Anatomical aspects of angiosperm root evolution

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Background and Aims Anatomy had been one of the foundations in our understanding of plant evolutionary trends and, although recent evo-devo concepts are mostly based on molecular genetics, classical structural information remains useful as ever. Of the various plant organs, the roots have been the least studied, primarily because of the difficulty in obtaining materials, particularly from large woody species. Therefore, this review aims to provide an overview of the information that has accumulated on the anatomy of angiosperm roots and to present possible evolutionary trends between representatives of the major angiosperm clades.

Scope This review covers an overview of the various aspects of the evolutionary origin of the root. The results and discussion focus on angiosperm root anatomy and evolution covering representatives from basal angiosperms, magnoliids, monocots and eudicots. We use information from the literature as well as new data from our own research.

Key Findings The organization of the root apical meristem (RAM) of Nymphaeales allows for the ground meristem and protoderm to be derived from the same group of initials, similar to those of the monocots, whereas in Amborellales, magnoliids and eudicots, it is their protoderm and lateral rootcap which are derived from the same group of initials. Most members of Nymphaeales are similar to monocots in having ephemeral primary roots and so adventitious roots predominate, whereas Amborellales, Austrobaileyales, magnoliids and eudicots are generally characterized by having primary roots that give rise to a taproot system. Nymphaeales and monocots often have polyarch (heptarch or more) steles, whereas the rest of the basal angiosperms, magnoliids and eudicots usually have diarch to hexarch steles.

Conclusions Angiosperms exhibit highly varied structural patterns in RAM organization; cortex, epidermis and rootcap origins; and stele patterns. Generally, however, Amborellales, magnoliids and, possibly, Austrobaileyales are more similar to eudicots, and the Nymphaeales are strongly structurally associated with the monocots, especially the Acorales.

Key words: Anatomy, angiosperms, cortex, epidermis, evolution, roots, vascular tissue.

INTRODUCTION

Evolutionary origin of the root

A root is a highly differentiated multicellular axis found only in the sporophytes of vascular plants that typically has a rootcap, endodermis, pericycle and lateral roots. It is the main organ in vascular plants that anchors the plant body to its substrate and absorbs water and dissolved minerals to support growth and development. The free-living gametoophytes of bryophytes, lycophytes and monilophytes grow on moist environments, and anchorage is accomplished by a system of unicellular or undifferentiated multicellular rhizoids. Evolutionarily, the root seemed to be the last of the three main vegetative organs to evolve, perhaps since early land plants grew on or near the water and so much of their early innovations were geared toward maximizing photosynthesis through development of stems and leaves. Vascular plants evolved at least during the Silurian based on the oldest known macrofossil – Cooksonia (Lang, 1937; Edwards et al., 1992), but there is no record of specialized root axes in this Period (Gensel et al., 2001; Raven and Edwards, 2001). In the Early Devonian, several early land plants already had well developed stems and leaves, but only structures with considerable similarity to roots are known, such as those of Asteroxylon mackiei (Rayner, 1984; Li and Edwards, 1995; Gensel et al., 2001; Bennett and Scheres, 2010). Although no rootcap was observed from the rooting structures of lycophytes during the Early Devonian, they had sub-terranean parenchymatous axes which performed the functions of roots and so these plants have been considered to have possessed roots (see Raven and Edwards, 2001). Bennett and Scheres (2010) suggested that these horizontal axes later evolved rootcap meristems, and so rootcap formation was a separate innovation that allowed penetrating growth into the soil. There is no corresponding evidence for root-like structures for the eufyloophytes (monilophytes and seed plants) during the Early Devonian, and the earliest convincing evidence of root in this group is from Lorophyton goense, a Middle Devonian fern-like (cladoxylalean) plant (Fairon-Demaret and Li, 1993). Roots of extant lycophytes and eufyloophytes possess a rootcap that is derived from and functions to protect the root apical meristem (RAM), and root hairs are present on the epidermis of all major vascular plants (Jones and Dolan, 2012). Their endodermis ensures a one-way mode of water transport into the plant, while the pericycle is usually where the lateral roots originate.
It has been hypothesized that roots evolved at least twice, once in the lycophytes and another in the euphyllophytes (Bierhorst, 1971; Kenrick and Crane, 1997; Gensel et al., 2001; Raven and Edwards, 2001; Boyce and Knoll, 2002; Jones and Dolan, 2012; Pires and Dolan, 2012). Phylogenetic analysis that integrated key fossil taxa with extant lineages shows that the roots of lycophytes and euphyllophytes are analogous (Fig. 1) (Friedman et al., 2004). This suggests that the common ancestors of both lycophytes and euphyllophytes lack roots, and so the possession of roots must be the result of exhibiting the same developmental patterns in response to a similar selective pressure (Friedman et al., 2004). Therefore, there is strong support for the independent origin of roots in land plants. However, it is still not clear how many times roots evolved within the lycophytes and euphyllophytes. In plants with pyramidal root apical cells [e.g. monilophytes and some lycophytes (Selaginella)], lateral roots arise from the endodermis, whereas in plants with one or more superimposed initials [e.g. seed plants and some lycophytes (Lycopodium)], lateral roots originate from the pericycle. The orientation and origin of the embryonic root axis relative to the shoot axis are different in monilophytes and seed plants (Gensel et al., 2001; Raven and Edwards, 2001). Specifically, the embryonic root subtends the embryonic leaf in ferns (Cooke et al., 2004) and, although the root is formed on the basal pole of the embryo in angiosperms, it is derived from the hypophysis in eudicots and ground meristem in monocots (Bennett and Scheres, 2010). All these suggest multiple origins of the roots and therefore warrant further investigations.

**Developmental origin of the root**

The early vascular plants were composed of dichotomously branched parenchymatous axes or telomes based from the Rhynie Chert fossils. In the Zosterophylla of the Early Devonian, most of the branches were erect and functioned as the main organ for photosynthesis (shoot-like), whereas other branches were oriented horizontally and rested on the surface of the substrate. Some of the horizontal axes were directed downwards and were root-like in appearance. Based on this, the roots of extant lycophytes and euphyllophytes probably arose from an original dichotomizing axis, particularly from the horizontal branches that penetrated the substrate, anchored the plant body, and absorbed water and dissolved minerals. Thus, roots and shoots are considered homologous since they are both believed to have evolved from the same
dicotomously branched axes (Gensel et al., 2001; Friedman, 2004; Bennett and Scheres, 2010).

Molecular, developmental and genetic analyses are congruent with the fact that, at least in angiosperms, roots and shoots are derived from the same dichotomizing axes (Friedman, 2004). In Arabidopsis thaliana, these two organs share many developmental processes in common such as the: (1) formation of the endodermis which both require the expression of SCARECROW and SHORTROOT genes (Pysh et al., 1999; Nakajima et al., 2001); (2) regulation of radial patterning of the ground tissues during and post-embryo formation (Fukaki et al., 1998; Wysocka-Diller et al., 2000); (3) specification of epidermal cell fate, e.g. GLABRA2 in conjunction with WEREWOLF genes (in roots) and its functionally redundant parologue GLABRA1 (in shoots) are required for the differentiation between hair- and non-hair-bearing cells in roots and shoots (Dolan and Scheres, 1998; Schiefelbein, 2003; Bruex et al., 2012); (4) provascular cell fate commitment (ScarPELLa et al., 2000); and (5) similar mutations affecting both the RAM and the shoot apical meristem (SAM) (Ueda et al., 2004).

It is becoming clear that the production and maintenance of stem cells in the RAM and SAM involve the same developmental patterns (Mayer et al., 1998; Haecker et al., 2004) and expression of the same genes or class of genes including SCARECROW (Wysocka-Diller et al., 2000), WUSCHEL RELATED HOMEOBOX (Haecker et al., 2004), HALTED ROOT (Ueda et al., 2004) and the CLAVATA3/ENDOSPERM SURROUNDING REGION-related gene family (Miwa et al., 2009). The stem cells in the RAM and SAM are located in regions referred to as the quiescent centre and organizing centre, respectively, which are the signalling centres that make up the stem cell maintaining microenvironments in both meristems. These two regions are considered to be functionally equivalent (Baurle and Laux, 2003; Byrne et al., 2003; Haecker et al., 2004; Veit, 2004; Bennett and Scheres, 2010). There are many similarities in the organizations of the RAM and SAM, and the signalling components required for stem cell initiation and maintenance seem to be relatively conserved. This suggests that similar genes or classes of genes have been co-opted for use in both types of meristems (Friedman et al., 2004; Bennett and Scheres, 2010). Bennett and Scheres (2010) have proposed a mechanism for how the RAM and SAM of monilophytes and seed plants descended from the ancestral generic meristem of a dichotomizing axis.

Origin of genes involved in root development

The genome sequence of the model bryophyte Physcomitrella patens has provided the opportunity to probe the genes, gene expression patterns and development associated with the gametophyte phase of the plant life cycle. It also has allowed for the comparison of sequences and analysis of evolutionary trends across phyla and between the gametophyte and sporophyte generations of the plant life cycle. Molecular genetic analysis of root and shoot development does not only suggest homology of roots and shoots, but also that genes involved in the formation of gametophyte-related structures in ancestral plants were co-opted to perform regulatory roles in the formation of sporophyte-related structures in more evolutionarily derived plants. This is exemplified by the ROOT HAIR DEFECTIVE 6 (RHD6) and RHD6-LIKE 1 (RSL1) genes that control the differentiation of roots hairs in A. thaliana, and two similar genes (PpRSL1 and PpRSL2) that are involved in rhizoid development in P. patens (Menand et al., 2007). Rhizoids and root hairs both elongate by tip growth and fulfill similar functions (Jones and Dolan, 2012). However, rhizoids are gametophytic filamentous structures from the gametophytes, whereas root hairs are sporophytic tubular projections from root epidermis. It appears that genes that promoted the development of cells with rooting functions in the gametophytes of bryophytes were co-opted in the development of the sporophytes of vascular plants or their ancestors, perhaps through gene duplication and sub-functionalization. Once expressed in the sporophyte, they promoted the development of hairs on roots. It is also possible that changes in the cis-regulatory regions of RSL1 genes played a role in altering their expression patterns, i.e. promotion of their transcription in the sporophytes and repression of their transcription in the gametophytes (Jones and Dolan, 2012). The elaboration of the sporophyte generation of the vascular plants and particularly the large radiation of morphological forms that occurred during the Devonian may have been achieved in part through the recruitment of genes or genetic networks that had previously evolved and functioning in the gametophyte generation of early land plants or their ancestors. The demonstration that similar genes control the development of cells with a rooting function suggests that gene recruitment is an ancient mechanism (Edwards and Feehan, 1980; Menand et al., 2007). Therefore, RHD6 and RSL1 genes control the development of cells with rooting functions in bryophytes and angiosperms. However, it remains to be seen if these genes will exhibit the same function in lycophytes, monilophytes and gymnosperms. Also, it is likely that there are other genes involved in the development of roots and other structures in the sporophyte that were recruited from the gametophyte. Therefore, investigations into the molecular genetics of root structure and development in basal land plants will address the questions regarding homology of roots and shoots and multiple origins of the roots. Our understanding of the diversity of root anatomical structures will also help in formulating or substantiating possible evolutionary relationships.

Angiosperm root anatomy and evolution

Much work has been done on the anatomy and development of roots of extant angiosperms, particularly from model herbaceous species such as A. thaliana and Oryza sativa, and other species of economic importance such as Zea mays and Glycine max, plants that are considered to be more evolutionarily derived. To bridge the gap in our understanding of root structures, development and evolution, a review of our knowledge of this subject that includes the major clades of the angiosperms is necessary. Therefore, this article will cover root structures from plants representing the basal angiosperm lineages, such as Amborellales, Nymphaeales and Austrobaileyales (Heimsch and Seago, 2008), and representative magnoliids, monocots and eudicots. Data will be derived from the literature, including our articles, and from some new research,
with methods, including brightfield and epifluorescence photomicroscopy, from Seago et al. (1999b, 2005), Seago (2002), Soukup et al. (2005) and Heimsch and Seago (2008).

Formation of below-ground axes from rhizomes into roots has been considered with regard to various ecological and functional aspects of evolutionary phenomena (e.g. Cairney, 2000; Raven and Edwards, 2001; Brundrett, 2002; Sperry, 2003; Jackson et al., 2009; Pires and Dolan, 2012). However, more generally with regard to angiosperms, researchers have examined structural features such as the transition from tracheids to vessel elements and specific xylem cells associated with water/mineral conduction (Carlquist, 1975; Carlquist and Schneider, 2001, 2002, 2009; Schneider and Carlquist, 2002), RAMs (Phillipson, 1990; Barlow, 1994, 2002; Clowes, 2000; Groot and Rost, 2001; Groot et al., 2004; Heimsch and Seago, 2008) and, to a lesser degree, the roots (Bell and Bryan, 2008, p. 126; for nature of seminal cells associated with water and have been previously characterized (Seago, 2002; Seago et al., 2000b, 2005; Carlquist and Schneider, 2002, 2009). The salient traits are pentarch to polyarch stele in Nymphaeaceae (Fig. 3D, E) and often monarch stele in the extremely small roots of the Cabombaceae (Fig. 3C, two xylem cells) and Hydatellaceae (Fig. 3A; cf. Arabidopsis of eudicots, Baum et al., 2002). However, Conard (1905) described diarchy in Nymphaea species, but such a condition has not been confirmed because of the ephemeral nature of the primary roots. Distinctive expansigenous aerenchyma is present in the Nymphaeaceae and Cabombaceae. An endodermis with CBs and sometimes a small amount of SL, prominent astrosclereids of the Nymphaeaceae and their absence in other Nymphaeales, and uniseriate exodermis with CBs and SL (Fig. 3D, E; Seago et al., 2005) are also salient features of the Nymphaeales.

Trithuria filamentosa, a representative of the family Hydatellaceae, usually has one central tracheid surrounded by phloem (Fig. 3A) and pericycle (our results; Rudall et al., 2007), but we have also observed two tracheids in the stele; xylem of T. filamentosa lacks vessels, unlike the Nymphaeaceae and Cabombaceae (Carlquist and Schneider, 2009). We show here clearly that T. filamentosa has a cortex with an endodermis with CBs in anticlinal walls, and, like Cabomba, the SL are more prominent on the outer tangential walls (note: root endodermis is continuous with the stem endodermis). In the Hydatellaceae, the cortical aerenchyma is derived through schizogeny, followed by expansigeny (Fig. 3B, right insert). There is an exodermis with CBs and SL under the epidermis (Fig. 3A).

A brief depiction of the RAM and root tip of T. filamentosa is necessary; there is a closed, three-tiered meristem with a distinct, distal tier for the very small rootcap, a separate tier of ground meristem/protoderm for cortex and epidermis, and the proximal tier is for the procambium (Fig. 3B; Heimsch and Seago, 2008). Its RAM appears slightly more monocot like (tied basal angiosperm in Heimsch and Seago, 2008) than Cabomba-like (tied basal angiosperm in Heimsch and Seago, 2008). The root tip of T. filamentosa does not have a cleft separating the lateral rootcap from the epidermis, and the rootcap and its portion of the RAM overlie the protoderm/ground meristem tier (Fig. 3B). This is very similar to the closed or tiered RAM of the monocots under the characterization (and also Clowes, 2000). In T. filamentosa, the tier for ground
meristem/protoderm is most clearly aligned with the epidermis, and there is a very clear distinction between the rootcap initials and the ground meristem/protoderm. The images of newly germinated *T. submersa* primary roots in Rudall et al. (2009) and in Friedman et al. (2012; their fig. 6A) appear to be very similar to our image in Fig. 3B. Further, Fig 3B (left insert) shows a radial arrangement of cortical cell files that are like monocots derived from tiered monocot RAMs (Heimsch and Seago, 2008); this distinctive radial cortex is not usually found in the Cabombaceae (Seago, 2002; Seago et al., 2005; Heimsch and Seago, 2008). Strangely, the RAM and root tip of some Lemnaceae (Araceae, Alismatales) are very similar to the *Cabomba*-tiered basal angiosperm-type RAM with a cleft between the lateral rootcap and epidermis (see Landolt, 1998), except that its RAM has more pronounced tiered monocot cell lineages like *Trithuria*.

**Astrobaileyales**

*Illicium floridanum* is used here as a representative of the order. Like other members of this clade, it is terrestrial (Thomas, 1914; Metcalfe, 1987); its lateral roots are often small and diarch, with early-maturing phloem fibres and an endodermis with CBs and SL delimiting a very small cortex with some radial cell patterns and without an exodermis (Fig. 2E). Its adventitious roots are mostly diarch (Metcalfe, 1987), but tetrarch patterns are also found, and early secondary growth produces secondary xylem with vessel elements, a narrow phloem region without noticeable pericyclic development into phellogen, and a redivided endodermis, fully complete with CBs and even thick SL (Fig. 2F); and an extensive hypodermis which is probably exodermal. These roots show a much more extensive cortex with irregular cell patterns, typical of an open RAM origin for cortex, although...
lateral roots have monocot-like RAMs (Heimsch and Seago, 2008).

**Magnoliids**

This clade is represented by four orders, i.e. Canellales, Laurales, Magnoliidae and Piperales (APG, 2009). However, since there is no information on the roots of the Canellales, we will deal only with the representative families from the other three orders: Calycanthaceae, Lauraceae, Magnoliaceae, Annonaceae, Aristolochiaceae, Piperaceae and Saururaceae. The magnoliids are terrestrial and their primary and lateral roots vary from diarch to hexarch (present study; Thomas, 1914; Metcalfe and Chalk, 1950a, b; Metcalfe, 1987); they are

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**Fig. 3.** Basal angiosperms, Nymphaeales: (A) *Trithuria filamentosa* – monarch stele, nine-celled endodermis with Casparian bands (CBs) and suber lamellae (SL), mid-cortex with aerenchyma and collapsed radiating cells, exodermis and epidermis. Scale bar = 20 μm. (B) Median longisection of root tip with tiered basal angiosperm (TB) apical organization (arrow). Scale bar = 20 μm. Insets: left – radial cortical cell files, scale bar = 40 μm; right – aerenchyma lacunae expanded by cell expansion, scale bar = 35 μm. (C) *Cabomba caroliniana* – monarch stele, nine-celled endodermis, schizogenous–expansigenous aerenchyma, exodermis with double CBs; photograph from Seago (2002), with permission from the *Journal of the Torrey Botanical Society*. Scale bar = 65 μm. (D) *Nuphar lutea* – polyarch stele, astro sclereids in expansigenous aerenchyma, exodermis with CBs and SL; photograph from Seago (2002), with permission from the *Torrey Botanical Society*. Scale bar = 200 μm. (E) *Nymphaea odorata* – polyarch stele (seven xylem poles), astro sclereids, expansigenous aerenchyma. Scale bar = 60 μm. See Fig. 2 for list of abbreviations.
usually tetrarch or pentarch, but hexarch is also common (e.g. *Magnolia*, *Liriodendron*, *Asimina*, *Laurus*, *Aristolochia*, *Saururus*; Fig. 4A–D). For primary roots, *Calycanthus* and *Asimina* (see Thomas, 1914; Hayat and Canright, 1965; Metcalfe, 1987) are initially diarch and *Saururus* is tetrarch (present study; *Calycanthus* may also be tetrarch, Thomas, 1914). Adventitious and lateral roots in *Laurus* (Lauraceae) and *Aristolochia* (Aristolochiaceae) can also be tetrarch or diarch (with two distinct and widely separate protophloem elements at each pole). In the Piperaceae, Metcalfe (1987) reported diarchy and tetrarchy in *Piper* and polyarchy in *Calycanthus*. In most magnoliids, the centre of the stele often is occupied by metaxylem (Fig. 4B, C) or sclerenchyma (Fig. 4D). A pericycle delimits the stele, and the cortex is delimited on its interior by an endodermis which forms CBs and later SL; there is usually a uniseriate exodermis (Fig. 4B, D); depending on the state of early secondary growth, an epidermis may or may not be present in older roots after exodermis maturation. Endodermis and exodermis increase in cell number during secondary growth. All magnoliids so far reported have common initials in their RAMs (Heimsch and Seago, 2008).

**Monocots**

Most monocot roots are adventitious and arise endogenously usually from stems or leaves (Tomlinson, 1961, 1969, 1982; Metcalfe, 1971; Ayensu, 1972; Keating, 2002; Bell and Bryan, 2008). In mature roots, the stele is often polyarch (six

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**Fig. 4.** Magnoliids, Magnoliales. (A) *Magnolia soulangeana* — young root, hexarch stele, endodermis. Scale bar = 65 μm. (B) Secondary xylem with vessels, redivided endodermis with suberin lamellae (SL) and passage cells, exodermis. Scale bar = 30 μm. (C) *Aristolochia* sp. — tetrarch stele with early secondary xylem, endodermis with Casparian bands (CBs) and early suberin deposition, early stage of exodermis wall deposition. Scale bar = 55 μm. (D) *Saururus cernuus* — tetrarch root with early secondary growth, endodermal CBs, exodermis with CBs and partial SL. Scale bar = 115 μm. See Fig. 2 for list of abbreviations.
or more poles of xylem and phloem; Fig. 5A–H, J), but three xylem and phloem strands can be found, especially in wetland or aquatic plant roots such as *Hydrocharis* (Fig. 5I; Seago et al., 1999a). Tomlinson (1982) noted reduced numbers of poles for plants with floating roots in the Alismatales. Even *Acorus*, usually reported with six to nine poles of xylem and...
phloem (Keating, 2002; Soukup et al., 2005), may have only five poles (Soukup et al., 2005). Very high numbers of strands (≥20) occur in plants with large and/or aerial roots such as in Pandanaceae and Areaceae (Fig. 5F, G, J; Tomlinson, 1961, 1982; French, 1987a, b).

Figure 5 shows other features of some diverse monocots from the Acorales to the Zingiberales. The endodermis is uniseriate and most often has cell wall stages I, II and III (e.g. Fig. 5C, D, F, H, J; see Meyer et al. (2009) for endodermal cell wall stages). The middle cortex has great variability, but one of the most unique features is the occurrence of sclerenchyma bundles in roots of many aerial plants (Fig. 5F; Tomlinson, 1961; Keating, 2002). Aerenchyma is a common feature in the many families with aquatic or wetland species (Fig. 5A, B, D). In basal monocots (Acorus), aerenchyma is expansigenous, and in derived monocots it is often of varying schizo-lysigenous to lysigenous types (Fig. 5I; Seago et al., 2005; Jung et al., 2008). However, aerenchyma patterns in most angiosperms are still not well represented (Jackson et al., 2009; Van der Valk, 2012), except that the members of Cyperaceae are well known for their unusual tangential lysigeny (Seago et al., 2005; see also Metcalf, 1971). Often, in plants with aerial roots, especially large roots, the air spaces in the cortex are described as cavities (Tomlinson, 1961) because they are not developed or organized like typical aerenchyma. A hypodermis ranges from uniseriate to multiserial exodermis (Fig. 5A, C–H, J), and in many plants is dimorphic with long and short cells (Fig. 5K; Shishkoff, 1987). A velamen occurs in some aerial roots, especially in orchids (Fig. 5H; Ayensu, 1962; Zankowski, 1987; Keating, 2002).

The single most distinctive feature of monocots, as reported extensively by Clowes (2000) and Heimisch and Seago (2008), is the developmental association between ground meristem and protoderm in the RAM.

Eudicots

As early as 1914, Thomas compared the vasculature of many seedling roots that are now classified as either magnoliids or eudicots; he found them to vary from diarch to octarch, but in Metcalf and Chalk (1950a, b) and Metcalf (1987) very few species’ roots are heptarch and octarch. In the basal eudicot Ranunculaceae, there is diarch (Aquilegia, some Ranunculus; Thomas, 1914), tetrarchy (other Ranunculus, Berberis; Thomas, 1914) and pentarchy (Podophyllum). Thus, while vascular tissues of the stele in most eudicots have two to six poles or strands of alternating xylem and phloem (Fig. 6A, E–I; Metcalf and Chalk, 1950a, b), importantly, some groups near the base of the eudicots (Proteales) and core eudicots (Gunnerales) often have polyarch steles. Nelumbo lutea (Proteales, Nelumbonaceae; Fig. 6B) is aquatic, but Gunnera (Gunnerales, Gunneraceae) grows both in aquatic and terrestrial habitats, and its species often have large differences in numbers of xylem poles even in terrestrial plants, as we have collected (cf. G. perpensa, Fig. 6C, and G. killipiana, Fig. 6D), even though the roots may be similar or dissimilar in size; tetranch steles are aparently characteristic of these basal species (J.L. Seago, pers. obs.; A. Soukup and E. Tylova, pers. comm.; Wilkinson, 2000). Roots of species across the eudicot spectrum may have diarchy (Fig. 6I; e.g. Thomas, 1914; Hayward, 1938; Baum et al., 2002), especially when those roots are small, as in secondary root stages (Seago, 1973; Byrne et al., 1977). In many Fabaceae, primary roots are often triarch to hexarch (Fig. 6E), although in Glycine lateral roots originate in the diarch condition (Ambler et al., 1971) and then develop tetrarchy (Byrne et al., 1977); tetrarchy is very common in eudicots (Metcalf and Chalk, 1950a, b; Seago, 1971).

The cortex is delimited internally by the endodermis which varies as much in eudicots as it does in monocots, and often passage cells with CBs are opposite protophloem and SL cells are opposite the protophloem (Fig. 6A, B, G); stage III cell walls appear to be less common in eudicots. Air spaces in the form of aerenchyma are found most commonly in aquatic eudicots (e.g. Fig. 6G, H). There is an exodermis in many eudicots (e.g. Fig. 6A–C, H), but it is typically lacking in nodule-producing roots with an open transversal RAM such as legumes (Heimisch and Seago, 2008). Many eudicots, especially the many trees and shrubs, have secondary root growth, even if very limited as in small herbaceous plants (e.g. Fig. 6F; Metcalf and Chalk, 1950a, b). Secondary root growth is probably accompanied by a dilated endodermis and exodermis in many species, as in Gentiana (Fig. 6J; Sotniková and Lux, 2003) and Medicago (our observations).

Resin canals in roots are known but relatively little studied (French, 1987a). Laticifers are more a feature of eudicots (e.g. Ipomoea purpurea; Seago, 1971; and Lactuca sativa, J.L. Seago, pers. obs.; see also Metcalf and Chalk, 1950a, b; Metcalf, 1967) than of monocots where they are rare (Metcalf, 1967). Root laticifer development has been studied (e.g. Seago, 1971), and crystalliferous and tanniniferous cells, especially in rootcap or cortex, are also well known (e.g. Seago and Marsh, 1989).

All eudicots have a RAM with the protoderm/epidermis associated developmentally with the lateral rootcap (Clowes, 2000; Groot et al., 2004; Heimisch and Seago, 2008).

Air spaces in roots

Aerenchyma types have been presented by several researchers (e.g. Justin and Armstrong, 1987; Evans, 2004), but the explanations for the development of intercellular spaces into aerenchymatous lacunae by Seago et al. (2005) are the only adequate explanations for the roles of cell division, cell expansion, cell separations and cell deaths that can account for the types of root cortical aerenchyma: expansigenous, schizogenous and lysigenous. Based on this feature, Seago et al. (2005) and Jung et al. (2008) best provide the possible evolutionary path from basal angiosperms to monocots or eudicots. Clearly, the earliest root aerenchyma in angiosperms was most probably by expansigenous (Fig. 7A – Acorus; Nymphaeales of basal angiosperms and Acorales of monocots; Seago et al., 2005; Soukup et al., 2005), the lacunae arising by cell division and cell expansion, not by schizogenous (Fig. 7B) or lysigenous (Fig. 7C, as noted by Jackson et al., 2009). Particularly in monocots, various kinds of lysigenous arose in more derived families of several orders. The occurrence of diaphragms across aerenchymatous lacunae has been noted and even studied in detail (e.g. in Cabombaceae and
FIG. 6. Eudicots. (A) *Ranunculus repens* – tetrarch stele, endodermis with passage cells, cortex non-aligned, exodermis Casparian bands (CBs) and suberin lamellae (SL); brightly fluorescing epidermis. Scale bar = 85 μm. (B) *Nelumbo lutea* – polyarch stele with sclerified pith, endodermis with passage cells and suberized cells, exodermis suberized. Scale bar = 115 μm. (C) *Gunnera perpensa* – polyarch stele (seven poles), endodermis, mid-cortex with expansigenous spaces, endodermis with CBs and SL. Scale bar = 190 μm. (D) *Gunnera killipiana* – polyarch stele with 18 xylem strands, bundles within pith and parenchyma. Scale bar = 200 μm. (E) *Medicago sativa* – typical legume triarch stele, non-radial cortex, no hypodermis/exodermis. Scale bar = 95 μm; inset: somewhat unusual tetrarch stele observed in very few primary roots. (F) *Fraxinus americana* – secondary xylem, but pentarch primary xylem visible. Scale bar = 140 μm. (G) *Rumex crispus* – pentarch stele with partial sixth pole and central metaxylem, endodermis with CBs, expansigenous aerenchyma, no exodermis. Scale bar = 135 μm. (H) *Nymphoides crenata* – pentarch with pith, endodermis with CBs only and exodermis with CBs and SL, astrosclereids in mid-cortex with aerenchyma. (I) *Artemisia lavandulaefolia* – diarch primary root, no pith, endodermis with CBs only, faint CB staining in hypodermis; photograph courtesy of Chaodong Yang. Scale bar = 80 μm. (J) *Gentiana asclepiadea* – root with dilated endodermis and exodermis in early secondary growth; photograph courtesy of Alexander Lux. Scale bar = 45 μm. See Fig. 2 for list of abbreviations.
Nymphaeaceae of the Nymphaeales, Seago et al., 2005; the Hydatellaceae do not appear to have diaphragms, possibly a consequence of having only cell expansion and no further cell divisions contributing to the lacunae). The presence or absence of diaphragms has not been widely studied across monocots and eudicots (Seago et al., 2005).

In legumes, vascular cavities can be found in the pith of some triarch Pisum roots (Fig. 7D) under flooded conditions (Niki and Gladish, 2001). Legumes do not have the cortex development or structures that allow easy formation of aerenchyma (Seago et al., 2005) or exodermis (Heimsch and Seago, 2008). Such cavities are not considered aerenchyma. Secondary aerenchyma, aerenchymatous phellem derived from phellogen, can also occur in wetland plants (Seago et al., 2005). A hypodermis is the outermost cell layers of the cortex derived by periclinal divisions (e.g., Baylis, 1972; Brundrett, 2002).

DISCUSSION ON SELECTED ASPECTS OF ROOT ANATOMY

Root apical meristem

From the concepts of Barlow (1994, 2002) on increasing complexity and quiescence, to Clowes (1994) on epidermis origins, the possible evolutionary path of RAM organization has been presented in three major studies by Clowes (2000), Groot et al. (2004) and Heimsch and Seago (2008). The latter authors presented an analysis of RAMs with several manifestations of closed and open types and reported that some specimens of Amborella trichopoda and the magnoliids contain common initials for most meristematic tissues of the root. As stated above, Heimsch and Seago (2008) further related the open and closed RAMs (with cortex and epidermis association) in the nymphaealean families (Cabombaceae, Nymphaeaceae and now the Hydatellaceae) to the monocots. In Friedman et al. (2012), fig. 6 corroborates our findings herein for T. filamentosa that its primary, adventitious and lateral roots have a tiered monocot-type RAM (sensu Clowes, 1994, 2000; Heimsch and Seago, 2008), and the pattern of cortical development from a tiered RAM further illustrates a monocot-like root.

In overcoming some of the questions which Les and Schneider (1995) posed about the lack of solid evidence for nymphaealean and monocot phylogenetic connections, we argue that there is no stronger anatomical evidence for a Nymphaealean—monocot connection than the RAM and cortex, because such a type of anatomy is not found in Amborellaceae, magnoliids and eudicots. These patterns clearly arose in the ancestors of monocots, i.e., various ancestral, early basal angiosperms, as the patterns of epidermis and lateral rootcap connections characteristic of some basal angiosperms and all eudicots must have separately so arisen.

Further, there appears to be a clear association between RAM organization and the patterns of lateral rootcap cells and their sloughing (Hamamoto et al., 2006). Open RAM produces more cells and releases individual living border cells, whereas closed RAM releases sheets or groups of dead cells. The fate of lateral rootcap cells in the tiered or closed RAMs of Cabombaceae and Hydatellaceae, as well as the open transversal RAMs of Nymphaeaceae, need to be examined to determine if the same relationships holds for RAMs of these basal angiosperms. The differentiation of epidermal cells, especially in simple tiered RAMs, has received enormous attention in just a select few species (Bruex et al., 2012; Jones and Dolan, 2012), and this needs to be expanded.

Cortex: endodermis and hypodermis

The endodermis is a well-defined structural feature of angiosperm roots (Kroemer, 1903; Van Fleet, 1950; Wilson and Peterson, 1983; Seago and Marsh, 1989; Seago et al., 1999b; Soukup et al., 2005; Meyer et al., 2009), except possibly in holoparasites (Kuijt and Bruns, 1987). A hypodermis is the outermost cell layers of the cortex derived by periclinal divisions in the outer ground meristem (Seago and Marsh, 1989). When CBs are present and SL are also always present, the cell layer(s) is termed endodermis (Kroemer, 1903; Wilson and Peterson, 1983; Perumalla et al., 1990; Peterson and Perumalla, 1990; Seago et al., 1999b). Multiseriate hypodermis is much more common in monocots than in eudicots (Seago et al., 1999a, b; Peterson and Perumalla, 1990). Two different cell types can occur in eudicots – long cells and short cells; Shishkoff (1987) reported no dimorphic hypodermis in Nymphaeaceae (see also Seago et al., 2000b) and Laurales, but found them in Magnoliaceae and in basal eudicot Ranunculales (not in the Papaveraceae, however). Dimorphic hypodermis as seen in Allium cepa (Fig. 5K) is fairly common in monocots. The exodermis and its passage cells can have major effects on root–fungus associations (e.g., Baylis, 1972; Brundrett, 2002).

There have been analyses of root structures with regard to their application to systematics (e.g., French, 1987a, b;
Vascular tissues

The similarities between members of the Nymphaeales and the Acorales have been noted with regard to xylem cell structure (Schneider and Carlquist, 1995, 2002; Carlquist and Schneider, 1997) as well as cortex structure (Seago et al., 2005). Monocots have far more aquatic/wetland species and families (Les and Schneider, 1995; Van der Valk, 2012) than do eudicots, and their roots, mostly adventitious, are often polyarch. In the basal angiosperms, two of the families, Cabombaceae and Hydatellaceae, have predominantly monarch roots, while the Nymphaeales are dominated by species with mainly polyarch roots, as are Acorus (Acoraceae), sister to the rest of the monocots, and the Araceae (Keating, 2002). Most of the remainder of the monocots are polyarch, except for aquatic families such as Hydrocharitaceae (Seago et al., 1999a) and Lemnaceae (Landolt, 1998), plants with tiny roots which have triarch or monarch steles, respectively.

According to Metcalfe and Chalk (1950a, b), Popham (1966), Esau (1977) and Metcalf (1987), eudicots are generally depicted as having two to six poles or strands of primary xylem and phloem (often, apparently, diarch in young lateral roots; Byrne and Heimsch, 1968; Byrne, 1973). It seems that diarchy is more common in primary roots (derived from radicles), at least in the basal angiosperms. That some wetland eudicots at the base of the core eudicots (Gunnera) and near the base of the basal eudicots (Nelumbo) are strikingly polyarch, such as Nymphaeales and the vast majority of monocots, raises interesting questions. Possibly, plants evolving in aquatic/wetland conditions retain and express the genes necessary for adventitious root production and polyarchy more frequently than non-aquatic plants. The greater the number of poles or strands of xylem and phloem (heptarchy and above), the less likely it is that secondary growth may occur, whereas diarch to hexarch patterns can lead more easily to secondary growth.

Primary and adventitious roots

Since so many species, especially among basal angiosperms (including Nymphaeales, e.g. Friedman et al., 2012) and eudicots, have two cotyledons with a diarch vascular pattern in primary and other roots, except in the nymphaealean Trithuria, leading to the possibility that diarchy is strongly related to the dicotyledonary condition, then one might expect that monocot primary roots might have a monarch primary or seminal root. Such is clearly not the case; and, too many basal angiosperms and magnoliids have patterns other than diarchy.

Another aspect of development and structure which should be examined more closely is the state of embryo development and structure at the time of maturation and germination. Amborellales, Nymphaeales and Austrobaileyales have very small embryos with little differentiation (Martin, 1946; Tobe et al., 2000; Friedman et al., 2012; see also Taylor et al., 2006, for fossil Nymphaeaceae). This might be important to the balance between a primary root system and adventitious root systems, to the relative state of development in primary roots vs. adventitious roots and to differences in origin of monocots and eudicots from basal angiosperm ancestors.

Most eudicots, when producing adventitious roots, form them from more or less typical eudicot vascular patterns in stems, bundles in one ring with a remnant procambial strand or incipient vascular cambium. Most monocots, on the other hand, form adventitious roots from stems with scattered vascular bundles or two or more rings of vascular bundles, so that one could argue that it is the number of available vascular bundles that produces the greater number of xylem and phloem poles in monocot roots. Thus, vascular patterns in embryonically produced roots might reflect vascular bundle distributions of their stems. The contributions of molecular genetics will have a major impact on our understanding of evolution of vascular patterns in roots (see Scarpella and Meijer, 2004).

Root symbioses, mycorrhizae and nodules

On the matter of mycorrhizae, after Baylis (1972), Simon et al. (1993) and Cairney (2000), Brundrett’s (2002) thesis features needed to accompany the evolutionary pathways. Mycorrhizal roots can sometimes be extremely modified (e.g. Imhof, 1997, 2001). For nodules, the study of Solis et al. (1995) confirmed that there are only two eudicot families (Fabaceae, Ulmaceae) with Rhizobium nodules and eight families (Betulaceae, Casuarinaceae, Elaeagnaceae, Myricaceae, Rhamnaceae, Rosaceae, Datiscaceae and Coriariaceae) with Frankia actinomycetes. A recent study by Markmann et al. (2008) suggests how the evolutionary path may have involved the same genes in both nodulating bacteria, but the root structural paths have not been well addressed. Heimsch and Seago (2008) and Seago et al. (2005) briefly discussed the ramifications of RAM and cortex structure, respectively, in relation to nodule formation. It should be noted that these families with nodulating roots are not closely associated with basal angiosperms or basal eudicots, and none is found in the monocots where epidermal origin is associated with cortex, not lateral rootcap; roots with bacterial symbioses seem likely to represent a derived condition in angiosperms.

In summary, root anatomy offers many interesting perspectives on developmental patterns, systematics and evolutionary relationships but, since their structure can vary depending on the type of experimental conditions, their importance is often less appreciated. However, when roots are examined based on their typical habitats, they can be useful when comparing groups of plants. Therefore, based on the information presented in this overview, there appears to be a general trend in angiosperm root structure (see summarized
<table>
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<tr>
<th>Taxa</th>
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<th>Types of stele</th>
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<th>Endodermis</th>
<th>Mid-cortex pattern</th>
<th>Aerenchyma</th>
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<th>RAM</th>
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<tr>
<td>Amborellales</td>
<td>Terrestrial</td>
<td>Adventitious; lateral</td>
<td>Diarch; triarch</td>
<td>Primary; secondary</td>
<td>All with CBs and SL</td>
<td>Non-radial</td>
<td>None</td>
<td>All with CBs and SL</td>
<td>Epidermis-lateral rootcap; common initials, irregular epidermis</td>
</tr>
<tr>
<td>Nymphaeales</td>
<td>Aquatic</td>
<td>Adventitious</td>
<td>Monarch polyarch</td>
<td>Primary only; secondary</td>
<td>Some CBs; some CBs and SL</td>
<td>Radial; non-radial</td>
<td>Expansigenous</td>
<td>All with CBs and SL</td>
<td>Epidermis-cortex; common initials, irregular epidermis</td>
</tr>
<tr>
<td>Austrobaileyales</td>
<td>Terrestrial</td>
<td>Adventitious; lateral</td>
<td>Diarch tetarch</td>
<td>Primary; secondary</td>
<td>All with CBs SL; some dilated</td>
<td>Non-radial</td>
<td>None</td>
<td>All with CBs and SL</td>
<td>Epidermis-cortex; common initials, irregular epidermis</td>
</tr>
<tr>
<td>Magnoliids</td>
<td>Terrestrial</td>
<td>Primary; adventitious; lateral</td>
<td>Diarch to hexarch</td>
<td>Primary; secondary</td>
<td>All with CBs and SL; some dilated</td>
<td>Non-radial</td>
<td>None</td>
<td>All with CBs and SL; some dilated</td>
<td>Common initials, irregular epidermis</td>
</tr>
<tr>
<td>Monocots</td>
<td>Aquatic</td>
<td>Adventitious; lateral</td>
<td>Polyarch Triarch in a few</td>
<td>Primary only</td>
<td>Some only CBs; some CBs and SL; many CBs, SL and secondary walls</td>
<td>Radial; non-radial</td>
<td>Expansigenous; schizogenous; lysigenous</td>
<td>None; some CBs and SL; many CBs, SL and secondary walls</td>
<td>Epidermis-cortex</td>
</tr>
<tr>
<td>Eudicots</td>
<td>Terrestrial</td>
<td>Primary; lateral</td>
<td>Polyarch in some basal aquatics</td>
<td>Primary; mostly secondary</td>
<td>Many CBs; some CBs and SL; some CBs, SL and secondary walls</td>
<td>Radian; non-radial</td>
<td>Schizogenous; lysigenous; expansigenous</td>
<td>None; some CBs and SL; some CBs, SL and secondary walls; some dilated</td>
<td>Epidermis-lateral rootcap</td>
</tr>
</tbody>
</table>
information in Table 1) and, in general, we note that the Amborellales and magnoliids have many root structural features like those of eudicots, whereas the Nymphaeales roots are strikingly similar to those of the monocots, especially basal monocots such as Acorales. The Austrobaileyales are enigmatic and have root structural features which do not align easily to either monocots or eudicots. Clearly, the basal angiosperms require far more anatomical examination to corroborate the findings of molecular phylogenetic analyses.

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LITERATURE CITED


