

An evolutionary perspective of plant adaptations to dry environments

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Abstract

Plants transitioned from an aquatic to a terrestrial lifestyle during their evolution. On land, drought became one of the major problems they encountered, as it impacts correct cell functioning necessary to support life. The evolution of morpho-physiological and molecular adaptations to cope with and tolerate drought was undeniably useful to survive on land. Some of these adaptations appeared repeatedly in phylogenetically distant species, showing a signature of convergent evolution. Details of this convergent evolution are now being assessed thanks to recent developments on high throughput phenotyping and whole genome and transcriptome sequencing. Phylogenomic (comparative genomic) and comparative transcriptomic analyses are revealing complex, well-coordinated and intricate gain and loss of genes and co-option of gene regulatory networks underlying cell and tissue specific adaptations to moderate and extreme drought in phylogenetically distant species. Here we review recent research on signatures of convergent evolution of regulatory networks underlying carbon concentrating mechanisms such as C4 and CAM photosynthesis, desiccation tolerance in seeds and resurrection plants, and impermeabilization of root exodermis.

1 Introduction

The transition of an ancestor aquatic green algae to a terrestrial environment, termed terrestrialization, was a major event in the evolution and diversification of the land plant flora. About 500Ma after the first plant ancestor colonized the land, a multitude of adaptations were developed allowing plants to cope with several problems such as water scarcity (Becker & Marin, 2009; Delaux, Nanda, Mathé, Sejalon-Delmas, & Dunand, 2012; Kenrick & Crane, 1997; Wodniok et al., 2011). Some of the major adaptations to terrestrial lifestyle include modification of the life cycle, divergence of the plant body into roots and shoots, the appearance of complex phenolic compounds (e.g. lignin and flavonoids), vascularization, and the development of specialized cells (such as stomata) (Delaux et al., 2012). As they colonized land, exposure to high radiations and drought became a recurring problem encountered by multiple plant lineages, and common adaptations emerged in diverging plant clades.

For example, photosynthesis under high light and low water availability conditions became possible thanks to the recurring evolution of carbon concentrating mechanisms (CCMs) across plant lineages. CCMs involve either temporal or spatial separation of the initial carbon fixing from the photosynthetic carbon fixing via anatomical adaptations (Edwards & Ogburn, 2012). Studies have shown that all of the enzymes necessary for the temporally separated CAM (Crassulacean Acid Metabolism) and the spatially separated C₄ metabolic pathways are present in all plants and function in other processes (Burgess et al., 2016; Christin et al., 2013; Dunning et al., 2019; Heyduk, Ray, et al., 2019; Ming et al., 2015; Rondeau, Rouch, & Besnard, 2005; Yang et al., 2017). The co-option of these enzymes for the appearance of the CCMs in angiosperms was based on regulatory neofunctionalization of preexisting genes, including those involved in C₃ photosynthesis, and rewiring of ancestral gene expression patterns (Figure 1) (Ming et al., 2015; Yang et al., 2017).

Another clear example of convergent evolution of adaptations to dry environments is desiccation tolerance

(DT), which is the ability to survive extreme drying and remain alive in the dry state (Alpert, 2000; Leprince & Buitink, 2010; Oliver, Tuba, & Mishler, 2000). It has been long hypothesized that DT mechanisms present in the vegetative body of primitive bryophytes became confined in small reproductive structures (such as spores, pollen and seeds) during the evolution of tracheophytes (Figure 1) (Alpert, 2000; Oliver et al., 2000). Some plants were able to colonize extremely dry environments by redirecting seed DT mechanisms into their vegetative body parts, the so-called resurrection plants (Artur, Costa, Farrant, & Hilhorst, 2019; Farrant & Moore, 2011). This co-option hypothesis has been recently assessed at the genomic level thanks to the availability of whole genome sequences of resurrection plants (Costa et al., 2017; Giarola, Hou, & Bartels, 2017; VanBuren et al., 2018; VanBuren, Pardo, Man Wai, Evans, & Bartels, 2019; VanBuren et al., 2017). Comparative genomics has recently revealed gene family expansion and network rewiring underlying the convergent evolution of DT (Artur, Zhao, Ligterink, Schranz, & Hilhorst, 2019; Oliver et al., 2020; VanBuren et al., 2019).

Our final example is how hydrophobic extracellular biopolymers (such as lignin, cutin and suberin) contribute to cell permeability and water transport control, and are utilized also for critical drought tolerance adaptations that have convergently evolved in plants. For example, it was found that the ancestral green algae and red-algae were able to produce “lignin-like” compounds (Delwiche, Graham, & Thomson, 1989; Labeeuw, Martone, Boucher, & Case, 2015; Martone et al., 2009) and that lycophytes and spermatophytes independently developed the ability to produce monomers for lignin (Renault et al., 2017; Weng et al., 2010; Weng, Li, Stout, & Chapple, 2008). Cutin and suberin seem to have also independently evolved in different plant clades, as homologues of genes encoding enzymes necessary for the biosynthesis of their precursors were absent in ancestral non-angiosperm species (Cannell et al., 2020; Philippe et al., 2020; Pollard, Beisson, Li, & Ohlrogge, 2008). Furthermore, these biopolymers can be utilized in different cell types. In some plant lineages suberin can play a role as a barrier for water movement in the root exodermis in response to drought (Ejiri & Shiono, 2019; Enstone, Peterson, & Ma, 2002; Kreszies et al., 2020; Líška, Martinka, Kohanová, & Lux, 2016; Reinhardt & Rost, 1995; Taleisnik, Peyrano, Cordoba, & Arias, 1999). The exodermis possibly first appeared in early land plants (lycophytes), and may have convergently evolved in flowering plant lineages (Angiosperms) (Figure 1) (Perumalla, Peterson, & Enstone, 1990).

The evolutionary hypothesis underlying the evolution of exodermis and its role on drought adaptation, as well as DT and CCMs are largely benefiting from recent advances in whole genome sequencing technologies and comparative functional genomics. In this review, we provide an overview of the current knowledge about how convergent evolution contributed to the appearance of these adaptations to dry environments.

2 Carbon Concentrating Mechanisms: CAM and C₄ photosynthesis

Dry habitats are often also sunny, placing further specific restraints on plant physiology and specifically photosynthesis: high light intensity and high temperature. As plants acclimate to drought by restricting their water loss through closing their stomata, they also restrict CO₂ uptake, reducing the available CO₂ for photosynthesis. High light becomes damaging if the energy obtained from light absorption is not used by the photosynthetic electron transport chain due to the absence of CO₂. Simultaneously, high temperature reduces the solubility of gases so that RuBisCO activity shifts away from carboxylation towards more oxygenation, increasing photorespiration and leading to wasted energy (Edwards, 2019). To adapt to these constraints, carbon concentrating mechanisms (CCMs) have evolved in numerous lineages to enable efficient photosynthesis in dry, hot, and high light environments while improving water use efficiency.

CCMs work by separating the initial carbon fixing away from the photosynthetic carbon fixing (Calvin cycle) either temporally across the diurnal cycle, or spatially across different cell types or compartments. Both the temporal separation, as seen in Crassulacean Acid Metabolism (CAM), and spatial separation, as seen in C₄ photosynthesis, have evolved independently in over 60 lineages (Edwards & Ogburn, 2012). The evolutionary paths to both CAM and C₄ photosynthesis have been recently reviewed and discussed in great detail (Bräutigam, Schlüter, Eisenhut, & Gowik, 2017; Chen, Xin, Wai, Liu, & Ming, 2020; Edwards, 2019; Heyduk, Moreno-Villena, Gilman, Christin, & Edwards, 2019; Niklaus & Kelly, 2019; Schlüter & Weber, 2020; Sedelnikova, Hughes, & Langdale, 2018) and in short, both require two main aspects: 1)

an anatomical adaptation and 2) co-option of the carbon concentrating metabolic pathway to the correct spatiotemporal location. The main anatomical adaptation for CAM is enlarged storage vacuole to store the malate synthesized during the night and enable the day-night CCM (Luttge, 1987). In many C_4 plants, the spatial separation is across two cell types, mesophyll and bundle sheath, and to achieve this, C_4 leaves adapt with so-called Kranz anatomy with enlarged bundle sheath cells with increased plastid numbers and increased vein density (Haberlandt, 1904). The regulation of Kranz anatomy is proving to be a complex process (Sedelnikova et al., 2018), and although it has readily evolved convergently in some plant clades, it is starting to appear that not all plant clades are pre-conditioned for the C_4 photosynthesis to evolve (Edwards & Ogburn, 2012). Conversely to complex leaf anatomy and its regulation, all the enzymes required for both the CAM and C_4 metabolic pathways, such as phosphoenolpyruvate (PEP) carboxylase and malate dehydrogenase, are present in all plants serving other functions. To co-opt these enzymes for CCMs, the expression and regulatory patterns have evolved to be spatially and temporally specific (Brown et al., 2011; Burgess et al., 2016; Christin et al., 2013; Dunning et al., 2019; Gowik et al., 2004; Heyduk, Ray, et al., 2019; Kajala et al., 2012; Ming et al., 2015; Rondeau et al., 2005; Schulze et al., 2013; Williams et al., 2016; Yang et al., 2017).

Whole genome sequencing has enabled a level of understanding of how these CCMs evolved in plants and contributed to their drought tolerance. The first C_4 (*Sorghum bicolor*) (Paterson et al., 2009) and CAM (*Phalaenopsis equestris*) (Cai et al., 2015) genomes provided insights about redirection of genes involved in C_3 photosynthesis and expansion of ancient and recent gene families. Recent genome sequences and transcriptomic approaches are also offering new evidence about convergent evolution of genes and regulatory pathways underlying these CCMs. For example, the genome and temporal transcriptome sequencing of the CAM species *Kalanchoë fedtschenkoi* revealed that the independent emergences of CAM from C_3 have been based on rewiring of diel gene expression patterns along with protein sequence mutations (Yang et al., 2017). Furthermore, the pineapple genome (*Ananas comosus* (L.) Merr.), another CAM species, indicated that the transition from C_3 to CAM was based on regulatory neofunctionalization of preexisting genes and regulation of circadian clock components through evolution of novel *cis*-regulatory elements (Ming et al., 2015).

To resolve how gene expression patterns and regulatory networks have evolved in convergent C_4 lineages, comparative leaf transcriptomics have been utilized, including comparisons of C_3 , C_4 and intermediate C_3 - C_4 leaves, developmental gradients, specific cell types and environmental cues (Aubry, Kelly, Kümpers, Smith-Unna, & Hibberd, 2014; Bräutigam et al., 2011; Burgess et al., 2016; Gowik et al., 2004; Li et al., 2010). Transcriptomic comparison across the monocot-dicot divide revealed deep evolutionary conservation of C_4 leaf development pathways and that certain homologous cell type-specific regulators were co-opted during the independent evolutions of C_4 photosynthesis (Aubry et al., 2014). The understanding of C_4 enzymes' regulatory networks in ancestral C_3 state was elucidated also by a transcriptomics approach: comparison of how light and chloroplasts regulate C_4 enzymes in closely related C_3 and C_4 plants. This linked the C_4 enzymes into a pre-existing C_3 regulatory network, explaining the readiness of C_4 to evolve at the molecular level (Burgess et al., 2016).

Counter-intuitively, C_4 and CAM can exist in the same leaf. Earlier this year, a transcriptomic approach was taken to dissect the behavior of both C_4 and drought-induced CAM in the same plant, *Portulaca oleracea*, offering insight how the regulatory networks of shared enzymes might be able to coexist while responding to different environmental and temporal cues (Ferrari et al., 2020). With more C_4 and CAM genomes and transcriptomes becoming recently available (at least six C_4 and three CAM genomes at the moment - Phytozome v.12.1, Goodstein et al. (2012)), more information about the basis of convergent evolution of CCMs and other parallel drought adaptations in these species will become possible in future research.

3 Desiccation tolerance in seeds and resurrection plants

As some plant lineages colonized drier lands, they developed desiccation tolerance (DT), which is the ability to survive the loss of almost all their cellular water without irreversible damage that would cause death (Alpert, 2000; Leprince & Buitink, 2010; Oliver et al., 2000). DT differs from drought tolerance as, based on critical water levels, drought tolerance refers to survival after moderate dehydration (approximately 0.3

g H₂O/g dry weight) while DT generally refers to survival after further dehydration (below 0.3 g H₂O/g dry weight) (Hoekstra, Golovina, & Buitink, 2001). Desiccation tolerant organisms are able to survive almost total dehydration and enter into an ‘anhydrobiotic’ state of low metabolic activity (Hoekstra et al., 2001). To enter into the anhydrobiotic state a coordinated series of molecular events associated with prevention of cellular damage takes place (Hoekstra et al., 2001; Oliver et al., 2000).

It is believed that the initial appearance of DT features in vegetative tissues of primitive plants was a crucial step for their colonization and diversification on land (Oliver et al., 2000). As plants became more complex organisms and started to colonize harsher environments, DT was lost from vegetative tissues and became confined to reproductive structures, such as pollen and (orthodox) seeds (Alpert, 2000; Farrant & Moore, 2011; Oliver et al., 2000). A group of about 300 species dispersed across 13 lineages of the plant phylogeny are known to display common physiological, biochemical and molecular signatures of DT in their vegetative structures, and are called ‘resurrection plants’ (Artur, Costa, et al., 2019; Oliver et al., 2020; Oliver et al., 2000). Resurrection plants present two major strategies to tolerate desiccation: ‘homoichlorophyllous’ resurrection plants display leaf curling, rolling or folding, what provide protection against photo-damage, while ‘poikilochlorophyllous’ resurrection plants undergo chlorophyll breakdown, chloroplast disassembly and synthesis of anthocyanin (Alpert, 2000; Artur, Zhao, et al., 2019; Charuvi et al., 2019; Radermacher, du Toit, & Farrant, 2019). The recurring appearance of these DT strategies across plant phylogeny gives a strong support for the hypothesis of convergent evolution of DT, however, the features underlying this phenomena were until recently unknown.

Recent developments in whole genome sequencing have facilitated the assessment of the history of genes and regulatory pathways underlying the evolution of DT in plants. In the past five years, at least eight whole genomes and several transcriptomes of desiccation tolerant plant species from distinct phylogenetic groups became available (Artur, Costa, et al., 2019; Oliver et al., 2020). Comparative genomic studies are now enabling the discovery of features underlying the recurrent evolution of DT in plants (Artur, Zhao, et al., 2019; Costa et al., 2017; Pardo et al., 2020; VanBuren et al., 2019). Comparison between desiccation tolerant and desiccation sensitive genomes have revealed loss of genes associated with the aquatic lifestyle of the ancestor green algae, and the expansion of gene families and high expression of genes necessary for light and dehydration protection (Khraiweh et al., 2015; Rensing et al., 2008; VanBuren et al., 2019; Xu et al., 2018). The latter is clearly exemplified by expansion of late embryogenesis abundant proteins (LEAs) and early-light induced proteins (ELIPs) families (Costa et al., 2017; Khraiweh et al., 2015; Rensing et al., 2008; VanBuren et al., 2019; Xu et al., 2018). LEA proteins were discovered in cotton seeds as accumulating at the later stages of embryo development during the maturation drying phase (Dure III, Galau, & Greenway, 1980; Dure et al., 1989; Galau, Hughes, & Dure, 1986). These proteins together with sugars, form intra-cellular glasses that contribute with stabilization of membranes, organelles and the cytoplasm (Artur, Rienstra, et al., 2019; Buitink & Leprince, 2004; Crowe, Hoekstra, & Crowe, 1992; Wise & Tunnacliffe, 2004). LEAs belong to a large protein family divided into eight groups (Artur, Zhao, et al., 2019; Hundertmark & Hinch, 2008). The evolutionary analysis of the LEA families has revealed expansion of specific subgroups in resurrection plant genomes, suggesting that LEAs may have contributed with the establishment of DT in these species (Artur, Zhao, et al., 2019; Costa et al., 2017; VanBuren et al., 2017). ELIPs are known to protect the cells against photooxidative damage under high light intensities (Hutin et al., 2003). The analysis of the genomes of resurrection plants from distant phylogenetic clades revealed a massive proliferation of ELIPs as tandem duplications, supporting the hypothesis of convergent evolution of DT in resurrection plants (VanBuren et al., 2019). ELIPs expansion may have been especially important for homoichlorophyllous species, contributing to their ability to protect chloroplast structure and chlorophyll during desiccation (VanBuren et al., 2019).

Usually, angiosperm resurrection plants respond to vegetative desiccation by inducing the expression of regulatory pathways typically related to seed DT (Costa et al., 2017; Giarola et al., 2017; Pardo et al., 2020; VanBuren et al., 2017). A recent study has shown, however, that despite conserved seed regulatory networks being activated in vegetative tissues of the poikilochlorophyllous resurrection plant *Xerophyta humilis*, the master transcription factors (TFs) upstream of these pathways in seeds are not activated in vegetative tissues (Lyll et al., 2020). This finding opens up novel hypotheses about the evolution of DT. For example,

it is likely that the activation of components of seed DT in vegetative tissues involved the appearance of alternative TFs that have evolved in a similar fashion in different resurrection plant genomes. A comparative genome and transcriptome study have recently shown that seed dehydration-related genes shared similar expression patterns among desiccation tolerant and sensitive grass species during drought, however, subsets of seed-specific genes were identified as expressing only in desiccation tolerant grasses (Pardo et al., 2020).

Altogether these studies show the fundamental role of comparative genomics and transcriptomics for the understanding of the evolution of DT in plants. With more genomes and transcriptomes becoming available, more information will be given for the co-option hypothesis of DT between seeds and resurrection plants, contributing to the understanding of how the underlying gene regulatory networks have convergently evolved. These data will also provide knowledge about key TFs working upstream of gene regulatory networks controlling DT pathways, which are of potential interest for engineering more drought tolerant crops.

4 Impermeabilizing biopolymers

The evolution of hydrophobic extracellular biopolymers was a critical innovation for plant terrestrialization, contributing to permeability and water transport control. Some of these compounds include complex phenolic-derived polymers such as lignin, cutin and suberin (Niklas, Cobb, & Matas, 2017). Lignin generally works in the reinforcement of secondary cell walls, cutin is commonly found as part of the impermeabilizing cuticle of aerial parts and was recently reported as being also part of the root cap cuticle, and suberin is found in many tissues including specialized root cells, tubers, fruit skin, and seed coat (Berhin et al., 2019; Niklas et al., 2017; Philippe et al., 2020; Renault et al., 2017). In general these compounds function in mechanical support, defense against pathogens and herbivores, and in the control of the movement of water, nutrient and gases (Niklas et al., 2017; Philippe et al., 2020; Renault et al., 2017; Pei Wang et al., 2020).

Convergent evolution has also played an important role on the appearance and shaping of the biosynthetic pathways of some of these biopolymers across diverging plant taxa. For example the ancestral green algae and red-algae present the ability to produce “lignin-like” compounds, leading to the hypothesis that ancient biosynthesis pathways have been rewired in the vascular plant lineage (Delwiche et al., 1989; Labeeuw et al., 2015; Martone et al., 2009). Furthermore, it has been identified that lycophytes and spermatophytes have independently developed the ability to produce monomers necessary for lignin biosynthesis and assembly (Renault et al., 2017; Weng et al., 2010; Weng et al., 2008).

It is possible that convergent evolution also played a role on the appearance of cutin and suberin across distantly related plant species. Both cutin and suberin share similar initial biosynthetic steps and lipid precursors, however different enzymes belonging to the same superfamily and with different mechanisms of action work on their modification and assembly (Philippe et al., 2020; Pollard et al., 2008). A recent large scale comparative genomic study has revealed that the ability to synthesize precursor molecules of cutin and suberin emerged prior the evolution of land plants, however the subsequent steps of the biosynthetic pathway may have evolved independently across land plants (Cannell et al., 2020). Still, little is known about how the other parts of the pathway, such as polymerization and distribution in plant cells, evolved in land plants, what opens up a myriad of possibilities for future research (Niklas et al., 2017; Philippe et al., 2020; Pollard et al., 2008).

4.1 Cell type adaptation to drought: Root endodermis and exodermis

The diversification of biosynthetic pathways leading to the production of impermeabilizing hydrophobic compounds has also contributed to the evolution of distinct cell types important for adaptation to terrestrial life and for resilience to drought. For example, the roots of all vascular plants contain an endodermis surrounding the vascular tissues (Doblas, Geldner, & Barberon, 2017; Enstone et al., 2002). The endodermis cell layer forms a diffusion barrier for water, gases and nutrients due to the presence of two cell wall modifications: the Casparian strip and suberin lamella (Barberon et al., 2016; Doblas et al., 2017; Seago & Fernando, 2013; Vishwanath, Delude, Domergue, & Rowland, 2015). Casparian strip is composed of lignin, deposited in the walls of endodermal cells at their junctions, dividing the layer into outward and inward polarities and forming an effective barrier to the apoplastic movement of molecules into the stele and preventing their backflow

(Barberon, 2017; Enstone et al., 2002; Roppolo et al., 2014; Roppolo et al., 2011). The suberin lamella is a secondary cell wall modification deposited in the inner surface of the primary cell walls, usually after the Casparian strip is formed in the mature endodermis (Barberon, 2017; Enstone et al., 2002). Different from Casparian strip, the suberin lamella may not form in every root nor in every endodermal cell (the so-called passage cells) (Andersen et al., 2018; Barberon et al., 2016; Enstone et al., 2002). Despite debate in the past years, the role of suberin lamella as an apoplastic barrier for water and nutrient uptake from the apoplast to the endodermis cytoplasm has been demonstrated (Barberon et al., 2016; Ranathunge & Schreiber, 2011; Peng Wang et al., 2019).

The roots of several species also develop an exodermis below the epidermis, which is a specialized type of hypodermis with Casparian bands and suberin lamellae depositions (Enstone et al., 2002; Perumalla et al., 1990). The exodermis function as a dynamic barrier not only against water loss under drought and salinity, but also against loss of oxygen under anoxic conditions, against penetration of ions and heavy metals, and against pathogen infections (Aloni, Enstone, & Peterson, 1998; Damus, Peterson, Enstone, & Peterson, 1997; Ejiri & Shiono, 2019; Enstone et al., 2002; Liška et al., 2016; Namyslov, Bauriedlová, Janoušková, Soukup, & Tylová, 2020; Ranathunge, Lin, Steudle, & Schreiber, 2011; Tylová, Pecková, Blascheová, & Soukup, 2017). At the same time, the development of exodermis barriers has its downside as it may impair the uptake of nutrients and interaction with beneficial microbes (Kamula, Peterson, & Mayfield, 1994). To cope with this problem, many plant species developed the ability to induce an exodermis dynamically in response to abiotic stresses, such as drought (Enstone et al., 2002; Kreszies et al., 2020; Liška et al., 2016; Reinhardt & Rost, 1995; Taleisnik et al., 1999). Interestingly, the development of the exodermis may vary among closely related species displaying distinct stress response phenotypes (Ejiri & Shiono, 2019), indicating that this cell type contributes to plant plasticity and acclimation and may also help plants to adapt and colonize dry environments.

Regardless of its adaptive role, the evolution of exodermis in plants still remains untangled. Perumalla et al. (1990) surveyed 181 species from 53 families of plants from different ecological groups (hydrophytic, mesophytic, and xerophytic) to determine the presence of hypodermis with Casparian bands (exodermis). As the majority (156) of the species assessed presented an exodermis with suberin only (hypodermis) or with both suberin and lignin, the authors hypothesized that the presence of a modified hypodermis is ancestral to flowering plants, and has been retained in many species (Perumalla et al., 1990). Furthermore, the authors found that festucoid grasses lack Casparian bands despite presenting cells with similar shape and packing as species with hypodermal Casparian bands, leading to the hypothesis that their recent ancestor may have lost the trait. Interestingly in seminal roots of modern cultivars of barley (a festucoid species) the exodermis fails to develop even upon severe osmotic stress (Kreszies et al., 2019), while in wild barley the exodermis is induced in response to osmotic stress (Kreszies et al., 2020). On the other hand, in other crop grasses (non-festucoid), such as rice and maize, an exodermis is present and develops faster in response to stress (Ranathunge, Schreiber, Bi, & Rothstein, 2016; Schreiber, Franke, Hartmann, Ranathunge, & Steudle, 2005).

Understanding how the exodermis evolved in plants can help in the identification of the underlying regulatory networks responsible for its induction in response to drought. To obtain more knowledge about the evolution of exodermis, we compiled the current information about their presence in plant species based on literature search (Figure 2) (Bani, Pérez-De-Luque, Rubiales, & Rispail, 2018; Barrios-Masias, Knipfer, & McElrone, 2015; Barykina & Kramina, 2006; Brundrett, Murase, & Kendrick, 1990; Calvo-Polanco, Sánchez-Romera, & Aroca, 2014; Damus et al., 1997; Demchenko, Winzer, Stougaard, Parniske, & Pawlowski, 2004; Eissenstat & Achor, 1999; Ejiri & Shiono, 2019; Enstone et al., 2002; Ghanati, Morita, & Yokota, 2005; Kosma, Rice, & Pollard, 2015; Liu et al., 2019; Perumalla et al., 1990; Ranathunge et al., 2017; Reinhardt & Rost, 1995; Ron et al., 2013; Schreiber, Franke, & Hartmann, 2005; Schreiber, Hartmann, Skrabs, & Zeier, 1999; Shiono & Yamada, 2014; Thomas et al., 2007; Zhang, Yang, & Seago Jr, 2018).

Based on this analysis, the exodermis with suberin first appeared in early land plants (lycophytes) but it is missing from other seedless vascular plants and all but one gymnosperms (Damus et al., 1997). Interestingly, four species in the lycophyte genus *Selaginella* contain exodermal with lignified Casparian strips (Damus et

al., 1997). Most flowering plants contain an exodermis with suberization only (hypodermis), while a lignified exodermis appears in about a third of the species. The scattered appearances of the exodermal lignification indicates that it has evolved independently multiple times, suggesting a high evolutionary pressure and pre-conditioning for the characteristic to arise. Species with no exodermis have been identified in seven clades (in purple, Figure 2), and the most parsimonious explanation for presence/absence of exodermis is the loss of the cell type in these lineages. However, the evolutionary hypotheses are restricted by the sparse sampling in families of interest. This is highlighted by the relevant literature containing contradictions (e.g. pea Bani et al. (2018); Perumalla et al. (1990); Taleisnik et al. (1999)), likely due to the dynamic nature of the exodermis.

Evolutionary studies focused on characterizing the exodermis, e.g. by staining suberin and lignin or using barrier property assays (Supplementary Table 1) will contribute with important information about how this cell type has appeared or disappeared multiple times across the plant lineages. Coupling that with comparative genomics and transcriptomics of phylogenetically close species (e.g. from the same family) but with different phenotypes (e.g. non-exodermal, constitutive and stress-inducible exodermis) will be key to identify the origin of the regulatory networks and how master regulators underlying exodermis development and suberization in response to drought evolved. It is possible that the the sporadic appearance of exodermis during plant evolution was possible through rewiring regulatory networks of Casparian strips and suberin lamellae formation in endodermis or similar lignin and suberin biosynthetic pathways from other cell types. Recent studies are showing the importance of distinct clades of the MYB transcription factor family as conserved regulators of suberin deposition in response to osmotic stress in different cell-types and in phylogenetically distant plants, linking their evolution with colonization of dry terrestrial environments by early land plants (Capote et al., 2018; Cohen, Fedyuk, Wang, Wu, & Aharoni, 2020; Gou et al., 2017; Kajala et al., 2020; Kosma et al., 2014; Lashbrooke et al., 2016; Legay et al., 2016; To et al., 2020; Wei et al., 2020). Two of the possible scenarios are: (1) osmotic stress-inducible regulation of suberization diversified from pre-existing developmental pathways, (2) the regulation of suberization in response to drought was re-activated as plants colonized drier environments. Taking that into account, further evolutionary/phylogenetic study of exodermis is needed and selection of a good clade(s) to dissect the gain/loss events is key for understanding exodermis development and how it evolves so readily.

6 Conclusions and perspectives

The appearance and diversification of adaptations to dry environments and their underlying gene regulatory networks have been tightly shaped at cell, tissue and organismal levels during evolution. These adaptations were crucial for plants to colonize dry lands and survive variations on water availability in the environment. Carbon concentration mechanisms, desiccation tolerance and root impermeabilization are among the main plant adaptations to dry environments and share a similar pattern of convergent evolution in phylogenetically distant organisms. Detailed physiological and histological studies combined with recent genome and transcriptome sequencing techniques will allow to access how these adaptations evolved through millions of years. This information in combination with novel methods to simulate drought conditions (e.g. Marchin, Ossola, Leishman, and Ellsworth (2019)) and technologies for plant genetic modification (e.g. CRISPR/Ca9) will have the power to help on the improvement of plant performance in dry environments, specially in the current scenario of increasing climate change.

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Figure legends

Figure 1 Overview of the evolution of plant adaptations to dry environments. The recurring appearance of carbon concentrating mechanisms (CCMs), desiccation tolerance (DT) and root impermeability in Viridiplante lineages are shown. Root impermeability shows endodermis (yellow), exodermis with suberin only (bright turquoise) and exodermis with suberin and lignin (blue).

Figure 2 Exodermis (hypodermis) evolution across land plants. The inner ring represents the exodermis phenotype and the outer ring the major clades. The phylogenetic tree was generated using NCBI Common Tree (www.ncbi.nlm.nih.gov/Taxonomy/CommonTree/wwwcmt.cgi) and edited using iTOL (itol.embl.de/).

Tables

Supplementary Table 1 Histochemical tests for barrier cell-type identification:

Visualize	Stain type	Reference
Suberin	Sudan IV	Johansen (1940)
	Fluorol yellow	Brundrett, Kendrick, and Peterson (1991)
Lignin	Phloroglucinol HCl	Jensen (1962)

Visualize	Stain type	Reference
Barrier property	Berberine-aniline blue	Brundrett, Enstone, and Peterson (1988)
	Calcofluor white M2R (Cellufluor)	Perumalla et al. (1990)
	Propidium iodide	Pecková, Tylová, and Soukup (2016)

* In general the exodermis, similar to the endodermis, is auto fluorescent and resistant to acid digestion, and these properties have been used as a basis to characterize the presence of an exodermis in different species (Perumalla et al., 1990).



