaceae	OEM
	Cannaceae	TM, ETM
	Zingiberaceae	OEM, ETM
Commelinales	Commelinaceae	TM
	Pontederiaceae	TM, ETM
Poales	Typhaceae[ap]	TM
	Juncaceae	TM
	Cyperaceae	TM, ETM
	Poaceae[ap]	TM, ETM
Basal eudicots		
Ranunculales	Berberidaceae[ap]	OD, OTvD
	Ranunculaceae[ap]	OCD, OD
	Papaveraceae	TD
Buxales	Buxaceae	OD
Proteales	Nelumbonaceae[a]	TD, HTD
Basal core eudicots		
Gunnerales	Gunneraceae[a]	OTvD, OD
Caryophyllales	Plumbaginaceae	OD, TrD
	Polygonaceae	TD, HTD
	Simmondsiaceae	TD, TrD
	Caryophyllaceae	TD
	Amaranthaceae	TD
	Chenopodiaceae	TD
	Aizoaceae	TD
	Phytolaccaceae	TD
	Portulacaceae	TD
	Basellaceae	TD
Saxifragales	Hamamelidaceae	TrD, OCD
	Haloragaceae	OCD, TD
	Saxifragaceae	TD

TABLE 1. Continued.

Angiosperm group	Family	RAM type(s)
Basal rosids		
Geraniales	Geraniaceae	OD, OTvD
Myrtales	Onagraceae	TD
	Lythraceae	TD
	Punicaceae	TD, TrD
	Myrtaceae	OD, TrD, TD
Eurosid I		
Zygophyllales	Zygophyllaceae	TD, HTD, TrD, OTvD
Celastrales	Celastraceae	OD
Malpighiales	Salicaceae	OD
	Passifloraceae	OD
	Rhizophoraceae	TD
	Linaceae	TD, HTD
	Hypericaceae	TD
	Euphorbiaceae	OTvD
Oxalidales	Oxalidaceae	TD
Fabales	Fabaceae	OTvD, OD
Rosales	Rosaceae	OCD, TD, HTD, TrD
	Elaeagnaceae	OD
	Ulmaceae	OD
	Moraceae	HTD, TrD, OCD
Cucurbitales	Cucurbitaceae	OTvD, OCD
	Datiscaceae	TD
Fagales	Fagaceae	OCD
	Juglandaceae	OCD
	Betulaceae	TD, TrD
	Casuarinaceae	TD
Eurosid II		
Brassicales	Tropaeolaceae	OCD
	Caricaceae	OCD
	Limnanthaceae	HTD
	Resedaceae	TD
	Brassicaceae	TD
	Capparaceae	TD
	Cleomaceae	TD
Malvales	Malvaceae	OD, OCD
Sapindales	Anacaridaceae	TrD
	Meliaceae	OD
	Sapindaceae	OTvD, OCD
	Simaroubaceae	OCD
	Aceraceae	TD, HTD, TrD, OCD
	Hippocastanaceae	OCD
	Rutaceae	OCD
Basal asterids		
Cornales	Cornaceae	OCD
	Loasaceae	TD
Ericales	Balsaminaceae	HTD
	Polemoniaceae	TD
	Theaceae	HTD
	Ebenaceae	TD, HTD, TrD
	Primulaceae	TD
	Sarraceniaceae	TrD
	Ericaceae[al]	TrD
Euasterid I		
Unplaced	Boraginaceae	OTvD, TD
Gentianales	Rubiaceae	TD
	Hydrophyllaceae	TD
	Apocynaceae	TD, OCD
	Asclepiadaceae	TD, HTD, TrD
Lamiales	Oleaceae	TD, TrD
	Martyniaceae	TrD
	Plantaginaceae	TD, TrD
	Globulariaceae	TD
	Bignoniaceae	OCD
	Verbenaceae	TD
	Lamiaceae	TD

TABLE 1.   Continued.

Angiosperm group	Family	RAM type(s)
	Acanthaceae	TD
	Scrophulariaceae	TD
	Selaginaceae	TD
Solanales	Convolvulaceae	TD, HTD, TrD
	Solanaceae	TD, HTD, TrD, OD
Euasterid II		
Aquifoliales	Aquifoliaceae[al]	TrD
Apiales	Apiaceae	TD, TrD, OD
Asterales	Campanulaceae	TD
	Lobeliaceae	TD
	Menyanthaceae	TD, HTD
	Goodeniaceae	TD
	Brunoniaceae	TD
	Asteraceae	TD, TrD, OD
Dipsacales	Dipsacaceae	TD, TrD, OD

Notes: [a] = adventitious roots, [ap] = adventitious and primary roots; [al] = adventitious and lateral roots; [l] = lateral roots. RAM types: *CI* = common initial zone for epidermis, cortex, and rootcap; *OTvB* = open transversal meristem with cleft between epidermal initials and lateral rootcap in basal angiosperms; *TB* = closed, three-tiered meristem with little continuity across initials into tissues in basal angiosperms; *OEM* = in monocots, curved cortical and epidermal parts of initials into and over stelar initials, but boundary between epidermis and columella bridged; *OM* = cortex and epidermis open into columella with cortical and mostly epidermal files abutting or continuous into columella, monocots; *OTvM* = open transversal RAM with epidermis associated with cortex and files into columella base from cortex and epidermis; *CM* = closed RAM, cortical and epidermal initials abut sides of columella base that sits atop stelar initials in monocots with little radial cortex alignments; *TM* = three-tiered closed RAM with cortical-epidermal initials tier, or *ETM* = four-tiered closed RAM with separate cortical and epidermal initials; *OCD* = in eudicots, inner cortex tiered, outer cortex files into columella base; inner cortex radial, outer nonradial; *OD* = cortex and stele abut columella and some cortex continuous into columella edges; epidermis distinct; little radial alignments in cortex; *OTvD* = open, transversal meristem dicot, files from cortex into columella; lateral rootcap-epidermis distinct; almost no or sporadic radial files in cortex; *TD* = in dicots, three-tiered, closed RAM with one tier of cortical initials, or *HTD* four- or more-tiered, closed RAM with two or more tiers of cortical initials, the distal tier is hypodermis; *TrD* = in dicots, transitional RAM, cells bridging cortical and columellar initials border; cortex radially aligned.

Our interpretation is corroborated in transverse sections where at 50 μm behind the RAM abutting the cortex (Figs. 4, 12), there are no clear boundaries among the cortex, epidermis, and lateral rootcap, and the stele is also difficult to discern. There were no consistent radial alignments of cells across the cortex proximally or distally (Figs. 4, 5, 12), and the endodermis is not a uniform, single layer of innermost cortex surrounding the stele (J. L. Seago, unpublished epifluorescence work on *Amborella*).

Open transversal meristem in basal angiosperm (OTvB)—-Within the Nymphaeales, the Nymphaeaceae, as illustrated by *Nymphaea odorata*, have an open meristem with a more or less transverse zone of initials across the tip of the stele and cortex (Figs. 1B, 6). In *OTvB* RAMs, a ring of epidermal initials is associated with the periphery of the cortical initials, separated from the lateral rootcap and its initials by a cleft, beginning at the level of the initials (Fig. 6). The rootcap has a fairly well-defined columella initiated by columellar initials, abutting the epidermal and cortical region of initials (Fig. 6, right arrow). The cells at the tip of the stele, the stelar initials, abut the base of the central columella; they do not have curving cell files of pericycle extending into a narrow stelar tip.

The distal cell files of the epidermis in the *Nymphaea* meristem sometimes extend directly into the most peripheral files of columella, and the epidermis is usually not aligned to the ground meristem by any Körper patterns; we did not confirm this for any of the other members of Nymphaeaceae. The distal cells of the cortex generally abut the base of the columella, although some peripheral cell files appear to be continuous from cortex into columella. The columella and its initials in the transversal zone are generally distinct from the lateral rootcap.

The nymphaeaceous RAM yields a cortex with a nonradial organization and an aerenchyma with a distinctive pattern of origin via expansigeny (see Seago et al., 2000b, 2005). There is no definitive pattern of radial cell alignments across the cortex from the endodermis in *Nymphaea*; the arrangement is more or less random, although groups of 2–3 radially aligned cells can be found in root sections, indicating that there is little restriction of periclinal cell divisions to the endodermis (see Seago et al., 2000b, 2005). Overall, this manifestation is the clearest that there is no single tier of cortical initials in the RAM and that a unique open transversal RAM is present in the Nymphaeaceae.

Tiered or closed basal angiosperm (TB)—Our interpretation of the RAM in the Cabombaceae, as illustrated by both genera, *Brasenia* and *Cabomba*, is a closed RAM with three tiers of initials in basal angiosperm (*TB*) (Figs. 1C, 7) with the epidermis arising at the periphery of the tier or plate of cortical-epidermal initials. There is a very narrow cleft between the epidermis and lateral rootcap (Fig. 7, right arrow); i.e., epidermis and lateral rootcap are not appressed except at or very near the RAM. The rootcap is usually clearly divided into a columellar region and a lateral rootcap region, thus making the initials portion columellar initials and lateral rootcap initials, respectively. The cells of the tier of cortical-epidermal initials overlying the stelar initials are often imprecisely aligned to the cells of the cortex in longitudinal or transverse sections; it is a uniseriate tier of cells in which there are clear cell wall borders between the cortical-epidermal initials and columellar initials distally and stelar initials proximally. There may be an alignment between peripheral cells of the initial tier and the first protodermal and outermost ground meristem (proexodermal) cells via a Körper configuration; our interpretation of Clowes (2000) suggests that he found the same configuration, although he termed it closed like monocots (discussed later).

In transverse section, however, the epidermal cells and cells of the outermost layer of cortex are not usually radially aligned (Fig. 8, vertical arrow), as would be expected if there were a periclinally produced cell division with a Körper configuration for those two specific layers. Figure 8 (horizontal arrow) also shows that some sectors of cortex have radially aligned cells that suggest an endodermal origin, but other sectors do not. Thus, the cortex is not uniformly derived from the tier of cortical-epidermal initials by periclinal divisions of its innermost layer, the future endodermis or proendodermis.

Intercellular spaces are present near the level of initials, and the lacunae begin development by expansigeny immediately proximal to the initials zone and are accompanied by early diaphragm initiation (see Seago et al., 2000b, 2005). These observations for *Cabomba* differ from those that one of us (Seago, 2002) reported for mature roots, but our reexamination of Seago's (2002) epifluorescence specimens confirms our present finding.

Other RAMs in ANAs—The RAM of *Amborella* in the longisections of the 14 adventitious root tips we examined was variable, with some roots having a *CI* (Figs. 1A, 2) and other roots varying from an open *OD* RAM (Fig. 1K) to an open *OCD* RAM (descriptions of *OD* and *OCD* RAMs later under eudicots) with 2–3 irregular tiers of cortical initials and other cells of this zone associated with the columella and epidermis associated with lateral rootcap (Figs. 1J, 3). Transverse sections of the initials region with a lack of radial cell files (Fig. 4) clearly indicate that the cortex is not derived from a tier of initials and arises from multiple initials in cortical portions of the open RAM. Among the Austrobaileyales, we also found closed, monocot types of RAM (discussed later). In some adventitious roots and in all lateral roots of *Illicium* and *Schisandra*, there are separate tiers of initials for root cap columella, epidermis, cortex, and stele (Figs. 1H, 9) in a traditional, four-tiered *ETM* monocot RAM (e.g., Seago et al., 1999; Clowes, 2000); some specimens were *TM* with just three tiers. These findings seem quite unusual and were unexpected. Clearly, this order needs to be more thoroughly investigated.

Basal angiosperm commentary—The roots of basal angiosperms have a variety of RAM types. Within the most basal angiosperms, *Amborella* is somewhat different in that it has some common initials characteristic of conifers (Pillai, 1964) and some eudicot RAM features. The root of *Amborella* obviously needs to be examined in detail developmentally from the origin of the root in stem tissues until cessation of its growth. The Nymphaeales are clearly distinctive and have similarities to monocots. The assignment of *Amborella* and the Nymphaeales as sister groups to remaining angiosperms by Qiu et al. (2006) does not seem to be reflected in the organization of their root apical meristems. An apparent oddity is the monocot-like tiered RAM, along with roots with *CI*, found in species of the Austrobaileyales.

It is understandable why Conard (1905) and Pillai and Kavathekar (1975) interpreted Nymphaeales as having dicot-type transversal meristems. However, because some longisections showed cellular alignments between the initials of the epidermis and those of the cortex, Voronkina (1974: see Figs. 1, 2) interpreted both the nymphaeaceous (*Nymphaea* sp.*, Euryale, Victoria, Nuphar, Barclaya*) and cabombaceous (*Cabomba*) RAMs as being distinctly monocot-like (epidermis associated with cortex in origin), as did Guttenberg and Müller-Schröder (1958) for *Nuphar*. Clowes' (1994, 2000) drawings of even more members of Nymphaeales enabled him to amplify Voronkina's (1974) interpretation of the RAM organization. Their drawings clearly resemble our images (Figs. 6, 7) and the photographs of Seago et al. (2000b, p. 635; 2005, p. 568). Our designation of this RAM as a transversal *OTvB* RAM for the Nymphaeceae differs from Clowes (1994, 2000), but his drawings leave us uncertain about the configuration in *Nuphar* and *Barclaya*.

The unique *TB* RAM of Cabombaceae is not a typical closed RAM, sensu Clowes (1981, 2000) or Groot et al. (2004) because at the stages of development examined there is no clear cell lineage, via Körper patterns, between the layer of RAM cells, i.e., the cortical-epidermal initials, and the cortex. While there may have been a developmental association between the tier of initials and the hypodermal-epidermal tissues, examination of the drawings in Voronkina (1974) and Clowes (1994) can easily support our interpretation. Also, *Cabomba* does not have a separate tier of initials for the epidermis, as Clowes (1990) found for floating monocots.

Our inability to discern a clear eudicot-like RAM in any of the magnoliids is similar to the interpretations of Clowes (2000) in that we found epidermal RAM initials associated with cortex and/or lateral rootcap and that these are highly variable. RAMs with common initials are very difficult to interpret because one cell file may appear aligned by Körper or Kappe patterns proximally, but can be aligned with a different tissue distally. Undoubtedly, there are derivatives of cell divisions that bridge the tissue regions, influencing the appearance of the cell patterns and producing such *CI*. These differences at least partially explain Clowes' (2000) findings that the magnoliids either have monocot type or dicot type epidermal associations. In *Magnolia*, even though heavy lines were used to distinguish RAM regions in the drawing by Voronin (1964), the patterns of cells showed continuity between the distal tips of the cortex and epidermis and a common set of initials. And, Pillai et al. (1965, p. 271) described in detail that *Piper* of the Piperaceae (Piperales, magnoliids), as well as *Grevillea* of the Proteaceae (Proteales, eudicots) and *Amaranthus* of the Amaranthaceae (Caryophyllales, core eudicots), had a "common group of initials." These observations for the Piperaceae conflict very strikingly with Clowes (2000), who reported closed RAMs. Even though Clowes (2000) did not term these RAMs common initials, we interpret such magnoliid RAMs as consisting of common initials, more like some conifers than most other flowering plants (see examples in Wilcox, 1962; Pillai, 1964; Bogar and Smith, 1965).

Groot et al. (2004) used *Aristolochia* as an example of the angiosperm "ancestral intermediate-open type" of RAM. If one magnifies the *Aristolochia* image in their paper (Fig. 3A in Groot et al., 2004), the epidermal files therein may also be aligned with either cortex or lateral rootcap in one or more places, even though they outlined the epidermis with heavy lines. Earlier, Groot and Rost (2001) reported that *Magnolia* and *Drimys* had "intermediate open" RAMs. However, there is nothing "intermediate" about the open magnoliid RAM. For reconciling the discrepancies among the various studies, the magnoliids need to be examined in much greater detail to broaden our understanding beyond Pillai et al. (1965), Clowes (2000), and our surveys.

The monocots—In monocots, RAMs always have their epidermis associated with cortex and not with lateral rootcap production (Table 1; Figs. 1D–I, 13–22).

Open epidermal monocot (OEM)—In the open epidermal monocot RAM, there is a distinct set of stelar initials, delimited by the curving cell files of the pericycle. While the cortical-epidermal initials curve over the stelar initials and appear closed and tiered, there is no clear border between them and the columella of the rootcap. The epidermal part of the RAM abuts irregularly into and contributes to the columellar region of the rootcap, but the columellar initials are not distinct (Figs. 1D, 13), although the columella is usually clearly set off from the lateral rootcap. The lateral rootcap initials are outside the edge of the columellar region and overlying the epidermal initials (e.g., under the arrow in Fig. 13).

In transverse sections, the ground meristem, or young cortex, derived from the initials has only some radial cell files across the inner cortex, and the outer cortex is usually irregularly populated with nonaligned cells (Fig. 14). Even within the RAM zone, there are few radial alignments among cells. This type of RAM might be maintained during root elongation because it
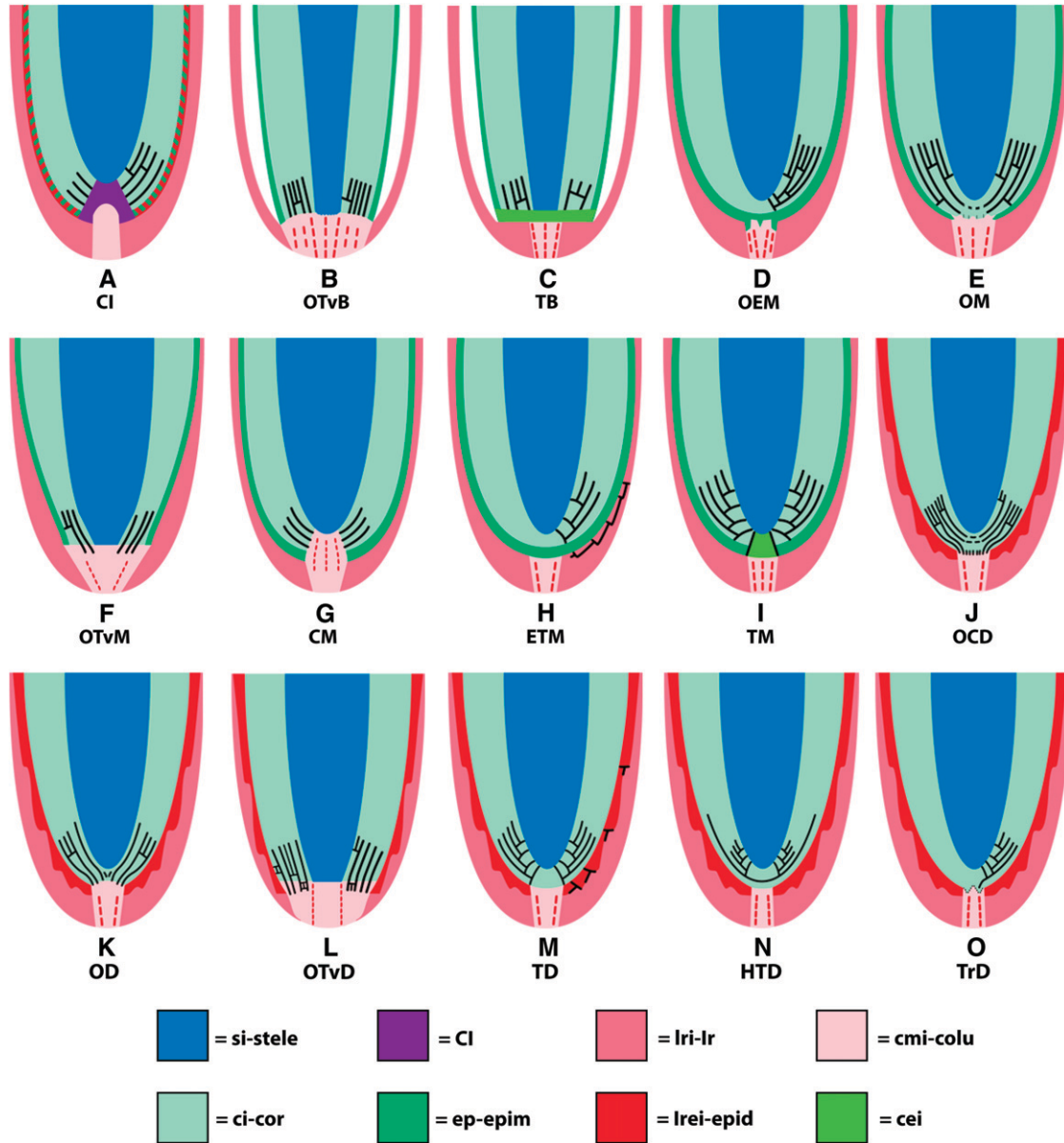
Fig. 1.   Diagram of the RAM types illustrated in the longisections in Figs. 2–35 with some cell lineage lines added. (A) *CI*, common initials. (B) *OTvB*, open transversal basal angiosperm. (C) *TB*, tiered basal angiosperm. (D) *OEM*, open epidermal monocot. (E) *OM*, open monocot. (F) *OTvM*, open transversal monocot. (G) *CM*, closed, untiered monocot. (H) *ETM*, closed, tiered monocot with separate tiers of cortical and epidermal initials. (I) *TM*, tiered monocot with tier of cortical-epidermal initials. (J) *OCD*, open cortical eudicot. (K) *OD*, open eudicot. (L) *OTvD*, open transversal eudicot. (M) *TD*, tiered eudicot with one tier of cortical initials. (N) *HTD*, tiered eudicot with two or more tiers of cortical initials, distal tiers are hypodermal initials. (O) *TrD*, transitional eudicot, a RAM that is changing from closed to open. CI = common initials, epidermal origin uncertain; cei = cortical-epidermal initials, basal angiosperms and monocots; ci-cor = cortical initials and cortex; cmi-colu = columellar initials and columella; ei-epim = epidermal initials and epidermis, monocots; lrei-epid = lateral rootcap-epidermal initials, eudicots; lri-lr = lateral rootcap initials and/or lateral rootcap; si-stele = stelar initials and stele.

has few cell divisions in the initials region that would lead to a complete opening of the meristem; it appears intermediate between typically open RAMs and tiered RAMs, as described later.

These RAMs are most common in several monocot groups (Table 1), especially the Acorales (see Soukup et al., 2005), Dioscoreales, and some Asparagales. Soukup et al. (2005) and Seago et al. (2005) have already shown how similar *Acorus* is to the Nymphaeales in other root traits. Similar RAMs have been shown in the Zingerberales (Pillai et al., 1961b; Pillai,

1963) and in the Musaceae (Pillai and Pillai, 1961b). Pillai (1963) suggested that the open epidermal RAM (our *OEM*) may be ancestral to the closed meristems in other members of the same order.

Closed, untiered monocot (CM)—Members of Pandanaceae, Antheriaceae, and Arecaeae have a RAM in which the columellar initials directly abut the stelar initials, and the ends of the files of epidermis and cortex form walls of a large cup of initials surrounding the base of the distinctive columella (Figs. 1G, 15).
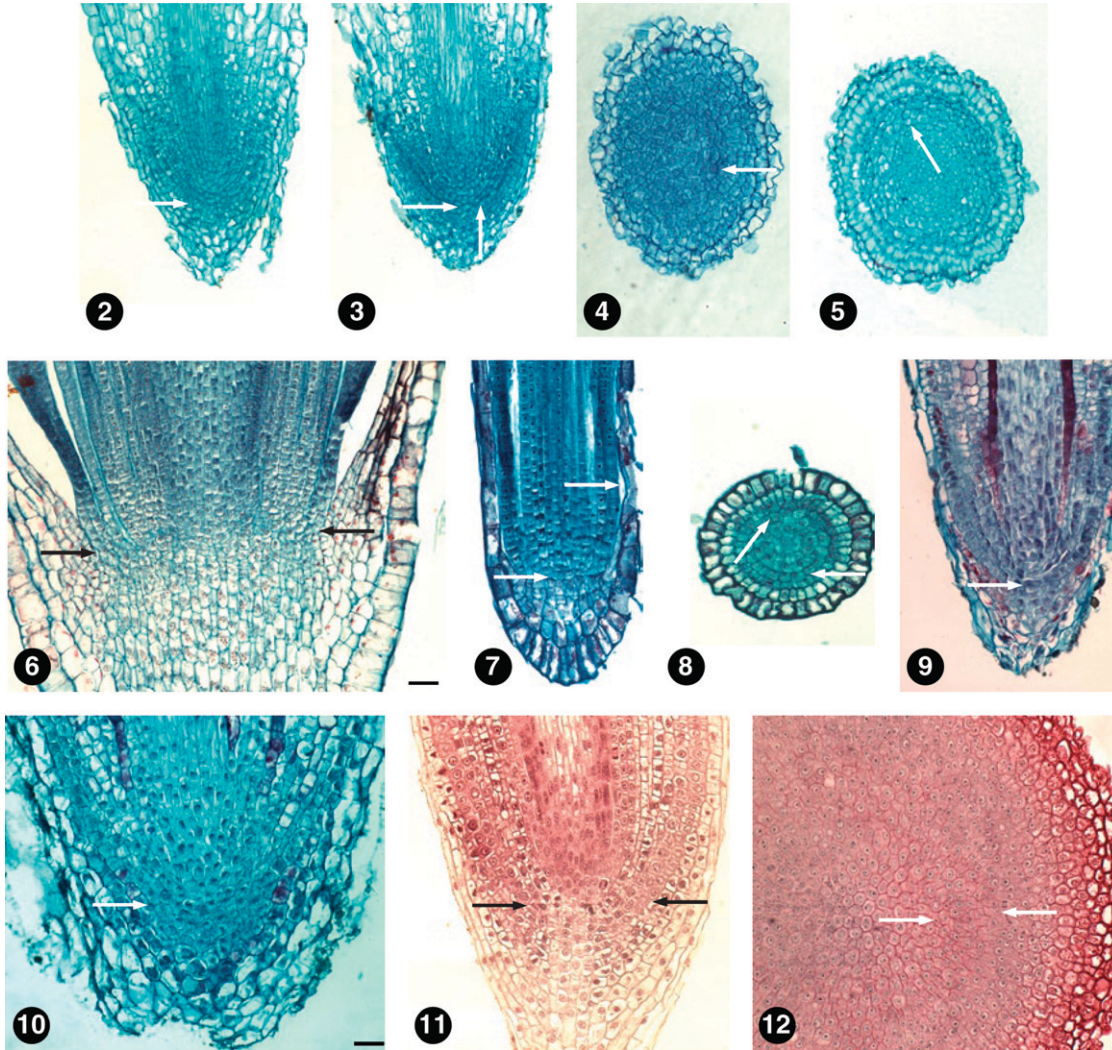
Figs. 2–12.   Light micrographs of root tips of basal angiosperms and magnoliids. All longitudinal sections are median. **2.** *Amborella trichopoda*, Ambo-rellaceae. *CI* RAM at arrow. **3.** *A. trichopoda*. *OCD* RAM. Horizontal arrow points to large group of cells in position of cortical initials, and vertical arrow points to zone where there is no boundary between cortical and epidermal-rootcap portion of RAM. Note, however, connection between epidermis and lateral rootcap above the right arrow. **4.** *A. trichopoda* . Transverse section, 50 μm behind initials. No good boundaries between tissues. Lack of radial arrangement of cells in cortex from endodermal region (arrow) into cortex. **5.** *A. trichopoda*. Transverse section, 200 μm behind initials. Arrow points from endodermis to lack of radial arrangement across cortex. **6.** *Nymphaea odorata*, Nymphaeaceae, Nymphaeales. *OTvB* between arrows. **7.** *Cabomba caroliniana*, Cabom-baceae, Nymphaeales. *TB* with lower arrow on tier of cortical-epidermal initials; note lack of orderliness to cells of tier and little alignment to cells of cortex or epidermis. Upper arrow at narrow cleft. **8.** *C. caroliniana*. Transverse section near level of cortical initials. Right arrow between packets of radially aligned cells; left arrow on boundary between epidermis and cortex and region lacking radial alignments. **9.** *Illicium floridanum*, Illiciaceae, Austrobaileyales. Lateral root *ETM* RAM with four separate tiers of initials for the stele, cortex, epidermis (arrow), and rootcap. **10.** *I. floridanum*. Arrow in region between cortical and columellar part of *CI*. **11.** *Magnolia grandiflora*, Magnoliaceae, Magnoliaceae. RAM with *CI*. Arrow at edge of *CI* where cortex, epidermis, and lateral rootcap all merge. **12.** *Calycanthus floridus*, Calycanthaceae, Laurales. Transverse section at 50 μm. Arrows at junctures between stele and cortex and between cortex and epidermis; note lack of boundaries. Scale bars = 35 μm; all photos at same magnification. Abbreviations as in Figure 1.

In effect, these are cortical initials and epidermal initials, although they are not tiered at all; the lateral rootcap initials reside outside the columella (Fig. 15, left arrow). This RAM is not open because the cortical and epidermal cells never appear as files into or bridge the boundary with the columella. The tips of the epidermis and most files of distal cortex are not well aligned with a discrete tier of initials, and they are distinct and separate from the columellar base. Often, the base of the columella has secondary columellar initials and columella (Fig. 15, upper right arrow). The stele is generally distinct. This RAM appears to be a characteristic of the adventitious aerial roots in some monocots and is, in effect, a different type of closed RAM, but one that is not tiered as defined by Clowes (1961, 1981, 1994, 2000; Guttenberg, 1968; Groot et al., 2004). We suggest that it is probably derived from a tiered, closed RAM (*TM* or *ETM*, described later) either by having the cortical initials and epidermal initials displaced peripherally by lack of cell divisions or by the cells in the positions of those initials being turned into columellar initials. It may be a temporary meristem condition, with active cell divisions restricted to an inverted cup-like region, or a characteristic of particular aerial root phases.

Open monocot (OM)—The Melanthiaceae, some Liliaceae, and other families possess an open RAM (Table 1; Figs. 1E, 16) in which the inner files of cortical initials curve into the RAM and outer files of cortical initials abut against or are even continuous with the columella base and its initials, or the files of cortical initials and the epidermal initials abut directly against the outer base of the columella edges. The lateral rootcap initials are peripheral to this ring of initials. The stelar initials are characterized by the curving files of pericycle into the tip. In transverse section, these RAMs have radial alignments across the inner cortex but rarely in the outer cortex (Fig. 17).

Open transversal monocot (OTvM)—*Clivia* (Amaryllidaceae: Asparagales) represents a RAM with a transverse plate of cells across the tip of the stele (Figs. 1F, 18), much like that described earlier for *Nymphaea* and for legumes (described later), except that the epidermal part of the initials is associated with the cortex, not with the rootcap, and there is no cleft between epidermis and rootcap. Files of cells all across the tip of the cortex can extend into the base of the columella, thus making cortical initials and columellar initials indistinguishable. The cell files into the stelar initials often abut the columella and its initials, and the pericycle does not curve over the tip of the other stelar cells in the typical curved pattern of other open monocot RAMs or the closed RAMs. Otherwise, it is similar to the *OM* RAM of the Liliaceae, for example.

Tiered, closed monocot (TM or ETM)—The tiered, closed monocot RAM has four (*ETM*; Figs. 1H, 19; stelar, cortical, epidermal, and columellar initials with lateral rootcap initials) or three tiers of initials (*TM*; Figs. 1I, 20; stelar, cortical-epidermal, columellar with lateral rootcap). The situation with separate epidermal initials, hence *ETM*, is prevalent in floating aquatic members of the Hydrocharitaceae, but it is not limited to floating aquatics because we also found such four- or more-tiered RAMs with separate epidermal initials in *Thalassia* and in emergent wetland irises, grasses, and cyperads, and in non-wetland *Musa* species and orchids (Table 1). This differs from the findings of Clowes (1990, 1994, 2000), who stated that only floating aquatics produce separate layer(s) of epidermal initials. Clearly, whenever cells in the tier of a RAM with cortical-epidermal initials divide perependicular to the long axis of the root or periclinal to the surface of the stele, a RAM with a proximal tier of cortical initials and a distal tier of epidermal initials would be produced; such an increase from one tier to two tiers was demonstrated by Seago and Wolniak (1976). We do not know whether this depends on age and stage of elongation. Except for damaged or experimentally manipulated roots (e.g., Feldman, 1975; Clowes, 1992; see also Clowes and Wadekar, 1989; Varney and McCully, 1991; Jiang et al., 2003) or, possibly, the situation described for *Elodea* by Kadej (1966) or our *Thalassia* findings (with epidermal and hypodermal initials), we have no indication that monocot roots with closed RAMs ever open, even as they age (see Seago and Wolniak, 1976; Seago and Marsh, 1989). The stele is characterized at its tip by curving cell files, bounded by the tip of the pericycle, which basically encloses the tips of cell files, which in turn are reduced in number. The rootcap often has two zones: the central region distal to the cortical-epidermal initials consists of columellar initials and the peripheral lateral rootcap initials; Fig. 1H illustrates Kappe patterns in the origin of a lateral rootcap without epidermal association, typical of monocots.

In transverse section, monocot roots with tiered RAMs show the beginnings of radial packets of cells at the level of the cortical-epidermal initials or cortical initials and epidermal initials (Fig. 21), and cell files are distinctly radial across the ground meristem and cortex (Fig. 22). These radial files of cells are indicative of a proendodermis dividing by periclinal divisions to produce all layers of the cortex, even the hypodermis initially (Kadej, 1963; Seago and Marsh, 1989).

Monocot commentary—The pattern found in the Acorales, sister to all other monocots, superficially looks closed but has an open configuration; this is also true in the Araceae, sister to other Alismatales (APG, 2003), which have RAMs with a similar configuration (see also Hinchee, 1981). All other Alismatales have tiered *TM* or *ETM* RAMs (see Scott, 1906, for Hydrocharitaceae, Butomaceae, Zosteraceae, and Alismataceae; Schade and Guttenberg, 1951, for Araceae, Hydrocharitaceae, and Lemnaceae; Lee and Chang, 1958, for Alismataceae; Clowes, 1985, for Hydrocharitaceae). Kadej (1966), however, has shown that *Elodea* (Hydrocharitaceae) roots change early in growth from an *ETM* RAM to one like our *OEM*, in which the epidermal tier part of the RAM expands by cell division to produce a secondary columella and then an open *OM*. The changes are so great and so rapid that a description of the RAM depends entirely upon the stage observed; this is not true for *Hydrocharis* (Hydrocharitaceae; see e.g., Seago et al., 1999).

Except for Pandanales, the remaining noncommelinid orders of monocots (APG, 2003; Soltis et al., 2005) have mostly open RAMs.

In the Asparagales, the Orchidaceae and most Iridaceae have closed RAMs with cortical-epidermal initials or cortical initials and separate epidermal initials; like Popham (1966), we found some Iridaceae to have open RAMs, here *OEM*. We might normally assume that the closed RAMs are derived and that the families are derived within the order (APG, 2003; Soltis et al., 2005). The closed RAM of the Orchidaceae has a distinctive bulging of the outer cortical initials into the inner sides of lateral rootcap, curving along the base of the columella (Blackman and Yeung, 1981; Zankowski et al., 1987; Raghavan and Goh, 1995). Most other Asparagales, including Alliaceae (*Allium,* Hoffman, 1933; Jensen and Kavaljian, 1958; Berta et al., 1990; García-Sánchez et al., 1991), Amaryllidaceae, Hyacinthaceae, and Agavaceae, have species with slightly varying open monocot RAMs. *Clivia* (Amaryllidaceae) is so distinctive with its open transversal *OTvM* RAM, even though this is probably derived from some other simpler open RAM. Interestingly, Clowes (2000) reported the RAM of *Allium* (Alliaceae) to be closed, but we state unequivocally that it is open; see also the drawings of Hoffman (1933) and Hayward (1938) in which the primary root looks closed but the adventitious root RAM is open. This could be a difference between primary and adventitious roots or roots grown in soil vs. roots grown in water, but ours were grown in a vermiculite–soil mixture. However, the photographs of *Allium* RAMs in Esau (1953), Jensen and Kavaljian (1958), Berta et al. (1990), and García-Sánchez (1991) strongly support our interpretation. Antheriaceae, however, have closed, untiered RAMs, in which the cortical and epidermal initials surround the sides and base of the columella; this is also characteristic of aerially rooted plants found in Pandanaceae and Arecaceae, but not in aerial roots of Orchidaceae. We disagree with the interpretation of the Pillai and Pillai (1961c) because there is a distinct set of stelar initials; their photographs and drawings, however, illustrate the characteristics we found.
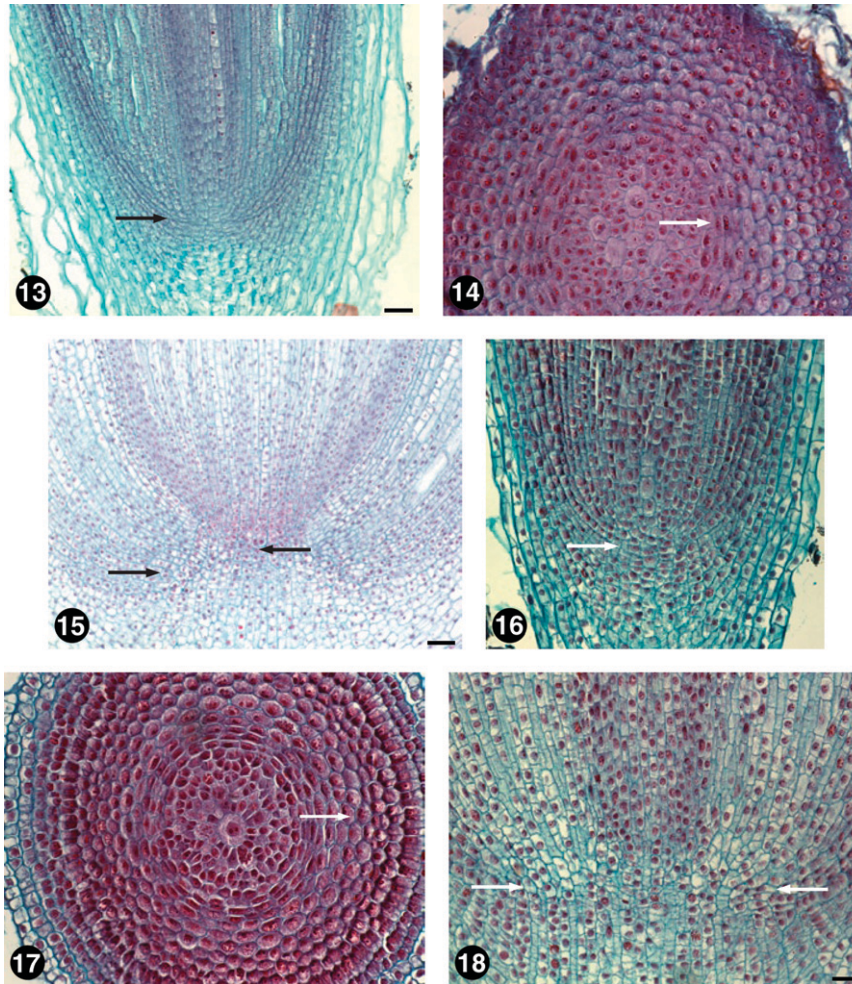
Figs. 13–18.   Light micrographs, root tips of monocots. **13.** *Acorus calamus*, Acoraceae, Acorales. *OEM* meristem with arrow at point of opening between epidermis, where it loses alignment with cortex, and columella. **14.** *Arisaema triphyllum*, Araceae, Alismatales. Transverse section at 250 μm. Arrow tip at endodermis; note radial files of cells only extend for 3–4 cells into cortex. **15.** *Pandanus utilis*, Pandanaceae, Pandanales. *CM* meristem. Arrow at left on tip of epidermis, and arrow on right on base of secondary columella. **16.** *Allium cepa*, Alliaceae, Asparagales. Arrow on edge of *OM* RAM at tip of epidermis. **17.** *A. cepa*. Transverse section, 200 μm behind initials. Arrow from endodermis to middle cortex; note lack of radial alignments beyond middle of cortex. **18.** *Clivia miniata*, Amaryllidaceae, Asparagales. *OTvM* meristem between arrows at tip of epidermis. Abbreviations as in Figure 1.

In the commelinids, most families have closed RAMs. Our representation of the palm root RAM as closed is quite different from that of all other authors, but Pillai and Pillai (1961c, p. 220, Fig. 2) drew a root apex that resembles very strongly our photograph of the unique closed RAM in which the cortical-epidermal initials do not cover the tip of the stele. They also argued that palm meristems evolved from open to closed; we suggest that the closed monocot meristem with surrounding cortical-epidermal initials may represent that earlier RAM. The drawing in Vallade et al. (1983, p. 64) also supports our interpretation, because their rhizodermis (epidermis) clearly abuts a "pericolumelle," which if heavy lines were redrawn, would give a closed but not necessarily tiered RAM.

Zingiberales have been well characterized by Pillai (1963), Pillai and Pillai (1961a), Pillai and Pillai (1961b), and Riopel and Steeves (1964), and there is a trend from an open RAM in *Musa* (Musaceae) and *Strelitzia* (Strelitziaceae) to a closed *TM* RAM in *Canna* (Cannaceae; Pillai and Pillai, 1961a; *Costus*,

Zingiberaceae; Pillai et al., 1961b) and to an *ETM* in *Maranta* (Marantaceae; Pillai, 1963), although our Zingiberaceae had both *OEM* and *ETM* (see also Pillai et al., 1961b).

For the rest of the commelinids, numerous investigators have documented the closed, tiered RAMs of these groups, e.g, Pontederiaceae: *Pontederia* and *Eichhornia* (Clowes, 1985; Seago et al., 2000a); Poaceae: *Hordeum* (Heimsch, 1951), *Saccharum* (Pillai et al., 1974a), *Zea* (Feldman, 1975; Seago and Wolniak, 1976; Jiang et al., 2003), *Avena* (Harkes, 1976), *Oryza* (Kaufman, 1959; Shimabuku, 1960); Xyridaceae: *Xyris* (Pillai and Pillai, 1961d); Typhaceae: *Typha* (Seago and Marsh, 1989); Cyperaceae: *Cyperus* (Kadej, 1963). Some of these also have a separate tier of epidermal initials, even though they are wetland plants with roots submerged in soil and not floating (cf. Clowes, 1990, 1994, 2000).

Eudicots—Root epidermal origin in eudicots is always associated with the lateral rootcap (Table 1; Figs. 1J–O, 23–35).
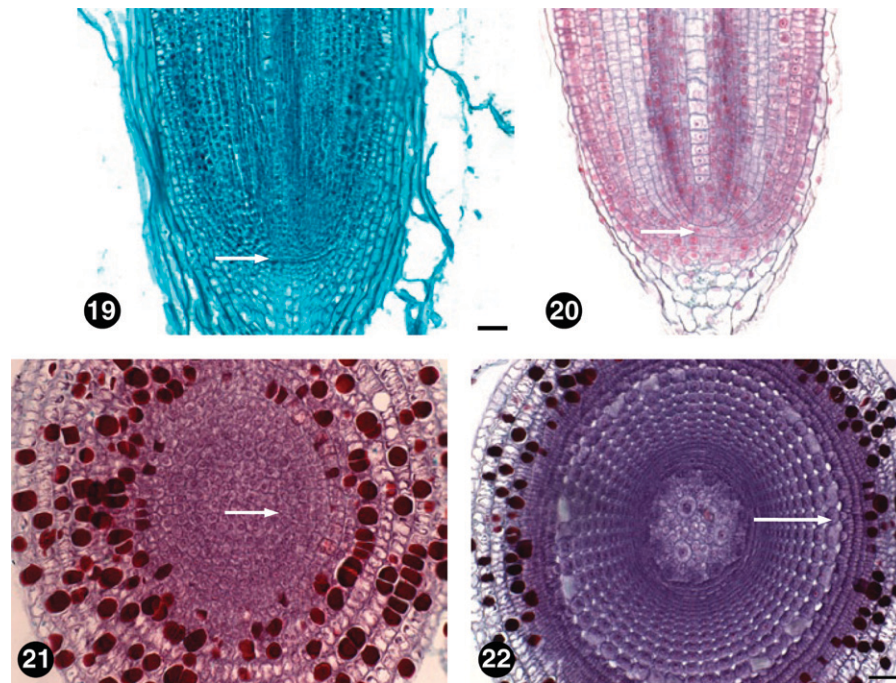
Figs. 19–22.   Light micrographs of root tips from monocots (commelinids). **19.** *Spartina alterniflora*, Poaceae, Poales. *ETM*, arrow on tier of epidermal initials overlying cortical initials. **20.** *Commelina communis*, Commelinaceae, Commelinales. *TM*, arrow on tier of cortical-epidermal initials. **21.** *Pontederia cordata*, Pontederiaceae, Commelinales. Transverse section at cortical initials. Arrow at edge of cortical initials where radial files of cortex are first visible. **22.** *Pontederia cordata*, Pontederiaceae, Commelinales. Transverse section, 200 µm behind cortical initials. Arrow across radial files of cortex to cell layer next to hypodermis. Abbreviations as in Figure 1.

Open cortical eudicot (OCD)—In many eudicots, the initials for the inner cortex overlie the tip of the stelar initials; the mid to outer cortex abuts the peripheral base of the columella or forms a group of initials that bridges the boundary between cortex and columella (Figs. 1J, 23). Figure 24 shows a transverse section in which the inner layers of cortex are radially aligned outside the endodermis, while the mid to outer layers of cortex are irregularly arranged. The stelar initials curve into a tip with a reduced number of files and are bounded by a pericycle. This RAM type is found scattered in several orders of eudicots (Table 1).

Open eudicot (OD)—Here, the cortical initials are an oblique, ringlike set of initials surrounding the base of the columella (Figs. 1K, 25), and the columellar initials abut the stelar initials. Many of these RAMs may be derived from the open cortical dicot RAM or the transitional type RAM during secondary columella formation (see Armstrong and Heimsch, 1976). The initials for the secondary columella may be very narrow as in some Ranunculaceae (Fig. 25) and Plumbaginaceae, quite variable in the Geraniaceae and Malvaceae (Fig. 26), or broad as in Gunneraceae (see Seago et al., 2005) and many legumes, in which they are very similar to the transversal meristem, but lack the truncated tip of the stele and cell patterns (see next section). Mitotic figures in a RAM might represent derivatives bridging any cortical initials and columellar initials boundary (Fig. 26). Many roots had such cell divisions in the cortical and columellar border region that produced derivatives forming a columella base; the lateral rootcap-epidermal initials are distinct from the columellar initials. These kinds of RAMs are very similar to those summarized by Byrne

(1974) for the Malvaceae (see also, Pillai et al., 1961a; Byrne and Heimsch, 1970), but are variable.

Open transversal eudicot (OTvD)—In longisection (Figs. 1L, 27), the *OTvD* forms a zone or plate of cells across the tips of the stele and cortex at the base of the columella; the cortical ground meristem cells, cortical initials, are in files or layers that are often continuous into peripheral portions of the columella (see Wenzel et al., 2001). There are few Körper patterns in the innermost layer or endodermis of the cortex. The central columellar initials abut directly against the initials of the stele, which usually terminate in a truncate, zig-zag cell pattern demarcating the boundary with the columella. The lateral rootcap-epidermal initials are clearly located around the periphery of the transversal meristem and produce the epidermis and lateral rootcap separate from the columella, which is very broad in most legumes and Cucurbitaceae. In transverse sections, the initials region has a broad zone of cells across the tip of the stele (Fig. 28) and no radial cell alignments across the cortex in differentiating (Fig. 29) and mature roots. Often, it is difficult to distinguish the stele from the cortex near the tip (Fig. 28).

This is the characteristic RAM organization of the legumes and some other families (Table 1) (Popham, 1955, 1966; Torrey, 1955; Sun, 1957; Hayat, 1963; Miksche and Greenwood, 1965; Pillai and Sukumaran, 1969; Pillai et al., 1974b; Pillai and Raju, 1975; Allan and Trewavas, 1986; Wenzel et al., 2001), although many legumes also have an *OD* RAM (Table 1) (Patel et al., 1975). The transversal RAM appears to be a modification of the open *OD* RAM, but we could not detail the subtle differences in organization reported by Patel et al. (1975). While Pillai and

Sukumaran (1969) and Patel et al. (1975) referred to a common initiating region for stele and columella, their illustrations clearly reveal separate stelar initials and columellar initials.

An interesting note here is that Esau (1953) even interpreted the legume RAM to be like the open coniferalean RAM, with two zones of initials, one for the stele and a common zone of initials for the cortex and rootcap. However, the legume transversal meristem has a distinctive set of lateral rootcap-epidermal initials, apart from columellar and cortical initials (Popham, 1955; Patel et al., 1975; Clowes, 2000; Groot et al., 2004).

Tiered or closed eudicot (TD or HTD)—A common type of RAM in many derived eudicots is the closed, three-tiered RAM (*TD*, Figs. 1M, 30) in which there are separate tiers of initials for stele, cortex, and columella, or multiple-tiered RAM (*HTD*, Figs. 1N, 32) in which a hypodermis has one or more separate tiers of initials in the cortical initials, stelar initials, and columellar initials ringed by lateral rootcap-epidermal initials. Figure 1M illustrates the Kappe patterns initiating an epidermis and lateral rootcap in eudicots (see Baum and Rost, 1996; Wenzel and Rost, 2001). The stelar initials are always distinctive, have few cells at the tip, and represent the tip of a pericycle, especially in narrow roots. The tier of cortical initials in the RAM (Fig. 30) has a characteristic pattern in which one file of cells becomes two files of cells after a periclinal division, as manifested by Körper configurations (Schüepp, 1917, 1926; Kadej, 1963). The slightly thicker cell walls abutting the stelar initials and columellar initials show that these cells probably had a common history (Clowes, 1961; Barlow, 2002). Wherever the *HTD* RAM is present (Fig. 32), the cortical initials comprise two or more tiers, and the distal tiers beyond the tier overlying the stelar initials produce a hypodermis under the epidermis, i.e., hypodermal initials. There are two tiers of cortical initials in families like Linaceae and multiple tiers of cortical initials in floating aquatics, e.g., Menyanthaceae (Seago and Seago, 2004); again, these findings are at variance with Clowes (1994, 2000).
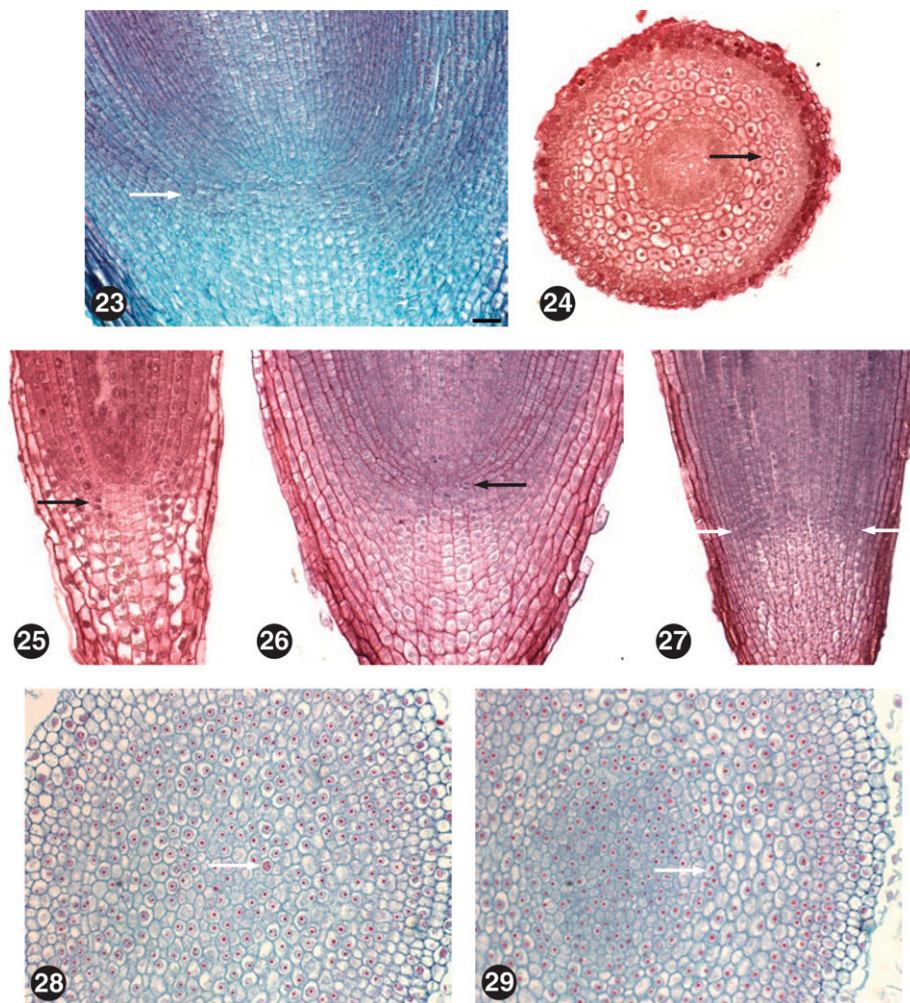
Figs. 23–29. Light micrographs of root tips from eudicots. **23.** *Quercus rubra*, Fagaceae, Fagales. Arrow points to edge of *OCD* meristem where cortex opens to columella base. **24.** *Carya illinoensis*, Juglandaceae, Fagales. Transverse section at 100 μm behind RAM. Arrow points to region where radial cell files in cortex stop. **25.** *Ranunculus muricatus*, Ranunculaceae, Ranunculales. Arrows on edge of *OD* meristem with mitotic figure at boundary of cortical tip and columellar base. **26.** *Hibiscus esculenta*, Malvaceae, Malvales. Open RAM with mitotic figures in cells that bridged boundary between cortical and columellar portions of initials. **27.** *Julbernardia fabiflora*, Fabaceae, Fabales. *OTvD* pattern of organization between arrows. **28.** *Pisum sativum*, Fabaceae, Fabales. Transverse section at level of *OTvD*. Arrow at boundary between stelar and cortical portion of *OTvD*. **29.** *Pisum sativum*, Fabaceae, Fabales. Transverse section, 200 μm behind position of *OTvD*. Arrow at early endodermis layer; note lack of radial cell alignments beyond the arrow. Abbreviations as in Figure 1.

The ground meristem or cortex in transverse section (Figs. 31, 33) has radial alignments of cell files across the entire cortex, except where these are interrupted by groups of cells like laticifers (Seago, 1971). This radial pattern is produced by a restriction of periclinal divisions to the proendodermal layer, the innermost layer of the cortex or ground meristem, as has been described for *Rubus* (Engard, 1944) and *Ipomoea* (Seago, 1971). This feature is important in the roots of flowering plants with closed RAMs, because it means that the proendodermal layer is derived directly from the tier of cortical initials and gives closed RAMs their most distinctive feature. The exception to this can be in the hypodermis in roots that usually have a very broad cortex, where the hypodermis is initiated as a separate tier or tiers (Fig. 33; Table 1; Seago and Seago, 2004, for *Nymphoides*: Menyanthaceae).

Transitional eudicot (TrD)—Many dicot roots have a transitional form of RAM (Figs. 1O, 34) in which the tiers of initials are disrupted at points along the boundary between the cortical initials and the columellar initials. Where some cells in the cortical initials bridge the boundary with the columella, one or more cells extend into the base of the columella. These may or may not be accompanied by transversely oriented cell divisions whose distal products become columellar cells (Fig. 34). The transverse sections, however, still show radial alignments of cell files across the cortex (Fig. 35), except for the hypodermis, which may not have such clear radial alignment. The designation, transitional, implies that a closed RAM with the cortical initials has formed a secondary columella by mostly horizontal cell divisions within cortical initials and is opening (Armstrong and Heimsch, 1976; see also Pillai et al., 1965). One can easily see the similarities between this transitional RAM (Fig. 34, Asteraceae) and the clearly open RAM, as manifested in the Malvaceae (Fig. 26).

Commentary on eudicots—Basal orders—For the families and species we surveyed (Table 1; Appendix S1, see Supplemental Data with online version of this article), we found that, in the sister order to other eudicots, the Ranunculales, the Berberidaceae and Ranunculaceae had open *OD*, *OTvD*, and *OCD* RAMs (Seago et al., 2000b) and Papaveraceae had closed, tiered RAMs, whereas Pillai et al. (1961a) reported a closed, tiered RAM for *Delphinium*, and Maxwell (1893) illustrated closed and transitional RAMs in Ranunculaceae. It is possible that the roots examined by Maxwell (1893) and Pillai et al. (1961a) were so short and young (i.e., newly germinated, 5 mm long) that they hadn't achieved the RAM organization that might be characteristic of slightly older and longer primary roots of the family, or that some species simply might retain radicular RAM organization. It might also be that *Delphinium* is more derived within the family.

The Buxales have open RAMs, but the member of the Proteales examined, *Nelumbo*, has closed RAMs with one (*TD*) or two (*HTD*) tiers of cortical initials. The basalmost tier in *Nelumbo* is not well aligned to the inner cortex; the outer tier does, however, become the hypodermis. We agree with Clowes' (2000) finding for *Nelumbo*.

Nonasterid basal core eudicots—*Gunnera* of the Gunnerales, sister order to other core eudicots (APG, 2003; Soltis et al., 2005) (Table 1), is characterized for the first time as having an *OD* or *OTvD* RAM. In the Caryophyllales, Plumbaginaceae have mostly open *OD* or transitional *TrD* RAMs. The remaining families through Basellaceae have closed *TD* RAMs. Pillai et al.

(1965b) reported open RAMs in *Amaranthus* (Amaranthaceae), whereas we found distinctly closed RAMs in *Achyranthes*. We agree with Clowes (2000) in these regards, in strong contrast to the images of Cactaceae in Freeman (1969) and Rodríguez-Rodríguez et al. (2003) that are like legumes in having *OD* RAMs wherein the pericycle delimits the tip of the stele.

However, in the Saxifragales (Table 1), Hamamelidaceae have open RAMs, Haloragaceae have both open (see also Groot et al., 2004) and closed RAMs (in aquatic members), and Saxifragaceae have closed RAMs.

Among the rosids, Geraniales (Table 1) have open dicot *OD* and transversal *OTvD* RAMs. In Myrtales, there is not a clear trend from open to closed RAMs because the Onagraceae, Lythraceae, and Punicaceae are closed, while Myrtaceae RAMs are open (*OD* and *TrD* in *Eucalyptus* and *Myrtus*) or closed (*TD* in *Psidium* and *Feijoa*). Pillai et al. (1965) also reported closed RAMs in Onagraceae, while Ellmore (1981) illustrated closed, two-tiered cortical initials (our *HTD*).

The eurosid I order Zygophyllales is one of our greatest dilemmas because *Zygophyllum* (Zygophyllaceae) has *OTvD* and *TrD* open and *TD* and *HTD* closed RAMs in its young primary roots; the extreme variability in one genus is unlike anything else we observed. Celastraceae have open *OD* RAMs with columellar initials abutting stelar initials.

In Malpighiales, some groups have open RAMs, but those of other families are mostly closed. In the Linaceae, consistent with previous reports, *Linum*, for example, produces a RAM with a *HTD* organization (Crooks, 1933; Deschamps, 1967; Byrne and Heimsch, 1968; Kadej et al., 1971), but the Euphorbiaceae have distinctly transversal RAMs (Pillai et al., 1961a; but see Raju et al., 1976). The Oxalidales sampled have closed RAMs.

Although Alfieri and Evert (1968) and Anderson and Postlethwaite (1960) claimed that certain legumes have tier-like apices, Fabales have long been known to have a characteristic open RAM (e.g., Janczewski, 1874b; Esau, 1953; Popham, 1955; Pillai et al., 1974b). Clowes (2000) described the legume RAMs as open, and Groot et al. (2004) described these RAMs as basic-open, except for *Trifolium*, which they found to be equivocal-open (cf. Wenzel et al., 2001). We found that legumes have both *OTvD* and *OD* RAMs, consistent with Hayat (1963) and Patel et al. (1975), the latter having illustrated patterns of the apical organization with which we basically agree, although our specimens generally had much broader meristematic portions without the "knees" that they found. Of course, we recognize that our *OD* and *OTvD* meristems may be variations upon a theme; however, the broad tip of the stele without a continuous pericycle makes the *OTvD* RAM different. There are other families that are characterized by this pattern of organization, the most prominent of which is Euphorbiaceae, but we also found it in Cucurbitaceae and in some specimens of Berberidaceae, Gunneraceae, and Geraniaceae, and scattered other families.

In the Rosales, we found many types of RAM (Table 1), some similar to the tiered RAMs in *Rubus* (*HTD*, Engard, 1947, fig. 389, p. 156); all families had species with open RAMs, but transitional *TrD* meristems were also found (cf. Hayward, 1938, and Miller, 1958). Cucurbitales varied from the *OTvD* and *OCD* in Cucurbitaceae (Guttenberg, 1968) to *TD* in Datiscaceae, and Fagales varied from open to closed (see also Clowes, 1954; Vecchierello, 1928; and Verdaguers and Molinas, 1999).

In the Brassicales (eurosid II), Tropaeolaceae (Guttenberg, 1940) and Caricaceae have *OCD* RAMs, but other families are
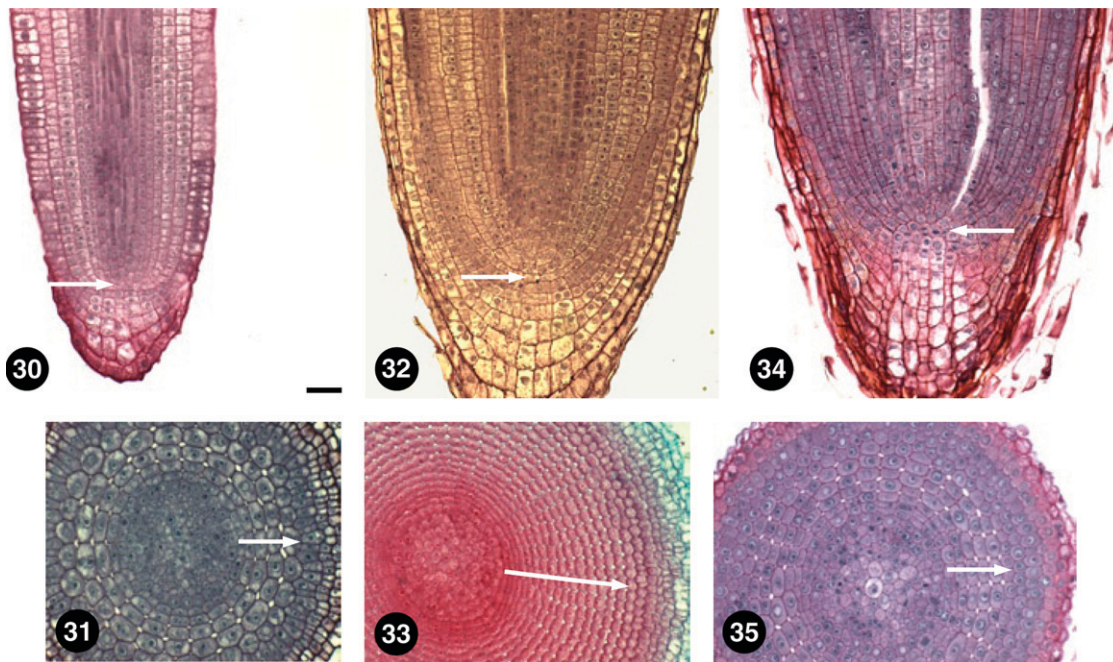
Figs. 30–35.   Light micrographs of root tips from eudicots. **30.** *Roemaria refracta*, Papaveraceae, Ranunuculales. RAM with *TD* apical organization; arrow on the single tier of cortical initials. **31.** *Raphanus sativus*, Brassicaceae, Brassicales. Transverse section, 100 μm behind cortical initials. Direction of arrow shows radial cell alignments across the narrow cortex; epidermis at arrow tip not radially aligned to cortex files. **32.** *Limnanthus douglasii*, Limnanthaceae, Brassicales. Arrow on distal tier of cortical initials, i.e., the hypodermal initials of the *HTD* meristem. **33.** *Nymphoides indica*, Menyanthaceae, Asterales. Transverse section, 100 μm behind cortical initials of *HTD*. Direction of arrow shows radial files; tip of arrow on hypodermis derived from outer tiers of *HTD*. **34.** *Helianthus annuus*, Asteraceae, Asterales. *TrD* meristem. Note the mitotic figures (arrow) producing derivatives bridging the boundary between the cortical and columellar parts of the RAM. **35.** *Xanthium pennsylvanicum*, Asteraceae, Asterales. Transverse section, 250 μm behind the *TrD* with radial cell alignments across cortex evident at arrow. Abbreviations as in Figure 1.

tiered. Malvales and Sapindales, on the other hand, are primarily open in organization (see Pillai et al., 1961a; Byrne and Heimsch, 1970), although Pillai et al. (1961a) also found closed apices. Except for the Sapindaceae (Groot et al., 2004), Clowes (2000) and Groot et al. (2004) are in agreement, although they covered few families.

Asterids—Among the major orders studied (Cornales, Ericales, Gentianales, Lamiales, Solanales, Apiales, Asterales, and Dipsacales), only one basal family, Cornaceae, is characterized only by an open RAM (Table 1), although in the Aquifoliales, Aquifoliaceae have transitional RAMs. The Loasaceae of the Cornales and the majority of other families have tiered, mostly *TD* RAMs. Convolvulaceae (Seago and Heimsch, 1969; Seago, 1971) have tiered or transitional apices, while some Solanaceae (Heimsch, 1960; Popham, 1966; Byrne et al., 1975) also have open RAMs. In Apiales, there is variation in Apiaceae from *OD* (e.g., *Foeniculum*) to *TD* RAMs (e.g., *Anethum*); Esau (1940) illustrated an *OTvD*-like RAM in *Daucus*. In Asterales, the Campanulaceae, Lobeliaceae, Goodeniaceae, and Brunoniaceae all have closed *TD* RAMs. Menyanthaceae, mostly floating aquatics, have *TD* or *HTD* RAMs. Thus, they have apices with 1–3 tiers of cortical initials (Seago and Seago, 2004), making them remarkably similar to floating, aquatic monocots like members of Hydrocharitaceae, even though the Menyanthaceae have columellar and lateral rootcap-epidermal initials, not separate epidermal initials as in monocots.

Interestingly, the most derived families, like Asteraceae and Dipsacaceae, also have species with transitional RAMs (*TrD*)

and with open *OD* RAMs; the occurrence of transitional and open RAMs comes as no surprise, given the findings of Armstrong and Heimsch (1976). Asteraceae vary from *TD* RAM (e.g., *Xanthium*, *Tithonia*, *Helianthus*, and *Ageratum*) through *TrD* (*Cosmos* and most other genera) to *OD* (some *Xanthium, Lactuca, Dahlia,* and *Helianthus* RAMs), much like stages shown by Armstrong and Heimsch (1976; see also Guttenberg, 1968, and Groot et al., 2004). In Dipsacales, Dipsacaceae include, for example, *TD* (some *Cephalaria*), *TrD* (*Scabiosa*), or *OD* (*Dipsacus*) RAMs. Our findings for many of the families in the various asterids are similar to those of Pillai et al. (1965a), Clowes (2000), and Groot et al. (2004). We have expanded the basis for understanding the RAM traits of many derived families.

DISCUSSION

While there is no typical RAM among the angiosperms and basal angiosperms have a broad array of RAM types, only basal angiosperms have common initials. The Nymphaeales have distinctive RAMs with a cortex-epidermis association, shared with some Austrobaileyales and monocots, and a cleft between epidermis and lateral rootcap.

In monocots, root cortex is produced either by tiered cortical initials, cortical-epidermal initials, or cortical portions of various open RAMs, and epidermis is always associated with cortex. In eudicots, cortex is produced by cortical initials or cortical portions of open RAMs, and the epidermis is always a part of

lateral rootcap-epidermal initials. Columellar initials are separate from lateral rootcap initials in monocots and eudicots. Initials for the stele are almost always quite distinct, although in roots with *CI*, *OTvM*, or *OTvD*, the stelar initials may seem to be continuous with columellar initials.

In our survey, tiered or closed RAMs are the only meristem type in 45% of angiosperm families (40% in ANA angiosperms, 0% magnoliids, 54% in monocots, and 45% in eudicots) and occur in 63% of all families; 36% of angiosperm families have only open RAMs, and open RAMs occur in 55% of angiosperm families. Closed RAMs are only slightly more common among families than are open RAMs. This is not the depiction of angiosperm root apices in most texts (e.g., Fosket, 1994; Raghavan, 2000; Leyser and Day, 2003; as contrasted somewhat with Evert, 2006) and in recent articles on molecular, developmental, and genetic aspects of angiosperm RAMs (e.g., Dolan et al., 1993; Benfey and Scheres, 2000; Groot et al., 2004; Ueda et al., 2005; cf. Clowes, 2000).

In addition to analysis of median longitudinal sections by cell lineages (Körper-Kappe patterns), analyzing cells and cell packets derived by cell divisions in or near the initials can lead to an understanding of cell lineages (Schüepp, 1926; Kadej, 1963; Seago and Wolniak, 1976; Barlow, 1987; Rost and Bryant, 1996). Transverse sections of roots aided in our demonstration of traits of open and closed meristems. Except under certain conditions, roots with open meristems have no uniform and regular radial cell file alignments across most of the width of the cortex, although they may have zones of radially aligned cell files, including the inner cortex of roots with some cortical initials overlying the tip of the stele, because the layer of endodermis may produce some layers of cells by periclinal divisions. Cortical initials in closed, tiered roots produce cortices with distinct longitudinal cell files and equally distinct radial files in transverse sections (Engard, 1944; Williams, 1947; Heimsch, 1960; Seago and Wolniak, 1976; Seago and Marsh, 1989). In some open RAMs, i.e., *TrD* and *OCD* meristems, the maintenance of the radial alignments (seen in transverse sections) at differentiating and mature cortical levels reflects the ontogenetic history of the cortex, which earlier was produced in a closed RAM (Seago, 1971; Armstrong and Heimsch, 1976); *OEM* RAMs are similar.

With regard to development and evolution of the cortex, we suggest that at some point there was apparently a restriction of cell additions by periclinal division to the innermost layer of the ground meristem, the future endodermis or proendodermis (Engard, 1944; Heimsch, 1960). This would have led to the characteristic Körper patterns in the root cortex and produced a cortex that, in transverse section, was occupied by radial files of cells (Engard, 1944; Williams, 1947; Heimsch, 1960; Seago et al., 2005; see also Cnops et al., 2000). This is not manifest in *Amborella*, magnoliids, or *Nymphaea*, and only somewhat manifest in the Cabombaceae (*TB* RAM). It is, however, seen in *Illicium* (*ETM* RAM) of the Austrobaileyales. In other words, the angiosperms considered sisters to all other angiosperms, *Amborella* and Nymphaeales (Qiu et al., 2006), do not have precise cell divisions in a tier of cortical initials or a layer of cortex (proendodermis) that can produce a whole cortex with precisely aligned, longitudinal cell files and transverse radial layers, even when the RAM has a tier of initials, as found only in Cabombaceae. This situation represents an ancestral condition.

Epidermal and rootcap origin—Clowes (2000) showed that a very important difference between monocots and dicots was the developmental relation between epidermis and cortex or rootcap. He concentrated, however, on the patterns of trichoblasts and atrichoblasts within the epidermis. Without considering root hair origins, we have shown a separation between columellar and lateral rootcap regions of the meristematic root tip for basal angiosperms, magnoliids, monocots, and eudicots. In eudicots, the columella has a separate set of columellar initials, and the epidermis is intimately associated with lateral rootcap origin (Baum and Rost, 1996; Wenzel and Rost, 2001; Wenzel et al., 2001; Rodríguez-Rodríguez et al., 2003); examination of a photo in Wenzel and Rost (2001, fig. 2a, p. 207) suggests that the sloughing remnants of lateral rootcap-epidermal initials in *Arabidopsis* are separate from the columellar derivatives.

These findings, combined with what we know about cortical and stelar origin, suggest that the initials zone of an angiosperm RAM is large. Barlow (1995) effectively expanded on the early concept of Clowes (1954, 1961) and the evolutionary approach of Hejnowicz (1980) and argued that, while the initials zone of the angiosperm root, or its minimal meristematic region, may be small (see also Guttenberg, 1968), rootcap production would require "initials of greater complexity" (Barlow, 1995, p. 16). We find that the initials zone is large under normal circumstances, but it is larger and far more complex than envisioned by Hejnowicz (1980). The production of the rootcap in differing substrates may well be the key (Barlow, 1995; Hamamoto et al., 2006). Further, the variations in RAM organization are, in part, related to developmental changes in the RAM that occur at different times in the growth of the roots, which in turn are undoubtedly related to environmental factors such as temperature, soil, water, and nutrient availability.

Primary roots from embryos and adventitious roots from shoots—It has been argued that primary root RAMs always derive from embryonic radicles that are closed (see Guttenberg, 1960, 1968; Groot et al., 2004). That is incorrect insofar as the "mature" radicular meristem in legumes, for example, is open (see Pillai and Sukumaran, 1969; Chiang and Tsou, 1974; Patel et al., 1975; Jones, 1977; J. L. Seago, personal observation); note that Sun (1957) shows a photo with a transitional RAM appearance in the radicle of *Glycine*. Many investigators show photographs of open RAMs in mature radicles of embryos, e.g., in *Malva* (Byrne and Heimsch, 1970, although they termed it closed); in *Quercus* (Vecchierello, 1928; Verdaguer and Molinas, 1999; J. L. Seago, personal observations); in legumes like *Pisum* (Reeve, 1948; Jones, 1977; J. L. Seago, personal observations, *OCD* RAM), *Phaseolus* (Chiang and Tsou, 1974), and *Arachis* (Pillai and Raju, 1975).

Many of the various types of RAMs are variations on developmental stages derived from the radicle of the embryo. Embryos often have closed RAMs; for example, there are tiered RAMs with columellar initials and one tier of cortical initials in eudicots like the Brassicaceae (Dittmer and Spensley, 1947; Maheshwari, 1950; Pillai and Pillai, 1977), while the Linaceae (Byrne and Heimsch, 1968) and some Asteraceae (Armstrong and Heimsch, 1976) have radicles with two tiers of cortical initials. In monocots, on the other hand, the epidermis is associated with the cortex in closed, tiered RAMs with cortical-epidermal initials or it is a separate tier of initials (e.g., Bonnett, 1961; Esau, 1977; Clowes, 1978). Lateral rootcap or lateral rootcap-epidermal initials are separate. There is need for detailed examinations of embryos and germinated roots in basal angiosperms and magnoliids.

However, many root tips also lose their closed nature early in embryogenesis when the hypophysis contributes to the cortical and/or epidermal zone as rootcap and other initials (Maheshwari, 1950). Maheshwari (1950) showed that any tiered or closed nature of the RAM can be lost shortly after formation of the meristem from the hypophysis. There, of course, would be early stages in which the meristem is so small that it might be interpreted as a closed meristem; however, some eudicots like *Ranunculus* may never produce a closed and tiered RAM from the hypophysis and cells forming the cortex (see Maheshwari, 1950). RAM types and even the quiescent center may be produced either in late embryogeny, early primary root elongation, or much later (see Clowes, 1961, 1978, 1981), and many types are probably developmentally related. Such examples as the Linaceae (Byrne and Heimsch, 1970) and Asteraceae (Armstrong and Heimsch, 1976) might illustrate how an initials zone, which has already divided transversely to increase tier number, could retain such divisions to open the meristem by secondary columellar production at germination. The timing of the ontogenetic changes may well vary less with phylogenetic position of plants than with habitat because angiosperms evolved more than a 130 million years ago (Barlow, 1995, 2002; Feild et al., 2003, 2004; Friis et al., 2004, 2005).

Opening of tiered apices—While the underlying mechanism of changes in planes of cell division may be the same, changes in planes of cell division (possibly related to hypophysis and radicular contributions to the embryo tip or to parenchyma contributions to adventitious root origin) could result in a second distinct tier of initials. In eudicots, it is the cortical portion of the RAM, the cortical initials (one-tiered or two-tiered), that undergoes cell divisions to open the RAM (Seago, 1971; Armstrong and Heimsch, 1976; Clowes, 1981). In monocots, which first have a tier of cortical-epidermal initials, the result of a change in plane of division in the cortical-epidermal initials results in two tiers of RAM, cortical initials overlain by epidermal initials (Kadej, 1963; Seago and Wolniak, 1976). When there are more than two tiers of cortical-epidermal initials, the intervening tiers are hypodermal initials. Except for Kadej's (1963) depiction of the *Elodea* RAM changes and despite our description of an *OEM* meristem, we know of no other examples of a monocot meristem actually opening from a closed RAM. If it occurs in monocots, it is the epidermal portion of the RAM that must initially undergo the opening, at least in large part, to produce new columella cells.

To proceed from a closed to open meristem, the cells of the RAM overlying the tip of the stele would be capable of dividing transversely and producing columellar cells instead of the usual plane of division that produces files of cells into the cortex. This situation is particularly interesting for monocots because epidermal files of cells arise so differently from epidermis in eudicots (Kappe configuration associated directly with lateral rootcap production) that one cannot attribute the same kind of mechanism to monocots in which those types of Kappe configurations occur only in lateral rootcap production; hence, the fundamental difference between monocots and dicots described by Clowes (1994, 2000). The restriction in the plane of cell division normally attributed to cortex expansion by periclinal divisions in the proendodermal layer of cortex is equally important in establishing a characteristic radially aligned cortex (closed, tiered RAMs), hence maintenance of tiered RAMs.

However, the closed RAM appears to be primarily a derived condition in monocots and eudicots. The closed RAM is prob-

ably a continuation of the radicular organization, maintained by quiescence (Clowes, 1978, 1981); transition to open RAMs via transitional RAMs occurs in early primary root elongation in many species (e.g., Seago, 1971) and much later in others (e.g., Baum et al., 2002).

Where the cortical initials overlying the tip of the stelar initials divide to produce the initials for a secondary columella in eudicots, the lateral rootcap-epidermal initials remain basically unchanged because they are already peripheral to the columella. In monocots, it does not appear that columellar initials are usually associated with lateral rootcap-epidermal initials at any stages of root growth, which means that initials for the columella are generally distinct from the initials for the lateral rootcap, regardless of whether or not it is associated with epidermis. In other words, the columellar initials are a distinct zone of initials in most angiosperm roots, as can be seen in Cactaceae (Rodríguez-Rodríguez et al., 2003).

Rootcap and habitat—Haberlandt (1914) had long ago noted the importance of the origin of the rootcap, vis-à-vis the other initials, in possible phylogenetic and ecological contexts; he regarded the closed RAM pattern with separate rootcap initials as the probable progenitor of the other types of meristem organization that he and earlier plant anatomists characterized for angiosperms. Recently, Hamamoto et al. (2006) observed a relationship between rootcap cell sloughing and RAM organization with regard to the open vs. closed patterns of RAM in selected eudicots. The open RAMs of many of the early angiosperms may have made it easier for them to slough rootcap cells (see Hamamoto et al., 2006), but what evolutionary advantage would accrue to closed meristems and their patterns of rootcap cell sloughing in soil? The association of rootcap origin and the nature of the sloughing cells need to be examined in the context of contributing initials.

On the one hand, many basal angiosperms and many basal eudicots have open RAMs. On the other hand, Asterales and Dipsicales have transitional and open RAMs (see also Armstrong and Heimsch, 1976; Groot et al., 2004), i.e., a return, evolutionarily, to the open meristem from the closed RAM. Do these factors relate to changed habitats? Important clues to the evolution of the first magnoliids, monocots, and eudicots and to the differences between closed and open root apical meristems with regard to rootcap structure and function may come from careful studies on the nymphaealean and amborellan roots, as well as from studies on plant groups such as Hydatellaceae, recently described as sister to the Nymphaeales (Rudall et al., 2007).

Evolution of angiosperm roots—Many roots of basal angiosperms have apices that tend to strongly resemble the open *CI* RAMs of many gymnospermous plants in which there are no clear boundaries between epidermis, cortex, and lateral rootcap, nor even clear sets of stelar initials (Esau, 1953; Pillai, 1964). This state appears to be the ancestral one. We suggest that the highly variable *Amborella* and open magnoliid RAMs might be ancestral to dicotyledonous RAMs with a lateral rootcap-epidermis association. Nymphaeaceous RAMs (*OTvB*, *TB*) could be ancestral to *Acorus* and araceous *OEM* type in basal monocots and closed *TM* or *ETM* RAMs of Austrobaileyales and Alismatales and so on. A plate of imprecisely aligned initials across the tip of the stele at the base of the rootcap could easily become a tier of cortical-epidermal initials or cortical initials. Irregular cell alignments in this plate could produce

ground meristem development, resulting in scattered radial organization across the cortex; i.e., there is no active proendodermis with periclinal divisions because there are no regular planes of division in this apparent tier of cortical-epidermal initials. Changes in planes of divisions, possibly during embryonic development (interaction between hypophysis and cortical-epidermal regions of the radicle) or root origin from rhizome tissues (in bundles or vascular cambium), could result in a distinct tier of cortical-epidermal initials to give a monocot *TM* RAM, or a distinct tier of cortical initials, overlain by columellar initials in the rootcap. The lateral rootcap initials would be peripheral, ringing the base of the columella; in eudicots, it would become lateral rootcap-epidermal initials. These could then produce the characteristic Körper-Kappe patterns associated with the root apex (Clowes, 1950, 1961; Kadej, 1963, 1966). These cell division patterns would have impacted the cortex, epidermis, and rootcap, especially in the orderly radial arrangement of cortex cells in transverse section and in lateral rootcap regions (see Hamamoto et al., 2006, for the implications of the variation in production of border cells).

Another aspect of the evolution of the root and its RAM is the habitat of the plants as the angiosperms were evolving. Feild et al. (2003, 2004) believe that the first angiosperms grew in moist, tropical/semitropical understory conditions and were probably highly adapted to disturbed situations. Meanwhile, the fossil record, while minimal, points to Archaefructaceae (Sun et al., 2002) and ancestors of Amborellaceae, Nymphaeaceae, Cabombaceae, Illiciaceae, Austrobaileyaceae, and Chloranthaceae as early angiosperms (e.g., Friis et al., 2001; Gandolfo et al., 2004; Soltis et al., 2005). Thus, among the first flowering plants were aquatics growing in full sunlight (Friis et al., 2004, 2005; Feild et al., 2005) or terrestrial plants in "dark and disturbed" habitats in moist soil (Feild et al., 2004). Therefore, if roots of extant members of these families are at all similar to their extinct precursors, then vegetative reproduction and adventitious rooting would have been very important, especially in basal angiosperms and monocots (Conard, 1905; Arber, 1920; Les and Schneider, 1995), and the monocot RAM, characterized by the association of cortical and epidermal origins, may have originated from roots with a meristem characteristic of the Nymphaeales, but without a cleft between epidermis and lateral rootcap. This cleft may represent an early aquatic plant adaptation that was lost in derived plants.

Which RAM type might be ancestral, the *Cabomba* tiered *TB* or *Nymphaea* open transversal *OTvB*? We do not know, but if early Cabombaceae preceded the early Nymphaeaceae evolutionarily (Qiu et al., 2006; Yoo et al., 2005), the *Cabomba* tiered *TB* RAM may be ancestral to the *Nymphaea* open *OTvB* RAM. As noted, this *TB* RAM does not have the cell patterns of a typical closed, tiered RAM of either monocots or eudicots. Rather, as we noted often earlier, the *Cabomba TB* RAM is just a plate of mostly nonaligned cells across the tip of the stele that is associated with cortex and epidermis. Thus, it might be easy to conceive of it as evolving into the *Nymphaea* type and/or into monocot types.

Because aerenchyma development in the Nymphaeaceae and Cabombaceae is quite similar, has unique cell divisions and enlargements leading to lacuna via expansigeny, and does not rely on a tier of cortical-epidermal initials with cell divisions restricted to a proendodermis (Seago et al., 2005), it is again plausible to conceive of a *TB* RAM developing into an *OTvB* RAM, although the reverse is also conceivable. Further, the developmental and structural similarities between roots of Nymphaeales

and Acorales, sister to the monocots (APG, 2003; Soltis et al., 2005), are now well established (see Clowes, 2000; Seago et al., 2000b, 2005; Seago, 2002; Soukup et al., 2005).

In conclusion, although the recently reclassified Hydatellaceae (Rudall et al., 2007) should be studied more (root organization is not apparent), we suggest that *Cabomba*, in particular, would be the appropriate plant for researchers interested in molecular development and genetics because it has very small aquatic roots with well-defined RAMs and tissue regions. More complete analyses of the *Cabomba* root could lead to a better understanding of the origin, development, structure, and function of root apices, at least in early angiosperms, if not also in monocots. Clearly, botanists will not understand angiosperm root evolution well until the roots of the basal-most members are studied from these perspectives.

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