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Control of root meristem establishment in conifers

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The evolution of terrestrial plant life was made possible by the establishment of a root system, which enabled plants to migrate from aquatic to terrestrial habitats. During evolution, root organization has gradually progressed from a very simple to a highly hierarchical architecture. Roots are initiated during embryogenesis and branch afterward through lateral root formation. Additionally, adventitious roots can be formed post-embryonically from aerial organs. Induction of adventitious roots (ARs) forms the basis of the vegetative propagation via cuttings in horticulture, agriculture and forestry. This method, together with somatic embryogenesis, is routinely used to clonally multiply conifers. In addition to being utilized as propagation techniques, adventitious rooting and somatic embryogenesis have emerged as versatile models to study cellular and molecular mechanisms of embryo formation and organogenesis of coniferous species. Both formation of the embryonic root and the AR primordia require the establishment of auxin gradients within cells that coordinate the developmental response. These processes also share key elements of the genetic regulatory networks that, e.g. are triggering cell fate. This minireview gives an overview of the molecular control mechanisms associated with root development in conifers, from initiation in the embryo to post-embryonic formation in cuttings.

Introduction

The root system, most of which usually exists below the soil surface, is essential for plant growth and survival because it provides anchorage and access to soil resources. Roots occurred as an early innovation in land plants that progressively acquired morphological complexity and enhanced functionality during evolution. They have additional vital functions, e.g. they serve as storage organs and can form symbioses with microorganisms and fungi. They are also sites of phytohormone synthesis. There are generally two types of roots: (1) those that are initiated during embryogenesis, such as the primary root; and (2) those that arise post-embryonically either from the primary root (lateral roots, LRs) or adventitiously from aerial organs (adventitious roots, ARs). The ability to form ARs is crucial for producing plants with genetic homogeneity, with this phenomenon being the basis for vegetative propagation of many plant species. Forest tree improvement efforts rely on vegetative propagation methods because the potential for obtaining better forests through sexual breeding is limited. Rooting of

Abbreviations – AGO, ARGONAUTE; AR, adventitious root; ESTs, expressed sequence tags; *GH3*, *Gretchen Hagen 3*; GRAS, Gibberellic-acid insensitive repressor of GAI1 SCR; *GUS*, *β-glucuronidase*; *HD-GL2*, *Homeodomain-Glabra2*; IAA, indole-3-acetic acid; LR, lateral root; NPA, 1-N-naphtylphthalamic acid; PAT, polar auxin transport; *PgAGO*, *Picea glauca AGO*; PIN, PIN-formed; QC, quiescent center; RAM, root apical meristem; SAM, shoot apical meristem; SAMS, S-adenosylmethionine synthase; SCL, SCARECROW-like; SCR, SCARECROW; SHR, Shortroot; *WOX*, *WUSCHEL-related homeobox*.

cuttings, together with somatic embryogenesis, are valuable methods that are extensively used to vegetatively propagate conifers in both breeding programs and the production of clonal forests.

Conifers are of great ecological and economic importance, as they are widely used in reforestation programs and are critical to preventing soil erosion, among other functions. They first appeared 250–265 m.y.a., and still dominate the forests of the northern hemisphere. Conifers are classified as seed plants and comprise two thirds of the extant gymnosperms.

Significant progress in developing and optimizing protocols for the induction of AR formation on stem or branch cuttings and LR formation in somatic embryos of coniferous species has been made during the last decades. In addition to being utilized as propagation techniques, in recent years somatic embryogenesis and adventitious rooting have emerged as versatile model systems for understanding the regulation of gene expression underlying embryo formation and organogenesis, respectively.

Here, we provide an overview of our present understanding of how root development, from initiation in the embryo to post-embryonic formation in cuttings, is controlled at the molecular level in conifers. Most of the results presented in this minireview benefited from *Arabidopsis thaliana* reference data, because, until very recently, the sequencing of conifer genomes had not been attempted due to excessive genome sizes. Furthermore, several molecular analyses of signaling pathways and processes (e.g. embryo maturation) have identified significant overlaps between conifer and angiosperm sequences, which indicates that many developmental pathways are conserved between plant species (Cairney and Pullman 2007). The recent release of three draft genomes from spruces and pines, along with advances in the methods for genetic transformation (reviewed in Uddenberg et al. 2015, Mackay et al. 2012), provide a robust framework for unraveling the unique regulatory networks underlying conifer developmental biology.

Conifer embryogenesis: root pole establishment

In seed plants, the body plan is delineated early during embryogenesis following the differentiation of the shoot apical meristem (SAM) and the root apical meristem (RAM). This differentiation occurs along both the embryo axis (apical-basal polarity) and the radial patterning that exists across the axis (adaxial-abaxial organization). Both gymnosperms and angiosperms share this body organization during embryogenesis, despite differences in early embryogenesis. Conifer embryos undergo a more irregular division pattern than the model plant *Arabidopsis thaliana* (Arabidopsis), with the zygote undergoing several rounds of nuclear duplication without cell division. Moreover, there is no clear asymmetric cell division that defines the apical and basal cell lineages. This results in a polar structure with a compact, globular embryonal mass (embryo proper) in the apical part that is characterized by proliferating cells, and an elongated structure in the basal part that contains several tiers of non-diving cells, called suspensor (Fig. 1; Stasolla and Yeung 2003, Cairney et al. 2006, Smertenko and Bozhkov 2014). The embryonal mass and the suspensor are subsequently separated by a layer of gymnosperm-specific cells called embryonal tube cells, which are derived from the proximal end of the embryo proper and move to the suspensor system, while the uppermost suspensor cell that becomes the root founder cell, called the hypophysis, produces the different root cell lineages that have been described in Arabidopsis. The young embryonal mass is divided into two regions: the region proximal to the suspensor in which cells undergo transverse divisions; and the region distal to the suspensor in which cells divide in all planes. The root cap differentiates from the proximal region, although the existence of stem cells at the basal part of the embryo proper in conifer embryos remains conjectural because a cell corresponding to the hypophysis has not yet been described (Fig. 1; von Arnold et al. 2016).

While Arabidopsis embryo morphology changes conspicuously, passing through 'globular', 'heart' and 'torpedo' stages, alterations in conifer embryo morphology are more subtle (Cairney et al. 2006). Nevertheless, embryogenesis in conifers can be divided into four phases: proembryogenesis, early and late embryogenesis, and maturation (Fig. 1). The root pole becomes visible between the 'early' and 'late' embryogenesis stages, and fully develops during maturation (Stasolla et al. 2003, Aquea and Arce-Johnson 2008).

Somatic embryogenesis has been used to study the regulation of embryo development in conifers, as the early and late stages of somatic embryogenesis are identical to the equivalent stages of zygotic embryogenesis (Aquea and Arce-Johnson 2008, Smertenko and Bozhkov 2014).

Embryonic *WOX* expression in conifers

Despite the differences in patterning during embryo development between conifers and angiosperms, recent results have shown that central parts of the regulatory network are conserved between both groups of plants (Palovaara et al. 2010a, Zhu et al. 2014, von Arnold et al. 2016). Embryonic patterning requires the establishment of distinct transcriptional domains that regulate

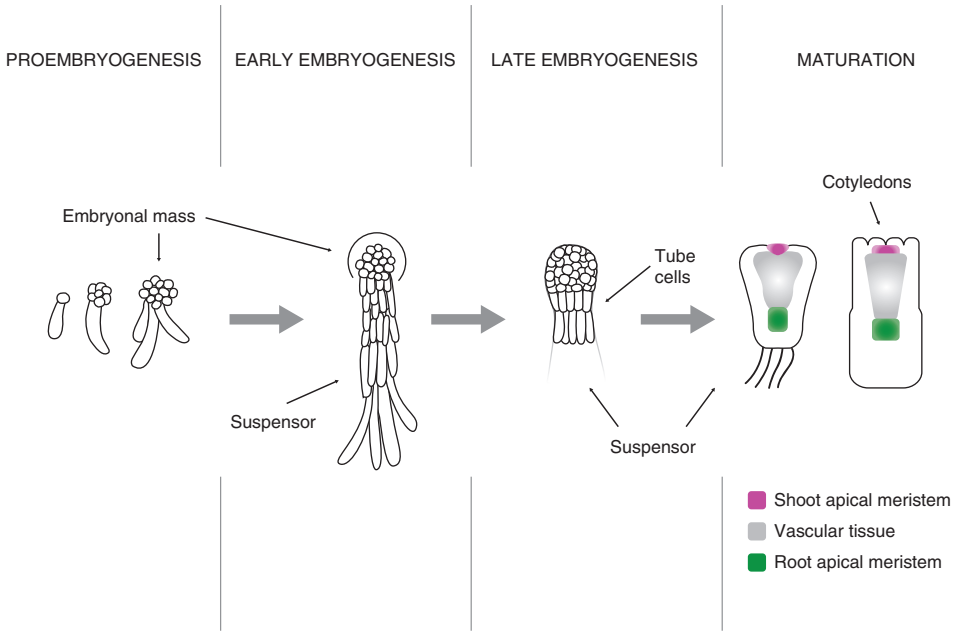

Fig. 1. Developmental stages during embryogenesis in conifers. The course of embryo development can be divided into four phases: proembryogenesis; early and late embryogenesis; and maturation. During proembryogenesis, a polar structure characterized by dividing cells on one pole of the embryo (embryonal mass) and large vacuolated cells on the opposite pole appears. The vacuolated cells give rise to the suspensor, which disappears as maturation progresses. Early embryogenesis is characterized by elongation of the suspensor. Subsequently, the embryonic mass is converted into the embryo proper, which separates from the suspensor by gymnosperm-specific tube cells. This stage (late embryogenesis) culminates with the specification of the protoderm (the outermost layer of the embryo proper) and the presence of cells in the proximal region of the embryo proper that divide periclinally to become the precursors for the root cap. As the embryo matures, shoot and root meristem is established.

spatiotemporal cell divisions. The orientation of the cell division plane determines not only the position but also the fate of the daughter cells (von Arnold et al. 2016).

In angiosperms, high expression of specific members of the *WUSCHEL-related homeobox* (*WOX*) gene family, which encode plant-specific transcription factors, has been linked to cell proliferation and specification during embryonic root formation. The Arabidopsis *WOX* genes, *AtWOX8* and *AtWOX9*, are expressed in the basal cell and its descendants. The *Picea abies WOX* gene, *PaWOX8/9*, which is orthologous to *AtWOX8* and *AtWOX9*, is mainly expressed during early and late embryogenesis, with expression declining in mature embryos (Fig. 2; Palovaara et al. 2010a, Hedman et al. 2013, Zhu et al. 2014). Using RNA in situ hybridization, Palovaara et al. (2010a) showed that *PaWOX8/9* is present throughout the early somatic embryo. As development progresses, expression becomes restricted to the future RAM and cotyledon initiation sites, with no transcripts detected in the SAM areas of the late somatic embryo (Fig. 2). In the mature embryo, *PaWOX8/9* is still detectable in the RAM and in the central area of the developing root cap, while no transcripts are found in the surrounding shoot apex and the epidermis (Fig. 2). The downregulation of *PaWOX8/9* expression during embryo development disturbs the orientation of the cell division planes within the basal part of the embryonal mass during early and late embryogenesis, resulting in an aberrant embryo morphology. This could be related to earlier findings that the downregulation of *PaWOX8/9* affects the transcription of several key cell-cycle regulating genes (Zhu et al. 2014).

Alvarez et al. (2017) recently reported high expression of two *Pinus pinaster WOX* genes, both of which are closely related to *AtWOX9*, in early and late embryos, with expression decreasing dramatically in germinated embryos. In addition, *PpWOX5*, which is orthologous to *AtWOX5*, shows maximum expression at the mature-embryo stage, with transcripts preferentially located to the root tip of seedlings. This, in combination with low expression levels in other seedling tissues, mirrors the expression pattern of *AtWOX5*, which has been shown to influence stem-cell function in Arabidopsis RAM (Alvarez et al. 2017).

Auxin regulates conifer embryonic root formation

The plant hormone auxin and its polar transport (PAT) are crucial for the correct formation of the embryonic

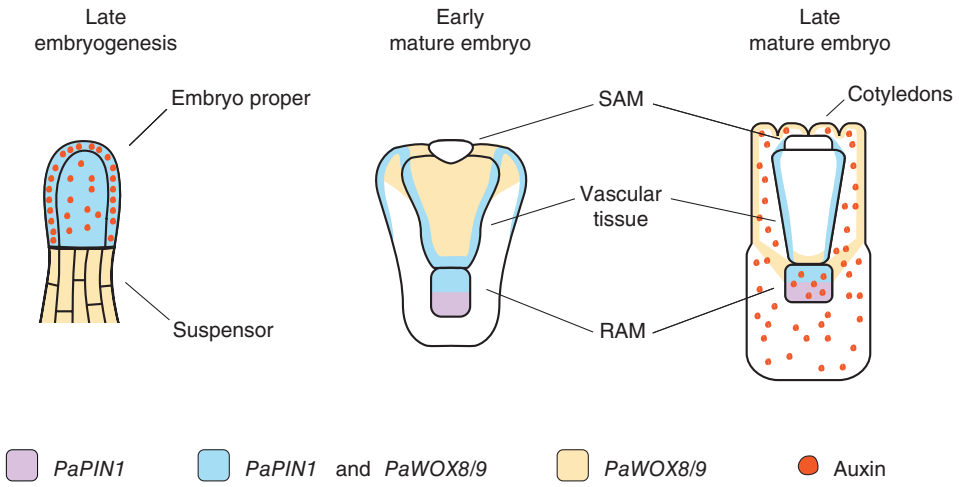

Fig. 2. Gene expression patterns and auxin distribution during the later stages of embryo development. Localized expression of *PaWOX8/9* and *PaPIN1*, as well as the establishment of auxin gradients, contribute to apical-basal axis formation during late embryogenesis and embryo maturation (Hakman et al. 2009, Palovaara et al. 2010a, 2010b). Auxin accumulates in the embryo proper, especially in the protoderm, during late embryogenesis. At this stage, the *PaPIN1* expression pattern follows auxin distribution, while *PaWOX8/9* expression can be detected throughout the embryo body, including the suspensor. In the early mature embryo, *PaPIN1* and *PaWOX8/9* expression patterns overlap in the procambium, the tips of cotyledon primordia, the epidermis and the central area of root apex, while no transcripts are present in the shoot apical meristem. At this stage, only *PaPIN1* can be detected in the root cap columella cells, while *PaWOX8/9* transcripts are present in the subepidermal cell layers of vascular tissue. The auxin distribution pattern during this embryo developmental stage remains unclear. In the late mature embryo, *PaPIN1* and *PaWOX8/9* exhibit a similar expression pattern, concentrated to the differentiating procambium and the root apical meristem. In addition, *PaWOX8/9* is detectable in the epidermis and the embryonic cortex, while *PaPIN1* transcripts accumulate in the root columella cells. At this stage, auxin accumulates in the epidermis, ground tissue and root apical meristem.

root. Various reports suggest that auxin-mediated events, which are key to establishing basic body organization, are of similar importance in both angiosperms and conifers (reviewed in Trontin et al. 2016). Auxin biosynthesis and PAT start during early embryo differentiation, while the auxin response machinery is activated at the beginning of late embryogenesis (von Arnold et al. 2016). Perturbation of PAT by treatment with the well-established auxin efflux inhibitor 1-N-naphtylphthalamic acid (NPA) has been shown to cause irregular cell division in RAM areas of developing Norway spruce somatic embryos (Larsson et al. 2008, Hakman et al. 2009, Palovaara et al. 2010b) and interfere with both hypophysis specification and root formation in Arabidopsis embryos (reviewed in Petricka et al. 2012). Depending on the embryo developmental stage, NPA treatment will have varying effects on the embryo morphology, with the first step of embryo maturation the most sensitive to PAT disruptions (Hakman et al. 2009). Nonetheless, NPA treatment during the early stages of spruce somatic embryo development leads to an increased concentration of the endogenous auxin indole-3-acetic acid (IAA), which subsequently shifts the balance between the embryonal mass and the suspensor (Larsson et al. 2008). The IAA accumulation patterns in developing spruce somatic embryos in the presence or absence of NPA have been investigated using IAA immunolocalization (Hakman et al. 2009, Palovaara et al. 2010b). A dynamic change in IAA distribution within early stage embryos was noted following NPA treatment, with the IAA signal appearing to be stronger in the protoderm/epidermis of untreated embryos than in that of NPA-treated embryos. No definite alterations in IAA localization were detected as embryos matured, an observation, implying that PAT mediates IAA relocation during the early stages of embryo development (Hakman et al. 2009, Palovaara et al. 2010b).

Later studies investigated whether blocking PAT influences auxin response and embryo development by monitoring the auxin response in a spruce transgenic line harboring a construct in which the *Gretchen Hagen 3* (*GH3*) promoter from soybean was fused to the *β-glucuronidase* (*GUS*) reporter gene (Larsson 2011). GUS activity is high in the suspensor cells and in the basal part of the embrional mass during early embryo development. This pattern of auxin response maxima is abolished by NPA treatment, with the lack of GUS activity in the embryonal mass in NPA-treated embryos. These results led to the formulation of a model to explain how auxin mediates embryo development in Norway spruce (von Arnold et al. 2016). According to this model, auxin moves from the suspensor cells to the embryonal

mass in early differentiating embryo. Blocked PAT during this developmental stage affects the differentiation of the embryonal tube and the suspensor cells, thus auxin transport controls embryo patterning. Afterward, the stream of auxin transport changes during late embryogenesis and auxin moves from the apical part of the embryo proper toward the basal part. The resulting basal accumulation of auxin is presumably triggering the specification of the future root meristem.

Efflux-facilitating proteins of the PIN-FORMED (PIN) family, AtPIN1, AtPIN4 and AtPIN7, accumulate in the hypophysis during the globular stage of Arabidopsis embryo development to establish an apical-to-basal auxin flux that triggers root pole specification. Palovaara et al. (2010b) reported that the expression pattern of *PaPIN1*, which is orthologous to both *AtPIN4* and *AtPIN7*, correlates with local auxin accumulation in early stage spruce embryos (Fig. 2) and its expression shares many similarities to *AtPIN1*. At early stages of embryo maturation, *PaPIN1* activity was observed in the pre-procambium cells of the embryo axis, the cotyledonary primordia and the root apex, while expression is restricted to the procambium and the RAM during later stages (Fig. 2). This evidence suggests that *PaPIN1* is involved in the specification of the procambium and the RAM also in conifers.

The expression pattern of a putative ortholog of the auxin response factor *ARF16* in pine embryos was reported to reflect what has been described for *ARF16* in Arabidopsis, where it represses *WOX5* transcription and restricts the expression of this gene to the quiescent center (Ding and Friml 2010, de Vega-Bartol et al. 2013).

Other important regulators for conifer embryonic root formation

Several other genes that are well established as important developmental regulators of root meristem have been shown to be expressed during somatic embryogenesis in conifers. One study focused on the role of a specific member of the ARGONAUTE (AGO) protein family in white spruce embryo development (Tahir et al. 2006). Members of the AGO family participate in post-transcriptional gene silencing by processing miRNAs, influence stem cell fate specification and regulate the balance between proliferation and differentiation in both plants and animals. *Picea glauca AGO* (*PgAGO*) expression was detected in cells at the future site of RAM already in early stage white spruce embryos, while its localization was restricted to the apical poles at later stage of embryo development. Moreover, embryos with reduced levels of *PgAGO* transcripts develop abnormal root meristems, as they fail to develop the large group of cells that occupies the central region of the root meristem in embryo with normal *PgAGO* expression. These cells, which are mitotically inactive during embryogenesis, are considered analogous to the quiescent center (QC) of angiosperms roots and, following the onset of germination produce new root cells through mitotic reactivation (Yeung et al. 1998). In addition, transcriptomic analyses suggest that *AGO* genes are preferentially expressed during late stages of embryo development in *Pinus taeda* and *Pinus sylvestris* (Oh et al. 2008, de Vega-Bartol et al. 2013). A genome-wide analysis of microRNAs in *Larix leptolepis* proposed that miR168s modulate *AGO1* mRNA during somatic embryo development, with the highest expression levels observed at the late/mature transition stage of embryogenesis (Zhang et al. 2012).

A gene belonging to the *Homeodomain-Glabra2* (*HD-GL2*) plant-specific family has been suggested as a marker for monitoring the radial pattern formation in the embryonic root in *P. abies*. *PaHB2* (for *P. abies Homeobox2*) transcripts are uniformly expressed in early somatic embryos, but become preferentially localized to the outer cortical layer and root cap in mature embryos. A similar *PaHB2* expression pattern exists during post-embryonic primary root development (Ingouff et al. 2003).

Another gene that is important for the early delineation of radial patterning in the embryonic root is *SCARECROW* (*SCR*) from the GRAS (GIBBERELLIC-ACID INSENSITIVE, REPRESSOR OF GAI1, SCR) transcription factor family. Two *SCR* genes are upregulated in white spruce embryos during RAM specification (Stasolla et al. 2003), suggesting that *SCR,* which confers endodermis identity in angiosperms, may also have a conserved role in conifers. The expression patterns of *SCR*, *SHORT-ROOT* (*SHR*) and several *SCR-LIKEs* (*SCLs*) are similar during the maturation stages of somatic embryos in *Pinus radiata*, with expression gradually increasing at the polarization stage and peaking during the subsequent differentiation stage (Fig. 3; Abarca et al. 2014). In *P. taeda*, *PtSCR* and *PtSHR* show basal levels of expression during all of the late embryo developmental stages, reaching a maximum level of expression characterized by an almost twofold increase at the differentiation stage (Jones 2011).

Mathieu et al. (2006) studied the role of a *GERMIN-like* gene (*LmGER1*) during somatic embryogenesis in hybrid larch (*Larix kaempferi* × *Larix decidua* = *Larix* × *marschlinsii* Coaz) by expressing the GUS reporter gene fused to the *LmGER1* promoter. They found that *LmGER1* encodes for a germin-like protein that localizes within the extracellular matrix, where it contributes to the oxidative scission of polysaccharides.

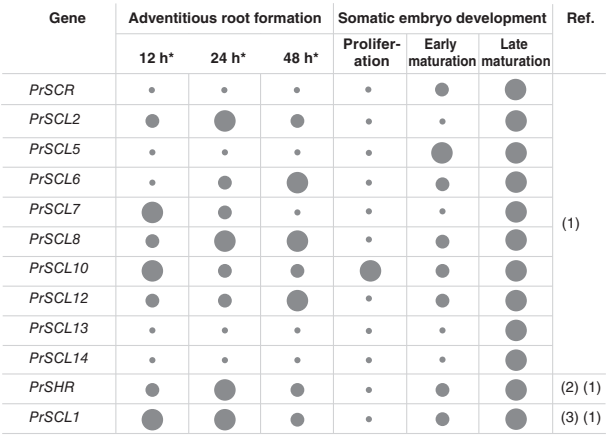

Fig. 3. Expression patterns of selected *Pinus radiata GRAS* genes during the early stages of auxin-induced adventitious root formation and somatic embryo development. The size of circles reflects relative gene expression levels (larger circles correspond to higher expression levels). *hours after the onset of the auxin treatment; SHR, SHORTROOT; SCR, SCARECROW; SCL, SCARECROW-LIKE. References: (1) Abarca et al. 2014, (2) Sánchez et al. 2007, (3) Solé et al. 2008.

LmGER1 was shown to be expressed in the region between the embryonal mass and the suspensor during early embryogenesis, and its transcription was still detectable within the same region once the embryonal root cap had developed during embryo maturation. Based on their observations in white spruce, Yeung et al. (1998) suggest that the embryonal root cap is important to development because of its storage function, containing proteins, lipids and starch. At later stages of embryo development, the starch grains disappear as the root initials become distinct.

AR development in conifers

Studying AR development can also provide information about how root meristems form. AR formation is a postembryonic organogenic process in which roots form from determined or differentiated cells that have been specified to develop a root at positions where they do not normally occur. This developmental process implies that a somatic differentiated cell reverts to a pluripotent or totipotent cell that can develop a root.

In the last decades, a simple and synchronized experimental system has been used to explore the molecular mechanisms that regulate AR development in conifers. This system is based on the ability of hypocotyl cuttings from young pine seedlings to form ARs after treatment with an optimal dose of auxin. Experiments from *P. taeda*, *Pinus contorta* and *P. radiata* confirmed that all three of these pine species demonstrate similar anatomical changes during AR initiation in hypocotyl cuttings (Díaz-Sala et al. 1996, Lindroth et al. 2001a, 2001b, Ricci et al. 2008). Cambial cells, which surround the primary xylem, share the competence to form ARs. When exposed to exogenous auxin, these cells exhibit rapid cell division and re-orientation of division planes to organize the root meristem and lose competence for de novo regeneration of roots with the age and tree maturation (reviewed by Díaz-Sala 2018).

Gene expression patterns during AR formation in pine

Sara von Arnold's research group has previously used *P. contorta* as a model species to study the molecular basis of physiological processes that occur during specific phases of AR development (Lindroth et al. 2001a, 2001b, Brinker et al. 2004). The expression of *PcCDC2*, which encodes for a cyclin-dependent kinase of the PSTAIRE class, increases during the first 12 days of the auxin-induced AR development in pine hypocotyl cuttings when compared to the untreated control. The expression pattern does not coincide with an S-phase correlated marker, suggesting that *PcCDC2* expression in hypocotyls could reflect the state of competence of cells that are capable of responding to auxin for AR development (Lindroth et al. 2001a).

In another study, the same authors noticed that S-adenosylmethionine synthase (SAMS) activity, which is required for the methylation of several substances, including nucleic acids, proteins, carbohydrates and membrane lipids, increases fourfold during the induced AR development when compared to untreated hypocotyl cuttings. They found that two *SAMS* genes, *PcSAMS1* and *PcSAMS2*, are differentially expressed during root formation. *PcSAMS1* expression appears to be restricted to the AR primordia and the protruding ARs, a pattern that is in sharp contrast to that of *PcSAMS2*, which is downregulated during AR development (Lindroth et al. 2001b).

An analysis of global gene expression changes in *P. contorta* using an array of expressed sequence tags (ESTs) from *P. taeda* revealed the timing of the molecular events that occur during AR development (Brinker et al. 2004). During the first 3 days, genes involved in protein synthesis are upregulated, while the expression of genes related to protein degradation decreases. This expression pattern switches during root formation and elongation so that the expression of genes associated with protein degradation increases while the expression of protein synthesis-linked genes decreases. Moreover, photosynthesis-related genes are downregulated, indicating that hypocotyl cells lose their photosynthetic potential early during AR formation. Cell

wall modifications also occur during AR development, as shown by downregulation of cell wall synthesis genes and upregulation of genes involved in weakening cell walls and cell adhesion during the first 3 days after auxin treatment. An opposite expression profile for cell wall-remodeling genes is evident during root primordia, root meristem and root formation phases (Brinker et al. 2004). Similarly, other authors have reported high expression of *α-EXPANSIN* genes, which are responsible for cell wall loosening, during the first 24 hours of auxin-induced AR development in *P. taeda* hypocotyl cuttings (Hutchison et al. 1999). Cell replication-related genes are strongly induced during the first 6 days, which confirms the *PcCDC2* expression profile during AR development described earlier. Furthermore, genes regulating meristem fate regulatory genes are differentially expressed during specific phases of root development. For example, the transcription of a *PINHEAD/ZWILLE-like* gene is induced during the earliest stages, suggesting a role in root meristem initiation.

Local auxin gradients are required for AR initiation in pine

In *P. contorta*, Brinker et al. (2004) observed that there is lack of active auxin transport at the beginning of root development, but this process is promoted during root meristem differentiation to activate the auxin response machinery. The authors suggest that exogenous auxin stimulates the activation of competent cells whereas endogenous auxin stimulates meristem establishment. Several other studies have also investigated how PAT and local auxin distribution influence AR formation (Díaz-Sala et al. 1996, Greenwood et al. 2001, Abarca et al. 2014, Brunoni et al. 2014). A growing body of evidence suggests that the ability to respond to exogenous auxin triggers rapid cell division and re-orientation of division planes. However, the same hypothesis states that dividing-cells only commit to forming root meristem when they are competent (Díaz-Sala et al. 1996, Greenwood et al. 2001, Abarca et al. 2014). In this sense, auxin may non-specifically stimulate specific pre-established cell differentiation patterns and, therefore, the capacity to re-enter cell division is alone insufficient to reset the previous cellular state in non-competent cells (Abarca and Díaz-Sala 2009, Díaz-Sala 2014). Nevertheless, a unique asymmetric localization of endogenous auxin was observed in hypocotyl cuttings that can form ARs during the initial stages of root induction, supporting the concept that only competent cells retain the intrinsic capacity to accumulate auxin (Abarca et al. 2014, Brunoni et al. 2014).

In *P. radiata*, the establishment of AR meristem involves members of the GRAS family (Fig. 3; Sánchez et al. 2007, Solé et al. 2008, Abarca et al. 2014). The expression patterns of *PrSCL1* and *PrSHR* overlap during the initial stages of AR development. Following the onset of cell division, the transcription of both genes increases and they are specifically localized to the cambial region of competent cells, a phenomenon that mimics the asymmetrical auxin distribution reported earlier (Sánchez et al. 2007, Solé et al. 2008). A subset of pine *GRAS* genes is expressed during embryonic development, specifically at the polarization and differentiation stages, as well as during the initial stages of AR formation (Fig. 3; Abarca et al. 2014). The expression of these genes could be associated with an embryo type that reflects competence for adventitious organogenesis in cuttings.

Conclusions

Our understanding of root development in conifers has advanced over the past two decades mainly as a result of the integration of various types of data from studies of conifer embryo development and AR formation in pine cuttings. Similar to what has been described for Arabidopsis, root development in conifers is a dynamic process that includes interactions between transcription factors and plant hormones, principally auxin.

The sequence conservation observed across embryonic and post-embryonic root development-related genes from both angiosperms and conifers could indicate that conifer sequences are functional equivalents of Arabidopsis genes. However, as Cairney and Pullman (2007) highlight, different genes can encode proteins with similar functions while genes that share a high degree of sequence similarity could nevertheless have evolved different functions. In addition, it has been estimated that about 11.4% of the *P. taeda* transcripts found in developing pine embryos are novel and do not have counterparts in other plants (Cairney and Pullman 2007). Furthermore, several mRNAs with currently unknown functions were differentially expressed during AR formation in *P. contorta* hypocotyl cuttings (Brinker et al. 2004), which suggests that unique regulatory networks for root development exist in conifers.

Differences in root growth regulation across angiosperms and conifers could also involve alterations in gene regulatory regions, changes in chromatin status and epigenetic mechanisms that create specific nuclear architectures. The information gained from conifer genome sequencing will help us better understand the unique regulatory interactions that govern growth and development in conifers.

Author contributions

F.B. conceived the manuscript and prepared the figures. F.B. wrote the manuscript together with K.L. and C.B. All authors have reviewed, edited and approved the final manuscript.

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