Initiation of vascular cambium derivatives produces new branch roots in a woody parental axis: Effect on root architecture

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ABSTRACT

The anatomical differences of traces formed in woody parental roots enable the formation of two categories of branch roots: "primary branch roots" (PBR) and "secondary branch roots" (SBR). PBR are those roots hypothesized to be formed by primary tissues such as perycycle, endoderm or xylary parenchyma. SBR are roots hypothesized to be formed by cambium initials and/or phloem derivatives. After their formation the SBR primordia elongate through the secondary phloem, cortex, and phellogen before protruding externally from a swelling bark. A proteomic approach is used to produce a map of woody root axis, and to puts in evidence the occurrence of quantitative variations in a number of proteins in relation to SBRs production. The identities and possible physiological roles of these differentially expressed proteins is discussed in relation to root branching in woody roots. We suggest that SBRs emission improves the adaptation of woody plants to their rooting environment thorough a modification of root architecture.

KEYWORDS: root primordium, vascular cambium, lateral roots

1. INTRODUCTION

Root architecture plasticity is one of the most important factor enabling plants to colonize the extremely variable rooting environments present in the mainland. In this regard the literature has established that production and modification of root branching in the root system is obtained through regulation of the activities of primary tissues such: pericycle, endoderm, xylar parenchyma (Esau, 1965; Casson, 2003). In woody species, these tissues are present only in the proximal region (Esau, 1965) of each root axis and this portion is very limited in comparison to the total root length. On this basis one can assume that if an alteration of root architecture is needed that would occur only in the proximal portion of the root axis and this would contrast strongly with data accumulated in literature which report that new branch roots are observed everywhere along the root axis of a woody species (references in Chiatante et al., 2006). To explain this apparent contradiction, one must make the assumption that an unknown secondary tissue in woody roots, in analogy with the primary tissues, could provide initial cells responsible for originating a new lateral root primordium. At the present, the vascular cambium, originating in part from the pericycle cells (Esau, 1965) has been proposed for this role (Paolillo, 2006). This hypothesis is not unreasonable and requires only that some cambium initials retain the

competence to initiate a new root primordium as their cell lineage ancestors. The considerable implications involved in this event calls for further studies at anatomical, cytological, and molecular level. For this purpose we have investigated the emission of lateral roots from woody parental roots in poplar seedlings at cytological, anatomical, and biochemical level.

2. MATERIALS AND METHODS

2.1. Plant material and anatomical analysis

Populus nigra L. woody root cut in 0.5 cm clips, were fixed in Formaldehyde 4% in 0.1 M phosphate buffer (pH= 7) and then rinsed in 0.1 M phosphate buffer (pH= 7). Clips were first dehydrated in ethanol series (25%-75%-100%) at 4°C and then embedded in Technovit 7100 (Kulzer, Wehrheim, Germany). 8 μm thick cross-sections cut with a microtome (RM 2155 Leica, Germany) were double-stained with fast green and safranin red. Sections were mounted in Histovitrex and observed and photographed under a light microscope (DMRB Leica, Germany).

2.2. Biochemical analysis

Total proteins were extracted from poplar seedlings taproot following a phenol protocol (Mihr & Braun 2003) with minor modifications (Scippa et al. 2008). Phenol extracted proteins were re-suspended in IEF buffer (9 M urea, 4% w/v CHAPS, 0.5% v/v Triton X-100, 20 mM DTT and 1% w/v carrier ampholytes pH 3–10). For 2-D electrophoresis IPG strips (18 cm pH 3–10 nonlinear, Bio-Rad) were rehydrated overnight with 400 mg of total proteins in IEF buffer. Proteins were focused, reduced, and separated on 12% polyacrylamide gels (Scippa et al. 2008). Gels stained with 80% Comassie brilliant blue and 20% methanol, were analyzed by PDQuest 8.1 software (BioRad).

3. RESULTS

3.1. Anatomical investigations

Some branch roots showed a V-shaped trace extending internally toward the centre and penetrating deeply in the secondary xylem internally a wide medullar ray; others branch roots presented a V-shaped trace which did not penetrate within the secondary xylem but extended externally from the cambium zone pointing to the periphery. The branch roots with the first type of trace were called “primary branch roots” (PBR), the others were called “secondary branch roots” (SBR). These names derive from our hypothesis that PBRs originated by primary tissues (perycycle, endoderm or xylary parenchyma) whereas SBRs originated by cambium initials and/or its derivatives. The xylary components of PBR trace showed a radial orientation and the vascular cambium of the parental root was in contact with the vascular cambium of the PBR. The xylary components of SBR trace presented an axial orientation. Parenchymatic cells of PBR- and SBR-trace were always considerably replenished by amyloplasts. Analysis for full extent from end to end of several PBRs enable to divide PBRs in live (L-PBR) or dead (D-PBR. L-PBRs presented an intact bark layer, D-PBRs presented a degenerating bark. In the case of SBRs, we found a distinction between those protruding from the parental root without a defined pattern defined Random-SBR (R-SBR) and those found always in association with an existing PBR named Induced-SBR (I-SBR). The the secondary xylem thickening of the parental root was reduced in correspondence of the location of a L-PBR in comparison to opposite side. The trace of a L-PBR was normally bigger in the secondary xylem than in the secondary phloem and ended in one of the three arcs of the parental root primary stele. The vascular cambium of the parental root connected with the vascular cambium of the L-PBRs. D-PBRs showed all tissues being in a

decomposition state and the traces did not extend further from the secondary xylem of the parental root. Several R-SBRs were found at their initial developmental state when their length was still completely included within the parental secondary phloem. A R-SBRs examined from end to end (Fig.1) showed that a central cylinder elongated in the secondary phloem, and the basal tissues were in contact with the vascular cambium of the parent taproot. The vascular cambium of the parental root seemed to be strictly connected with the central cylinder of this R-SBR. When we investigated R-SBR at a further developmental state, then the meristematic tips of R-SBRs was found to be in contact with the bark and completely surrounded by a swelling of parenchymatic cells belonging to the parental root cortex. The I-SBR protruded always above an existing L-PBR along the same rank. The vascular cambium belonging to the parental root was in contact with the central cylinder of the I-SBR where primary tissues such as perycycle, endoderm and xylar-parenchyma were visible.

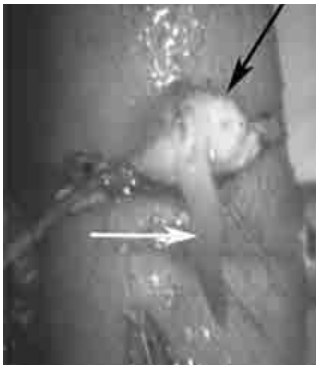

Figure 1. Random Secondary Branch Root .

3.2. Biochemical investigations

A map of the proteome of poplar woody root was produced by 2DE gel electrophoresis. As shown in the Figure 2 the woody root proteome was resolved in 300-350 protein spots. The most abundant protein spots (c.a 250) have been excised from the gel and are in progress to be sequenced. Moreover, antibodies raised against regulatory proteins controlling cell cycle are used in immunoblotting experiment to identify in the woody root proteome map factors involved in SBRs emission .

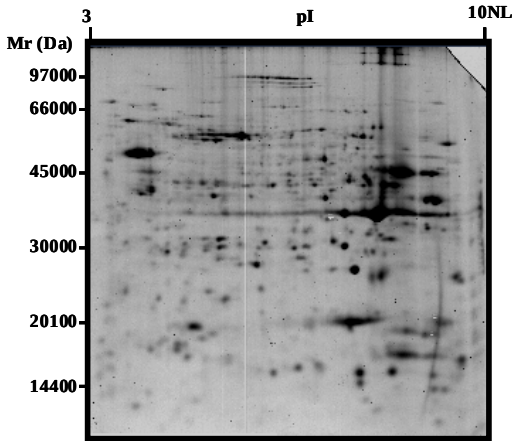

Figure 2. Two-dimensional gel electrophoresis of poplar seedling taproot.

4. DISCUSSION

Our data suggest that branching is possible even when the parental root has developed a secondary structure. This finding changes considerably our present understanding about root architecture deployment indicating the occurrence in woody species of two different branching mechanisms. The first active at early stage where primary tissues are present and is due to perycycle-, endorderm-, and/or xylar parenchyma-tissue; the second starts later where secondary tissues are present and is due to the induction of a unknown number of vascular cambium initials and/or derivatives. Both mechanisms would contribute to give the final root architecture of a woody species. According to this hypothesis branching of woody roots must be regarded as a common feature of all species hence and this fact calls for a revision of modelling being in use today to describe root architectures. Even the worldwide terminology used today requires an adjustment and the term “adventitious” (Paolillo, 2006) should be abandoned when describing branch roots originating from the vascular cambium. This term should be restricted only to root branching emerging from stem tissues (Esau, 1965; Fahn, 1990). For the same reason “accessory” (Chiatante, 2006) should be substituted with the term: “secondary branch root” (SBR). This new term satisfies two important considerations: 1) it states better when a root branches from “root” tissue; 2) it highlights that a root originates from secondary root tissues. For branch roots formed by primary tissues it would be more appropriate to use the term “primary branch root” (PBR) proposed by Paolillo (2006) because this term satisfies very well: 1) the lineage from root tissue; 2) the origin from primary tissues. A common element of all SBRs observed in this work is the continuity with their primary tissues (pericycle, endoderm and xylar parenchyma) with the taproot vascular cambium. This ensures a full integration between the two xylary systems representing the essential factor to grant the future growth for a SBR destined to become a persistent root. Interesting is the observations that in our samples some SBRs form above a PBR present along the same rank. This event has been observed also in other woody species and explains the occurrence of cluster roots (Paolillo, 2006). We have never observed the occurrence of cluster roots and moreover I-SBR are almost a rather sporadic event. The use of 2-D gel electrophoresis enabled the resolution of the proteome of poplar woody root, with approximately 250 protein spots in progress to be sequenced. The availability of a well resolved woody root proteome map, together with immunoblotting experiments and protein sequencing will allow the further identification of molecular factors controlling SBRs emission. In summary this work suggests in analogy with herbaceous plants that also woody plants change continuously their root architecture to adapt better to an extremely variable rooting environment. The possibility to form new branch roots on woody root axes gives to a tree the possibility to reduce the extension of the soil explored by returning cyclically to exploit the same volume of soil for nutritional and anchorage purposes.

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