Stefano Mancuso

Editor

Measuring Roots

An Updated Approach

Springer

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Preface

The real complexity of and adult root system can be barely conceived if we think that one single plant of rye excavated by Dittmer (1937) consisted of 13,815,672 branches and had a length of 622 km, a surface area of 237 m^2 and root hairs for 11,000 km. Furthermore, this complex network of widespread roots and subtle rootlets is laid bare in the soil and can be recovered from it only with great struggle. Thus, it is easy to understand why determining the position, the area, the degree of branching and other root characteristics has been for centuries a peculiarly difficult problem.

Roots represent half of the plant body: possibly the most interesting. This invisible part of the plant spreads widely through the soil and adsorbs the water and nutrients that, together with the carbon dioxide taken from the air, represent the material out of which the world's food supply is manufactured by. They give anchorage to the plant, frequently accumulate reserve foods and in some cases also represent a reproductive organ. Furthermore, according to Charles Darwin (1880), roots are the "anterior" pole of the plant, characterized by "brain-like" characteristics in opposition with its posterior end bearing the organs of sexual reproduction.

Despite the obvious importance for the whole plant, our comprehension of the root apparatus has been for long time annoyingly limited, mostly due to inadequacy in the techniques available. This situation just recently changed thanks to the advancement in visualization and measurement of roots that resulted in a significant progress of our understanding of the architecture and behaviour of the plant's hidden half. However, this information is spread across many specialized journals and, consequently "out of the sight" for many more applied-oriented scientists. On the contrary, many agronomy-based papers and books dealing with various aspects of root methods have been missed by more "academically oriented" colleagues. This book represents an attempt to combine both academic and practical component of this topic in one volume, making this book a universal handbook for any researcher or extension person interested in aspects of root methods. The most updated innovations in root visualization and analysis and the most advanced

techniques of observation, architecture and behaviour of the root are described in detail and discussed. *Measuring root* has been written for a rather broad audience, from professional academics to undergraduate students at tertiary institutions and extension people interested in practical aspects of growing crops.

The volume consists of 18 chapters grouped in two main parts, namely:

1. Lab methods
2. Field methods

which should answer the needs of a large audience.

In the end, the editor gratefully acknowledges the many contributors of the chapters here presented, the financial support given to the University of Florence by the *Fondazione Ente Cassa di Risparmio di Firenze* and the support of Dr. Andrea Schlitzberger, at Springer, for the invaluable guidance during the production of the book.

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Part I
Lab Methods

Chapter 1
Higher Plants: Structural Diversity of Roots

Lyudmila G. Tarshis and Galina I. Tarshis

Abstract At the present time, the necessity of accumulating information about structure diversity of roots and root systems of the species existing on Earth has occurred, due to development both of theoretical basis and methods of preservation of plant biodiversity. There are few publications on structural features of plant roots in botanical literature. Moreover, comparative anatomical studies of roots of higher plants are far behind the researches on structure of shoots. Due to this fact, so far there is an opinion among botanists about the structure uniformity of roots of higher plants, and root systems. We are not going to consider all the causes for lag of comparative anatomical studies on higher plant roots in botany. This was done as long ago as in 1960s by (Comparative plant anatomy, Chapter 7. Root. Holt, Rinehart and Winston, NY. pp 94–101), American anatomist. Let us remind just of the main causes:

- Weak knowledge about intraspecific variation of root structure
- Technical difficulties in collecting root samples, the same as shoot samples, etc.
- Deficiency of monographs on rhizology, with comparative anatomical studies of roots, similar to those of other plant organs

We had taken into account these causes in our long-standing rhizological researches from 1974 to 2010.

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1.1 Rhizological Research: Material, Methods, and General Principles

Base material for the analysis of structure diversity of roots of rhizophytes was collected in natural populations of species during our expeditions to the Baltics, Ukraine, Russia (Ural, Siberia, Altai, and Far East). Along with wild-growing plants, we examined root structural features of introducents, which grow in the Russian botanic gardens. We have analyzed a total of 1,200 species of higher plants, which belong to various taxa and biomorphs.

We extracted root systems from substrate using the method of dry excavation along the trench walls. Trench depth varied from 15 cm to 3 m, depending on the mechanical soil characteristics. We excavated roots of ten mature blossoming or sporificating plants of each species. The samples were described according to the biomorphological characteristics by Serebryakov's (1962). We washed the roots, sketched them or photographed them. After that we fixed them in 73% *ethanol* for further anatomical examination. Every new root sample was included into the collection of rhizomes.

In laboratory by microtome, or manually by razor, we made cross-sections of ten roots of each species through the basal zone, middle zone, and apical zone. We examined and prepared microslides under the optical microscope according to anatomy standards (Kivenheimo 1947; Voronin et al. 1972). We used the ocular micrometer to measure root microstructures. To evaluate microstructure variation we applied the variation coefficient CV%. This value helped to compare features with different characteristics. Along with that, we developed structure models of roots in a form of graphical schemes. We used map symbols to mark topographic zones, systems of tissues, and some specific root structures, which had been found during microscope examination of the cross-sections. To create schemes we used computational microscope and the "Paint" program. A brief description of anatomic features was provided for each scheme.

1.2 Root System of a Plant: Specifics of Root Variability Manifestation

All higher plants, rhizophytes, are characterized by structure variability of roots, though to a different degree. To a less degree structure variability manifests itself among primary homorhizic plants, which belong to the following divisions: Lycopodiophyta, Equisetophyta, and Pteridophyta. Conventionally, we named this form of intraspecific variability of root structures as endogenous variability. It means variation of structure features among the cognate roots of a specimen.

Among present-day spore-bearing plants the endogenous variability is most pronounced in the representatives of the division Equisetophyta. We have studied five species of horsetails from natural populations of various geographical areas of

Russia (*Equisetum arvense* L., *E. pratense* L., *E. sylvaticum* L., *E. hyemale* L., *E. fluviatile* L.), and have found a distinct dimorphism of adventitious roots. In all these species, the so-called extension roots and sucking roots can be distinguished in root systems of each specimen. These roots are conspicuous. Extension roots are thick, look longer, and have positive geotropism. They are few in number and vegetate one by one from plagiotropic rhizomes. *Sucking* roots are very thin and short; they vegetate in groups from rhizome nodes, and form small fibrils. Comparative anatomical analysis allowed to reveal at them characteristic structural features similar structure features, typical for these types of roots in all examined horsetails. Comparison of cross-sections of the extension roots showed that they are 3.5–3.7 times thicker than the sucking roots. This occurs due to the growth of wider multilayered primary cortex. It has external and internal zones with large aerenchyma cavities. For example, primary cortex in extension roots of *E. arvense* is 4.3–4.6 times wider than the sucking roots cortex (Fig. 1.1). The stele size is only 1.8–2 times bigger in the extension roots. That is why the number of xylem and phloem strands is 5–6 in steles of the extension roots, while it is not exceeding 4 in sucking roots. General amount of the tracheary elements reaches 12–14 in extension roots, and it is only 4–5 in sucking roots. Together with the distinctions between roots of two morphotypes, there are a number of similar identities among different species of horsetails. For example, all these roots develop the single-layered rhizodermis, whose cells have reddish membranes, and are divided into trichoblasts and atrichoblasts. All the horsetails roots have sparse, though long, root hairs. Their paucity seems to compensate by means of absorbing hairs, which appear on the epidermis cells of rhizomes. For example, in *E. arvense* a number of such tubular hairs varies from 15 to 22 (CV% = 11.7) along the perimeter of cross-section of the plagiotropic rhizome.

The representatives of Lycopodiophyta demonstrate root dimorphism less distinctly than horsetails. Root differentiation in a specimen root system has been found among species of the following genera: *Lycopodium* L. and *Diphasiastrum* Holub. (Tarshis 2007). The most specific anatomical features of roots of club moss are presented in the basal zone, where plagiotropic shoot produces the root. Here a root has a wide, multilayered cortex, differentiated into three zones, and

Fig. 1.1 Dimorphism roots in primarily homorhizophytic root system of a *Equisetum fluviatile* L

Fig. 1.2 A cross-section cut
of an adventitious root of
Lycopodium clavatum L
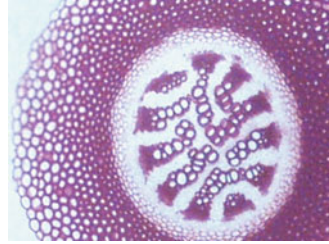
relatively small plectostele, which is similar in its structure to stele of shoot
(Fig. 1.2).

We have registered two types of different by origin adventitious roots, in a root
system of specimens, growing in the introduction conditions, which belong to the
genus *Selaginella Beauv.: S. apoda* (L.) Fern*., S. emmeliniana* Van Geert*.,
S. kraussiana* (G.Kunze)A.Br*., S. vogelii* Spring. These two types are shoot-borne
roots, which occur on the lower side of plagiotropic shoots, and rhizophore-borne
roots, which occur on the apical tips of the orthotropous rhizophores. It must
be emphasized that, notwithstanding the different origin, rhisophore- and shoot-
borne roots are anatomically identical and have specific structures: protostele,
tertiary structure of endodermis cells with wide Casparian strips, and long root
hairs on the thin-walled cells of rhizodermis.

Among the representatives of the division Pteridophyta, in general, endogenous
variability of roots manifests itself only in small variations of organs' thickness and
length, and in dimensions of certain microstructures. Hardly ever can we find more
significant differences in specimens of some species, which occur between thick
aerial roots and thin roots, which grow in substrate. We can observe this among
tropical epiphytic ferns, belonging to the genus *Platycerium* Desv.

The seed plants, allorhizophytes, as W. Troll called them (1949), show endoge-
nous variability in the most distinct way. Representatives of the divisions Pinophyta
and Magnoliophyta are known to undergo various underground root metamorphoses
extremely frequently. Their roots greatly differ in their exterior and microstructure.
As a rule, structure diversity of such roots is coming from their functions. For
example, aerial roots of many representatives of the families Orchidaceae and
Bromeliaceae are being developed in environment different than soil, and have
quite specific structure. Contractile roots, which grow from the perennial bulb stem
of *Lilium martagon* L., and draw it into substrate, differ as well. We will not recite
various metamorphoses of roots of seed plants, which are resulting from their long
adaptation to the certain environment. This problem was fully considered by many
morphologists before (Serebryakov 1962; Tarshis 1975). But it must be emphasized
that as well as root metamorphoses, seed plants undergo various shoot meta-
morphoses: rhizomes, stolons, tuber, and bulb. There are also metamorphoses
of mixed shoot–root nature in seed plants, e.g., caudices. Many of these organs
greatly resemble roots. That is why in the process of study of structure diversity of
roots we identified the morphology of underground organs, which comprise the root
system of each generative specimen, beforehand. For this purpose, we used method

of anatomical diagnostics. We also took into account that roots may vary themselves within a species' group of plants. Due to such a careful approach, we established the root system structure of two types in species belonging to the division Magnoliophyta: isomorphic and heteromorphic (Tarshis and Tarshis 1998).

For example, species of the subfamily Pyroloideae are found to have isomorphic type of root system structure, while species of the various taxa of the division Magnoliophyta are found to have heteromorphic type of root system structure.

Isomorphic type manifests itself most distinctly in four species: *Pyrola rotundifolia* L., *Orthilia secunda* (L.) House, *Moneses uniflora* (L.)A.Gray, and *Chimaphila umbellata* (L.)W. Barton. Isomorphic type was found in all the specimens from 35 populations under study, located in various regions of Russia. We found that four species, which grow in wide range of environmental conditions, develop the uniform secondary homorhizic root system, which consists of numerous adventitious roots, growing from the plagiotropic stolon-like rhizomes (Fig. 1.3). Structure features of both roots and rhizomes in these Pyroloideae are alike and unique. Anatomical features of the underground organs show great stability and low level of variation. Roots and rhizomes of this taxon species do not change their specific anatomical features, although demonstrate the miniaturization of structures, even in the Far North, on the Yamal Peninsula, in extreme environmental conditions. Due to this characteristic, it is possible to describe features of these roots, using just one single structure model. The uniformity of the inner structure of the stolon-like rhizomes is represented on the second structure model.

Another type of structure of root systems, heteromorphic, was found in natural populations among many Magnoliophyta species. For example, great structural diversity of root systems was registered in generative specimens in the populations of *Lupinaster pentaphyllus* Moench and *Sanguisorba officinalis* L. (Fig. 1.4).
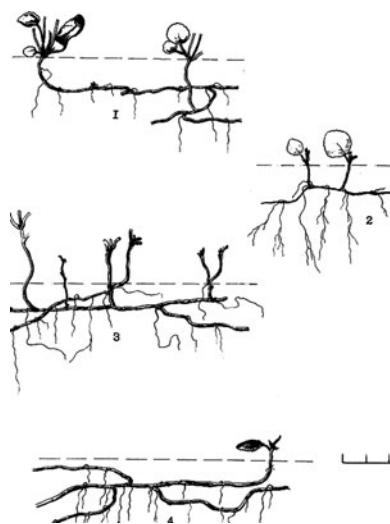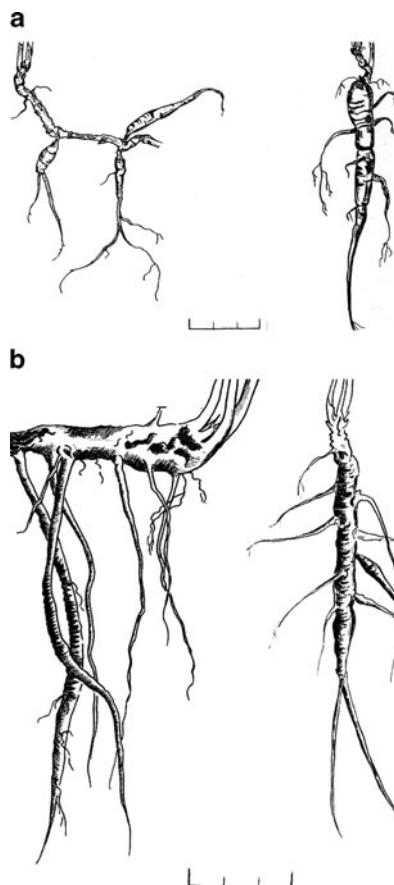

Fig. 1.3 Isomorphic type structural organization root system at species: (1) *Pyrola rotundifolia* L., (2) *Moneses uniflora* (L.) A.Gray, (3) *Orthilia secunda* (L.)House, (4) *Chimaphila umbellata* (L.) W. Barton

Fig. 1.4 Heteromorphic type
of the structural organization
of root systems at species:
(**a**) *Lupinaster pentaphyllus*
Moench, (**b**) *Sanguisorba
officinalis* L

For example, one group of generative specimens *L. pentaphyllus* had an allorhizic
root system, while another group, from the same cenopopulation, had a secondary
homorhizic root system. Moreover, these specimens showed great polymorphism of
underground organs, and some of the roots changed into tuber roots. There are also
two groups of generative specimens with allorhizic and secondary homorhizic root
systems in the nature cenopopulations of *S. officinalis,* distributed in different parts
of species area. In second group, there were big rhizomes and numerous adventi-
tious roots as well, turned into tuber roots. Microstructures of roots and root systems
showed great stability, alongside with the clearly pronounced intrapopulation
diversity of morphostructures. Detailed evaluation of variation of the anatomical
features of roots in seed plants, showed a very narrow range of manifestations of
individual variability (CV% = 2.2–13.2), registered in various phytogeographical
zones of Russia.

1.3 Roots and Root Systems: Species-Level Study of Structure Diversity

Comparative assessment of intraspecific variability level of anatomical features of the different taxa roots showed their significant stability. This fact convinced us that root anatomical features, due to their uniformity, can be used to distinguish the structure features complexes, which reflect their species characteristics. Sporophytes have the structure features complexes with minimal quantity of anatomical features, whereas spermatophytes have complexes with maximal quantity of anatomical features. For example, there are features of rhizodermis, primary cortex, and stele, which are the same in various environmental conditions; this peculiarity has been distinguished among the species belonging to the genera *Lycopodium* L. and *Diphasiastrum* Holub. The rhizodermis structure features are manifested in the root hairs quantity, which appear from the trichoblasts one by one, by two, by three, and so on. Thus, *Lycopodium dubium* Zoega plants have root hairs appearing by one at a time, while *Lycopodium annotinum* L. have root hairs appearing by two at a time. Usually, the structure features of a species' primary cortex are coming from its differentiation into zones, occurrence of aerenchyma cavities and their sizes, disposal of mechanical tissues (collenchyma and sclerenchyma). For example, primary cortex of the *Lycopodium clavatum* L. roots has three zones: exterior, middle, and interior. Exterior zone has 4–5 layers, and it is formed by small parenchymal cells. Middle zone is formed by thin-walled cells, stretched in radial direction; breakage of their membranes results in formation of aerenchyma cavities and/or air space between zones. Interior cortex has many layers. It surrounds the polyarch stele in the root center, like heavy similarly to a casing from sclerenchyma. It is necessary to note that stele of the basal zone in adventitious roots of the species, belonging to the genera *Lycopodium* and *Diphasiastrum*, has a strong resemblance to stele of plagiotropic rhizomes, where these roots grow from. In this type of stele, there are primary xylem and phloem strands, arranged in a form of peculiar curved bands. We have borrowed this term to denote similar structure models of steles of shoots and roots among the species of the class Lycopodiopsida and named them "plectostelic" (Tarshis 2003).

We have developed the "haplostelic model" for the aerial adventitious roots of the tropic *Lycopodium carinatum* Desv. A one-layer rhizodermis with numerous and very long (up to 700 μg). There are also cells with thickened and hardened membranes in the exterior zone of the cortex. Interior zone of the cortex is formed by thin-walled cells. There are two rounded steles, divided by a narrow parenchymal diaphragm in the center of most aerial roots. Inside of each stele there is a single, bend-like, curved strand of primary xylem, surrounded by the primary phloem cells from all sides.

We have found that plagiotropic shoots, orthotropic rhizophores, and adventitious roots of some species of the class Isoëtopsida have the most primitive stele structure. Species *Selaginella apoda* (L.) Fern., *S. emmeliana* Van Geert, *S. kraussiana* (G.Kunze) A.Br., *S. vogelii* Spring have central strand of xylem

tracheary elements, solidly encased by the primary phloem elements. All these species have a strong similarity in the inner structure of adventitious roots, shoots, and rhizophores. We have developed the "protostelic" structure model for them. There are vessels in metaxylem in roots with archaic protostele. Such a mixture of primitive and advanced root structures is considered to be a result of an early isolation of this group from other higher plants taxa during the evolutionary process.

Five wild species of the division Equisetophyta are found to have the minimal root structure diversity. Special feature of *Equisetum arvense* L., *E. pratense* L., *E. silvaticum* L., *E. hyemale* L., *E. fluviatile* L. is development of a thick, multilayered system of underground segmented shoots of two morphological types: plagiotropic and orthotropic rhizomes. It should be noted that all the species of horsetails are common to have poor developed adventitious roots of two morphotypes as well: thin sucking roots, and extension roots, which are thicker and longer. Roots of both types are incapable of secondary growth. All the roots are covered outside with one-layered rhizodermis, consisting of big cells, atrichoblasts, and short complanate trichoblasts. Cell membranes are brownish colored. All the species have long, but sparse root hairs, growing from trichoblasts. The primary cortex is built of parenchymal cells. Cell layers quantity varies from 4 to 10 in the root cortex of different horsetail species. Cells laying under rhizodermis (epidermis of root) have thick and lignification cellular walls other cortex cells layers are built of thin-walled parenchymal cells with light membranes. There are no mechanical tissue elements in cortex of all these species, but aerenchyma cavities occur. The endodermis is one-layered and consists of 10–11 rectangular cells with the Casparian strips. The stele is round, small, and inlaid with a narrow layer of pericycle cells. Primary xylem is exarch, and represented by a diarch ray or single tracheary element in thin roots. In thick roots a triarch, tetrarch, or pentarch xylem is developing in a form of rays, between which the phloem strands are arranged (Fig. 1.5). These root peculiarities of the species from the genus *Equisetum* L. are displayed in the "haplostelic" and the "actinostelic" models.
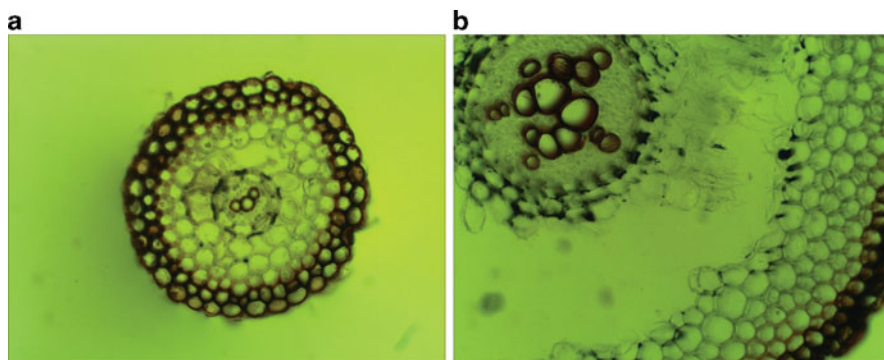

Fig. 1.5 Cross-section cuts of a roots horsetail (*Equisetum fluviatile* L.): (**a**) sucking root, (**b**) extension root

Analysis shows that the rhizome structure features, typical for the species, are more distinct than structure features of roots. Manner of the one/two endodermis layer arrangement in rhizomes is a very important diagnostic feature. For example, *E. silvaticum* L. has one sinuous endodermis layer, situated above the conducting bundles outside, while another one seems to wrap each of them inside. *E. fluviatile* L. has each conducting bundle, surrounded by own, or individual, endodermis. *E. arvense* L. and *E. pratense* L. have only one outer layer of endodermis in rhizomes. We have taken into account these particular structure features, when developing the three "artrostelic" model modifications for rhizomes of the various species of the genus *Equisetum* L.

We discovered that the division Pteridophyta species have the maximal diversity various microstructure features and feature complexes, which describe the specifics of the sporophytes' root structure. There were 80 species from 28 families of the classes Ophioglossopsida, Marattiopsida, and Polypodiopsida under study. Among them, samples of 37 species had been collected in natural populations, while samples of 43 species had been grown from spores. Fourteen structure models have been developed to describe the roots structure diversity of the division Pteridophyta species (Fig. 1.6). The following features have been included into the feature complexes:

- Presence or absence of root hairs and their characteristics
- Presence of mycorrhiza in the cortex cells
- Specifics of rhizodermis structure
- Specifics of primary cortex: differentiation manner, occurrence of aerenchyma and mechanical elements, quantity of layers, endodermis structure
- Specifics of stele: presence of pericycle and quantity of its layers, arrangement and quantity of the primary xylem and phloem strands

Roots of the species from the families Ophioglossaceae (R.Br.)Agardh and Botrychiaceae Nakai, belonging to the class Ophioglossopsida, are found to have
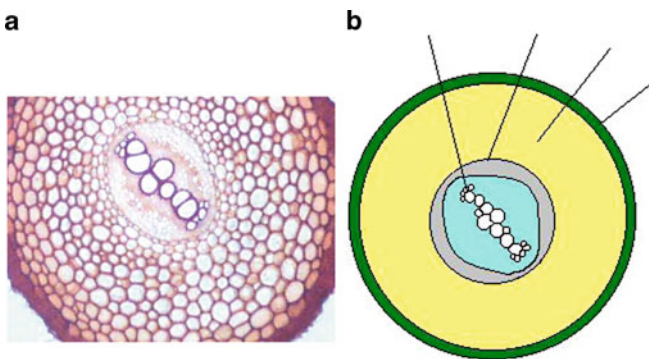

Fig. 1.6 A cross-section cut (**a**) and structural model (**b**) of a root fern *Matteuccia struthiopteris* (L.)Tod

a structure similarity, as well as great structure difference. Among the similarities there are the following:

- Absence of root hairs and presence of hyphae in the primary cortex cells
- Development of a wide, multilayered cortex, which is not divided into internal and external zones, etc.

Main differences are in the stele's structure features. For example, stele in the *Ophioglossum petiolatum* Hook. roots can be described as the simplest and the most primitive structure. In the stele center, which is called haplostele, there is an arcuated primary xylem strand, surrounded by the phloem continuous mantle. Other species, such as *Botrychium lanceolatum* (S.G.Gmel.)Ångstr., *B. lunaria* Sw., and *B. multifidum* (S.G.Gmel.)Rupr., have different structure, which is more specific; this structure is called actinostele, and is characterized by radial alternation of the primary exarch xylem and phloem. These differences became the basis for development of two structure models. A "haplostelic" model has been worked out for the roots belonging to species of the family Ophioglossaceae (R.Br.)Agardh, and "actinostelic" model has been worked out for the roots belonging to the species Botrychiaceae Nakai. Adventitious roots of the species *Angiopteris palmiformis* (Cav.)C.Chr., *A. polytheca* Tardien et C.Chr., and *A. crassipes* Wall., belonging to the class Marattiopsida, have a comparatively small stele and a very wide, multilayered cortex (32–45 layers). The stele has tubular structure, due to the pith's parenchyma cells in its central part, and primary xylem radial rays, whose quantity is up to 10–15. We have worked out a "siphonostelic" model for such a kind of root structure of the class Marattiopsida species.

The species of the class Polypodiopsida are found to have the greatest variety of root structures diversity. This class is known to have various life forms and ecological groups of plants, most of them grow at tropical and subtropical latitudes. That is why root structure peculiarities have been studied mostly in terms of species from the greenhouses of Russian botanical gardens. Twelve structure models have been developed during the interpretation of the results of comparative anatomical studies of roots. Root structure diversity of this taxon species manifests in as follows:

- Root hairs peculiarities, characteristics, and multitude
- Quantity of layers and differentiation of primary cortex
- Ratio of dimensions of cortex and stele
- Presence or absence of the coloration of cortex cells membranes with phlobaphenes
- Occurrence of aerenchyma cavities, mechanical tissues, and hyphae in cortex

Besides structure diversity, there are a number of similar features in the species of the class Polypodiopsida. Among these is presence of actinostele in the roots of all the species, belonging to this taxon. Most often a diarch actinostele was occurring (Fig. 1.7). It is common for these species to develop lateral roots from those endodermis cells, which are situated opposite to the primary xylem rays.

Among the representatives of this class, cortex without differentiation is being met in roots of the species *Osmunda vachellii* Hook., belonging to the order
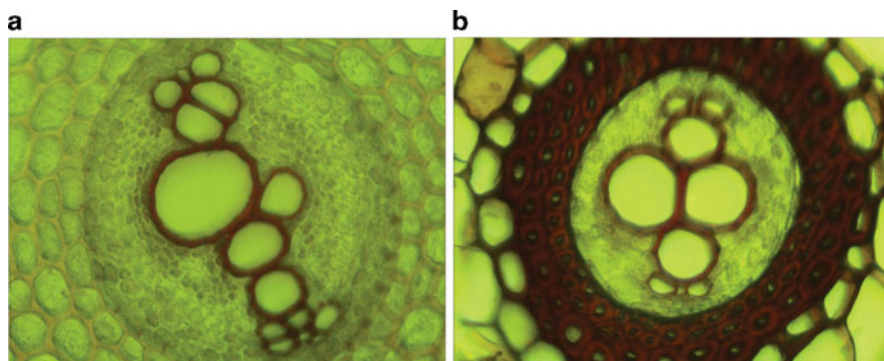
Fig. 1.7 Cross-section cuts stele of roots: (**a**) *Athyrium filix-femina* (L.)Roth, (**b**) *Pteridium aquilinum* (L.) Kuhn

Osmundales. On the contrary, in species of the order Schizaeales, root cortex divides into interior and exterior zones. Structure diversity is expressed most distinctly in structure features of water, morass, and epiphytic species of the order Schizaeales. For example, in roots of the genus *Ceratopteris* Brongn*., there are very large aerenchyma cavities, located in cortex and separated by narrow, one-row diaphragms, consisting of parenchyma cells. Different in shape hexagon stele has been met in the species *Lygodium japonicum* (Thunb.) Swartz. Such a form of stele is conditioned by peculiar structure of endodermis cells, which engirdle the stele. Strong thickening of cell membranes in the exterior zone of primary cortex is a characteristic feature of roots of the epiphytic species *Vittaria flexuosa* Fee and *Pellaea viridis* (Forsk.)Prantl.

In the four species of the genus *Platycerium* of the order Polypodiales, just primary cortex interior zone shows process of significant hardening (lignification), while the seven to eight layered cortex exterior cells have thin and light membranes. It must be emphasized that in most species of this order, cortex inner cells have the thickened membranes, and are saturated with phlobaphenes (reddish-colored tannins oxidation products), rather than lignin. In many species belonging to the order Cyatheales, root cortex is divided into two zones, though thickening and saturation with phlobaphenes occurs only in inner zone. It is necessary to note that some species groups, belonging to this order, such as *Blechnum brasiliense* (Desv.) T. Moore*, Doodia dives* Kunze*, etc., do not show differentiation of cortex at all. At the same time, in roots of *Onoclea sensibilis* L. there are cell membranes, which are thickening and browning in that cortex layer, which underlays the rhizodermis; it means that roots of this species may have exoderm.

Roots of amphibian and aquatic species from the family Marsileaceae are characterized by highest peculiarity. For instance, *Marsilea quadrifolia* L. entirely lacks root hairs, and there are numerous (up to 20) large (up to 140 μg in diameter) aerenchyma cavities. *Salvinia natans* (L.)All. do not have roots at all, which are reduced due to water environment adaptation. At the same time, mature specimens

of this species are noticed to have long multicellular hairs, which appear by one on the rhizome epidermis cells (Fig. 1.8).

On study of root structure diversity of seed plants species, first, we analyzed structure features of their root systems. Development of heterogenous root system was found in all examined species of the classes Cycadopsida, Ginkgopsida, Pinopsida, Gnetopsida, belonging to the division Pinophyta, and of the class Magnoliopsida, belonging to the division Magnoliophyta. As a part of such root system there are primary root, lateral roots, and adventitious roots, functioning at the same time.

Structure diversity of roots, comprising the species root systems of the division Pinophyta, was studied both in wild and cultured species. For example, one primary root and two shoot-borne roots were found in the biennial specimens *Cycas revoluta* Thunb., raised from seed. Ten-year specimens had primary root, lateral roots, coralloid roots, and about 20 adventitious contractile roots. They differ both in external and internal structure. For example, primary root and first-order lateral roots demonstrated secondary growth (Fig. 1.9). Coralloids have blue-green algae and other symbionts, found in the layer of parenchyma cells, which are situated between exterior and interior zones of primary cortex. All the examined species of the division Pinophyta are noticed to have great structure diversity of roots, comprising root systems. For this reason, on development of structure features complex, we compared the even-aged specimens' roots of the same origin and position in root system. To discover specific root features of seed plants in
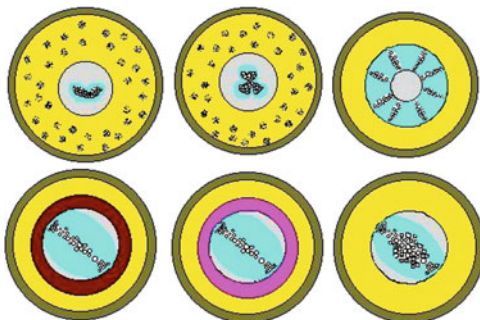

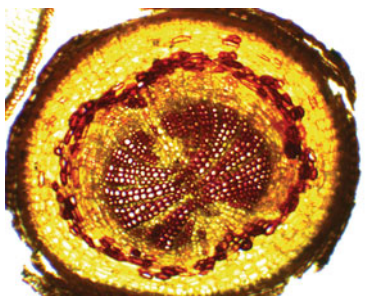**Fig. 1.8** Examples of schemes of structural models Polypodiophyta

Fig. 1.9 A cross-section cut of a lateral root *Cycas revoluta* Thunb

comparison with spore plants, we extended a set of anatomical feature for structure model development up to the following:

- Characteristics of the cambium layer development in roots, its configuration, and activity
- Phellogen location and compound of tissues, formed by phellogen
- Way of primary cortex dying off and casting
- Structure features of secondary conducting tissues – phloem and xylem; characteristics of inner bark and wood elements; location of certain specific structures (resin canals, aerenchyma cavities, etc.)
- Ratio of thickness of cork (phellem), secondary phloem (inner bark), and secondary xylem (wood)

Comparative analysis of cross-sections of the lateral roots in four species *E. horridus* (Jack.)Lehm., *E. altensteinii* Lehm., *E. lehmannii* Lehm., *E. trispinosus* (Hook.)R.A.Dyer, of the genus *Encephalartos* Lehm., showed that they have the same inner structure due to secondary growth. This growth is common for roots of gymnosperms and dicotyledon angiosperms, and manifests itself in emerging of secondary conducting tissues – phloem and xylem from cambium, and secondary ground tissue from phellogen. Perennial roots of all the examined species of the division Pinophyta have a thin cambium layer with rounded contours; outside it is engirdled by a wide pale circle of bark elements; inside of cambium layer there is a heartwood, which is segmented by parenchyma rays. For example, in roots of species, which belong to the genus *Encephalartos* Lehm., heartwood is bisected; between these two sectors there is a diarch primary xylem ray. Outside there is a multilayered cork, covering the perennial roots. It replaces rhizodermis and primary cortex, which are being thrown in the process of root thickening.

The same structure features are found in roots of the species belonging to the class Cycadopsida. Minor variations in secondary growth of roots are noticed in *Stangeria eriopus* (G.Kunze)Nash. It has heartwood divided on six small sectors, rather than 2, and there are aerenchyma cavities in the inner bark. Though, in general, structure variations of perennial roots of the species from the class Cycadopsida manifest themselves only in the ratio of thickness between phellema, secondary phloem, and xylem.

Specific allorhizic root system develops in 5-year specimens, raised from seed of *Ginkgo biloba* L., the only representative of the class Ginkgopsida. Primary and lateral roots, which are capable of secondary growth, are noticed to have the same structure features. In wood of both roots there is distinct annual growth, and rounded fringed pores on the tracheid walls.

Significant diversity of root structures has been discovered in species, which belong to the largest class Pinopsida, comprising about 600 species. Many species develop mixed allohomorhizic root system, which is characterized by occurrence of primary root, lateral roots of several orders, and adventitious roots. For example, in 5-year specimens of *Picea obovata* Ledeb. and *Larix sibirica* Ledeb., primary and lateral roots have secondary structure, while adventitious roots have primary structure (Fig. 1.10). There are specific resin canals in the roots' wood of the
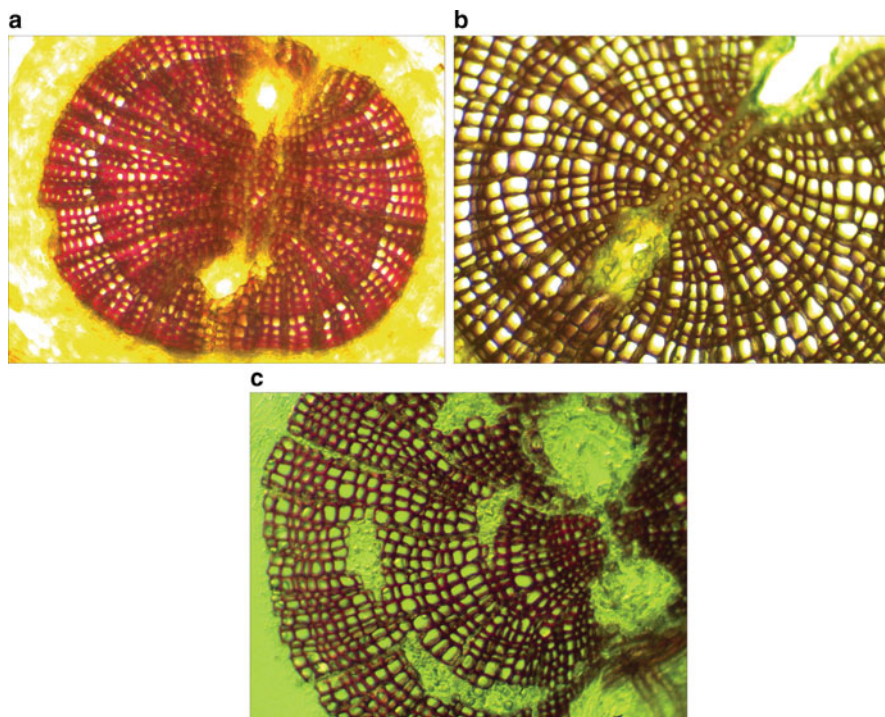
Fig. 1.10 Cross-section cuts of lateral roots of (**a**) *Picea obovata* Ledeb., (**b**) *Larix sibirica* Ledeb., (**c**) *Pinus sylvestris* L

species, belonging to the family Pinaceae Lindl. These specific structures are differently located in roots of species, belonging to different genera and families. Thus, peculiar vertical and horizontal resin reservoirs (cysts) are emerging in wood of roots of the species *Sequoia sempervirens* (D.Don)Endl., belonging to the family Taxodiaceae (Warm.)F.Neger. Perennial roots of the species of the genus *Metasequoia* Miki ex Hu et W.C. Cheng have large resin cysts, which are situated in wood opposite to the primary xylem rays. Such roots are noticed to have the high growth of wood, which is 1.8 times thicker than bark, and 6.4 times thicker than cork. Other more significant differences in root structure of most species of the family Taxodiaceae (Warm.)F. Neger have not been registered. The species *Sciadopitys verticillata* (Thunb.)Siebold et Zucc. does not have any resin canals or other resin reservoirs in wood of roots. Roots of *Callitris rhomboidea* R.Br. ex L.C.Rich. from the family Cupressaceae (A.Rich. ex Bartl.)F.Neger have bark fibers and sparse resin canals in bark and wood. Small ball-like resin reservoirs, which are situated in the phloem parenchyma, are discovered in roots of four species of the genus *Juniperus* L. It must be emphasized that lateral roots of these species show the same secondary anatomical structure, characterized by circular disposition of secondary ground and conducting tissues.