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The surprising anatomical diversity in the roots of African Restionaceae

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ABSTRACT

The root anatomy of 291 of the 350 species of the African Restionaceae, one of the ecologically dominant and taxonomically diverse elements of the Cape flora, is reported. There is substantial variation in the cortex (either collapses in older roots or persists as an aerenchyma), endodermis (in relative size, shape of the endodermal cells, and degree of wall thickening), pericycle (from 1 to 10 cells, varying from unthickened to massively thickened), and the metaxylem vessels (5 to more than 100, organized in a ring or scattered). Almost all root anatomical characters are phylogenetically constrained, similar to the culm anatomical characters. Although it is possible to, based on root anatomy, recognize groups of genera with broadly similar root anatomies, there are often also exceptions, indicating some evolutionary lability. Variation not phylogenetically controlled is significantly explained by differences in ground water availability and mean annual precipitation. The descriptive data are available in an online interactive key. The variation in root anatomy may contribute interesting insight into the evolution of this unusual group of plants.

1. Introduction

Belowground structures and organs of plants are poorly investigated, except for agriculturally important species. This is surprising, as in many regions access to water and nutrients may be more limiting than access to light and CO_2, and consequently we can expect that the belowground structures should be functionally and phylogenetically at least as important as above-ground vegetative organs. The importance of belowground structures is corroborated by the plants themselves, which may allocate upto 70% of photosynthate to roots (Poorter et al., 2012; Valverde-Barrantes et al., 2017). Variation patterns in roots, in both functional and morphological-anatomical traits, are complex, varying ontogenetically from young to old roots, architecturally within one plant, and at all phylogenetic scales. Simplistically, roots may be interpreted to consist of two functional domains. The "fine roots" or the root-hair zone, directly behind the growing root tip and so the youngest part of the roots, are actively involved in water and nutrient uptake. Fine root functional traits include root length, N content, weight, root hair development and fungal associations (Lambers et al., 2006), and are part of the "plant economic spectrum" that combines root and shoot traits (Freschet et al., 2015; Kramer-Walter et al., 2016; Pohl et al., 2011). Fine root functional traits show both phylogenetic and ecological signals, root traits may even be more phylogenetically constrained than leaf traits (reviewed in Valverde-Barrantes et al., 2017). This is formulated as the "root trait phylogenetic conservatism hypothesis". The older part of the roots is primarily a transport pipe, moving water from the root tips to the above-ground part of the plant, and nutrients and oxygen below ground to the fine roots, this transport is often through a hostile environment. Mature root functional traits include metaxylem vessel size (Lynch et al., 2014) and organization, as well as aerenchyma and the controls on radial oxygen loss (Connell et al., 1999).

It is unclear how much older root anatomical variation is phylogenetically or ecologically constrained, and consequently how useful this could be systematically. Although there is a general survey of root anatomical structure and variation across the monocots, as part of the Anatomy of Monocots project (e.g. Cutler, 1969; Metcalfe, 1960; Tomlinson, 1969), there have been relatively few detailed (e.g., almost complete generic coverage) systematic comparisons focusing on families or smaller groups. Seubert (1996a,b, 1997, 1998a,b) surveyed the roots of palms, and found little variation among congeneric species, but substantial variation between genera. Similarly, Carlquist (1966) found that in Rapateaceae congeneric species and related genera are quite similar in their root anatomy, but that there are systematic differences between groups of genera. Wilder (1986) also found that generally the genera of Cyclanthaceae could be grouped based on the root anatomy, but that some of these groupings were unexpected, based on other characters. That species groups within a genus can also be determined is evident from the study on the roots of *Allium* (Fritsch, 1992). However, a survey of bambusoid root anatomy revealed substantial anatomical variation, which was not taxonomically structured (Raechal and Curtis, 1990). This is not consistent with a global grass

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root anatomy survey, which suggested that there was a phylogenetic pattern in the variation (Goller, 1977). Such a phylogenetic pattern was also demonstrated by a careful study by Uma and Muthukumar (2014) in the Zingiberaceae. A very common problem, mentioned by almost all researchers working on a comparative analysis of root anatomy (e.g. Carlquist, 1966; Cutler, 1969; Metcalfe, 1960) is that the sampling, at species level, is too poor. Kauff et al. (2000) introduced the idea that root anatomy could be adapted to dry conditions in Asparagales. A convincing case study, with root anatomy well sampled at species level, and interpreted in both phylogenetic and ecological context, is needed to evaluate the systematic and ecological importance of mature root anatomical variation.

The absence of root information applies particularly to the Cape flora. This is surprising, as the species-rich *fynbos* flora is found in a seasonally arid climate on nutrient-poor soils, in an environment with no shortage of light. Rain falls in winter, when it is too cold to grow, whereas the warm summers are dry (Cramer and Hoffman, 2015). In such an environment competition should be largely belowground, and affect the roots: deep rooted plants can access water in summer, shallow-rooted cannot (West et al., 2012). The suggestion that competition may be largely belowground leads to the prediction that there should be substantial variation in root anatomy in the Cape flora clades. Root traits may be adaptive to extreme soil conditions, thus convergent (see Tanentzap and Lee, 2017), or they could be phylogenetically constrained. Restionaceae is highly diverse in the Cape flora (Linder, 2003) and one of the dominant elements of the typical *fynbos* vegetation (Rebelo et al., 2006). The species are often separated along hydrological gradients (Araya et al., 2012), and this has been linked to the presence of aerenchyma in the roots (Huber and Linder, 2012). The root anatomy of the Australian Restionaceae has been surveyed (Meney et al., 1999; Pate and Delfs, 1999). Shane et al. (2011) demonstrated the role of the sand-sheaths in *Lyginia* R.Br.. The *Lyginia* root system is quite remarkable, with 2–4 m deep roots reaching the permanent water-table, whereas shallower roots are summer-dormant (Shane et al., 2009). However, very little has been published on roots of the African Restionaceae (the monophyletic subfamily Restionoideae (Briggs and Linder, 2009), which we will refer to as restios): Cutler (1969) described the root anatomy of 18 restio species (Table S1), and Huber and Linder (2012) documented the distribution of aerenchyma.

Here we describe the anatomy of the roots of the restios. We first develop the descriptive terminology, and interpret the ontogenetic development of the root anatomical tissues. This is based on a small number of species to document the development of the anatomy from the root tip to the mature roots. Then we used a large sample of roots from herbarium specimens, and some freshly collected material, to document the variation in the root anatomy across the subfamily. We then map this variation across the phylogeny and test which traits are phylogenetically conserved. We test whether the phylogenetic signal differs from that of the culm anatomy. Restio culm anatomy has long been interpreted to be phylogenetically and systematically informative (Cutler, 1969; Gilg-Benedict, 1930; Gilg, 1891; Linder, 1984), and the culm anatomy has been scored from almost all restio species (Linder, 2001), making such a comparison possible and interesting. We use these root traits to diagnose the genera of the restios. Finally, in order to test whether species with similar root anatomy are found in similar environments, we simplified the root anatomical variation to four ordination dimensions, thus generated abstract root functional syndromes. We tested whether (and which) environmental variables significantly explained variation in these functional syndromes after phylogenetic correction.

2. Materials and methods

2.1. Sampling

In order to understand and map the root anatomical diversity of the restios, we followed three approaches. First, in order to explore the ontogeny of the root tissues, and so to establish the homologies among the roots, we studied 10 species (*Cannomois grandis* H.P.Linder, *Elegia elephantina* H.P.Linder, *E. fistulosa* Kunth, *Restio leptostachyus* Kunth, *R. paniculatus* Rottb., *Rhodocoma capensis* Nees ex Steud., *Thamnochortus bachmannii* Mast., *T. cinereus* H.P.Linder, *T. spicigerus* (Thunb.) Spreng. and *Willdenowia incurvata* (Thunb.) H.P.Linder) in detail. These were cultivated in the botanical garden of the University of Zurich, facilitating the collection of undisturbed 1st order roots. The plants were grown in normal potting soil and with a biweekly watering regime, under glass to prevent freezing, for at least two years to ensure that mature roots were present. The roots were fixed in formaldehyde:ethanol:acetic acid (4%:50%:5%) for at least 48 h, before being stored in 70% EtOH. Although sampling was constrained by the species available in cultivation, we were able to select pairs of closely related species, such that the species-pairs come from phylogenetically widely separated genera. Secondly, in order to control for sectioning artifacts, re-hydrated roots of 42 species from seven genera (harvested from herbarium specimens, see Table S1) were embedded in 2-hydroxyethyl methacrylate (Technovit7100; Heraeus Kulzer GmbH, Wehrheim, Germany), following the manufacturer's protocol, and sectioned at 3.5–10 µm with a Rotary Microtome HM355S. The sections were stained with Ruthenium Red and Toluidine Blue and mounted with Histomount.

Finally, to document the variation across the whole subfamily, we attempted to include all restio species. Roots were sampled from herbarium specimens in the combined herbaria of the University of Zurich (Z) and the Federal Technical University (ZT), as well as the Bolus Herbarium of the University of Cape Town (BOL). Where possible several collections per species were sampled, and in all cases the identity of the specimen was verified. We collected about 1 cm of dried root. Although we could not determine exactly where along the root the material was sampled, almost all samples were from the 1st order roots, and in the mature part (usually within 5 cm from the plant base). Because they are monocots without secondary thickening, it may not be so important to locate the exact places the root is sampled. We assume that if several phylogenetically closely related species show the same characters that this is characteristic of the species, and not a plastic response. In addition, we also field collected some species into FAA. This survey resulted in data for 298 of the 350 species, with all genera represented by at least one species (Table S1)

2.2. Sectioning methods

Herbarium material was incubated in a water-soap solution for three days, and preserved in 70% EtOH. Roots were sectioned transversally with a razor blade under a dissecting microscope, double-stained with Alcian blue (1% in H_2O) and Safranin (1% in EtOH) (2:1) (Tolivia and Tolivia, 1987). Alcian blue stains compounds with anionic groups blue and Safranin stains cellulose and lignin red. Sections were dehydrated through a sequence of increasing EtOH concentrations, washed in butanol, transferred to Histoclear, and mounted in Histomount on glass slides. Drying takes one month in the drying oven at 25 °C. The sections were visualized with a photomicroscope (Axioskop2 MOT, with AxioCam HRc) at magnifications of 40–200 times. Scale bars were added to the pictures with AxioVision4.8. The slides are in the Department of Systematic and Evolutionary Botany of the University of Zurich.

2.3. Data scoring

All slides were studied under an Olympus CH2, using bright light. Data were scored and organized in Delta (Dallwitz, 1980; Dallwitz and Paine, 1986). This allowed data to be kept as unordered or ordered multistate (categorical) data, as count values (meristic), or as real continuous data. Data were exported for phylogenetic analyses using