



## Biochemistry

# Auxins differentially regulate root system architecture and cell cycle protein levels in maize seedlings



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## ARTICLE INFO

## Article history:

Received 8 May 2014

Received in revised form

20 November 2014

Accepted 21 November 2014

Available online 5 January 2015

## Keywords:

Maize

Root development

Auxins

Cell cycle

Cyclins

## ABSTRACT

Maize (*Zea mays*) root system architecture has a complex organization, with adventitious and lateral roots determining its overall absorptive capacity. To generate basic information about the earlier stages of root development, we compared the post-embryonic growth of maize seedlings germinated in water-embedded cotton beds with that of plants obtained from embryonic axes cultivated in liquid medium. In addition, the effect of four different auxins, namely indole-3-acetic acid (IAA), 1-naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA) and 2,4-dichlorophenoxyacetic acid (2,4-D) on root architecture and levels of the heat shock protein HSP101 and the cell cycle proteins CKS1, CYCA1 and CDKA1 were analyzed. Our data show that during the first days after germination, maize seedlings develop several root types with a simultaneous and/or continuous growth. The post-embryonic root development started with the formation of the primary root (PR) and seminal scutellar roots (SSR) and then continued with the formation of adventitious crown roots (CR), brace roots (BR) and lateral roots (LR). Auxins affected root architecture in a dose-response fashion; whereas NAA and IBA mostly stimulated crown root formation, 2,4-D showed a strong repressing effect on growth. The levels of HSP101, CKS1, CYCA1 and CDKA1 in root and leaf tissues were differentially affected by auxins and interestingly, HSP101 registered an auxin-inducible and root specific expression pattern. Taken together, our results show the timing of early branching patterns of maize and indicate that auxins regulate root development likely through modulation of the HSP101 and cell cycle proteins.

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## Introduction

Roots are required for water and nutrient uptake, soil anchorage and are sites of interactions with biotic and abiotic factors that often determine crop productivity (Hochholdinger et al., 2004a;

López-Bucio et al., 2003). Most of our knowledge about the factors that determine root system architecture has been obtained from the dicot plant model *Arabidopsis thaliana*. The *Arabidopsis* root system is characterized by the primary root, from which lateral roots emerge and is regulated by different stimuli such as water and nutrient availability, microorganisms and abiotic factors (Grunewald et al., 2007; López-Bucio et al., 2003; Ortiz-Castro et al., 2008; Ramírez-Chávez et al., 2004). More recently, maize (*Zea mays*), an agronomic important crop, has been established as a model to generate information on the biology of cereals, and significant progress has been made in understating its root developmental program (Hochholdinger et al., 2004a,b; Strable et al., 2009). The maize root system architecture displays dramatic changes during development that are not well understood. In the first days after germination, the embryonic root system is formed by the primary root (PR) and later on adventitious and lateral roots (LR) proliferate and help for water and nutrient uptake and seedling establishment in soil (Hochholdinger et al., 2004b;

**Abbreviations:** AFBs, auxin signaling F-box; ARFs, auxin response factors; BR, brace roots; C, coleoptile; CDKs, cyclin dependent kinases; CDKA, A-type cyclin-dependent kinase; CKS1, cyclin-dependent kinase regulatory subunit 1; CLS, callus-like structures; CN, coleoptile node; CR, crown roots; CYCA, A-type cyclin; 2,4-D, 2,4-dichlorophenoxyacetic acid; ECL, enhanced chemiluminescence; HSP101, heat shock protein of 101 kDa; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; L, leaf; LBD, lateral organ boundaries domain; LR, lateral roots; MZ, meristematic zone; NAA, 1-naphthaleneacetic acid; PR, primary root; SN, scutellar node; SSR, scutellar seminal root.

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<http://dx.doi.org/10.1016/j.jplph.2014.11.012>

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Singh et al., 2010). Few weeks after germination, the maize root system is reinforced by the formation of crown roots (CR) and brace roots (BR), which are formed from the stem node, and more lateral roots can be formed on all types of these adventitious roots (Hochholdinger et al., 2004a,b). While this information shows the complexity of the maize root architecture, little is known about the timing of formation of adventitious and/or lateral roots and the physiological processes that determine the initiation and/or development of crown, brace or lateral roots.

Auxins are major regulators of plant growth and development. Indole-3-acetic acid (IAA), the predominant auxin in plants participates in embryonic and post-embryonic development (Perrot-Rechenmann, 2010; Teale et al., 2006). Auxin application to *Arabidopsis* modulates root system architecture mostly by repressing primary root growth and promoting lateral root formation depending on its concentration in the medium (Woodward and Bartel, 2005). Auxins also regulate maize root development as shown by Ferro et al. (2007), who demonstrated that many heterogeneous synthetic substances have auxin-like activity inhibiting growth of the primary root. 1-naphthaleneacetic acid (NAA) also inhibited primary root growth and promoted lateral and adventitious root development on maize mesocotyls (Han et al., 2012; Hetz et al., 1996; Woll et al., 2005). To date, however, there is limited information about how maize roots respond to compounds with auxin activity, which may be of great biotechnological application. Characterization of *Arabidopsis*, rice, and maize mutants altered in lateral root and/or adventitious root formation has helped to identify components of the polar auxin transport and auxin signaling at the level of transporters, receptors and transcription factors (Orman-Ligeza et al., 2013; Osmont et al., 2007). Although IAA is the most abundant auxin in plants, it is not routinely used in vegetative propagation programs, where indole butyric acid (IBA) or NAA are preferred. When supplied to cuttings, IBA efficiently promotes adventitious roots and it likely acts after its conversion to IAA (Schlicht et al., 2013; Strader and Bartel, 2009, 2011). Both passive diffusion and specific auxin influx and efflux transporter proteins are involved in the transport of all different auxins across the plasma membrane. Undissociated IAA molecules enter cells by passive diffusion, whereas the less lipophilic, and therefore less permeable, dissociated auxin anions (IAA<sup>-</sup>) are transported inside cells via auxin influx transporters of the AUX1/LAX family (Bennett et al., 1996; Swarup et al., 2008). In the more basic intracellular environment, IAA dissociates and requires active transport through the PIN (PIN1 to PIN8) or ABCB efflux transporter proteins to exit the cell (Mravec et al., 2008; Terasaka et al., 2005; Vieten et al., 2005; Zažímalová et al., 2007).

Important differences in transport among the auxinic compounds have been reported. Both IAA and NAA are good substrates for the efflux carriers (Delbarre et al., 1996). In contrast, 2,4-dichlorophenoxyacetic acid (2,4-D) is not effectively transported by the efflux transporters. Recent studies suggest that IBA, which has long been known to move great distances in plants, might use transporters that are distinct from those used by IAA. AUX1 acts as influx carrier for IAA but not for IBA. Similarly, PIN2, PIN7, ABCB1, and ABCB19 act as efflux carriers for IAA but not for IBA. In contrast, the PDR (PLEIOTROPIC DRUG RESISTANCE) family proteins ABCG36 and ABCG37 appear to transport IBA but not IAA (Strader et al., 2011). Intracellular auxin binds to its nuclear receptor from the TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX (TIR1/AFB) family of F-box proteins, which are subunits of the SCF E3-ligase protein complex. This leads to the ubiquitinylation and the proteasome-mediated specific degradation of auxin Aux/IAA transcriptional repressors. Subsequently, the auxin response factors (ARFs) are de-repressed and activate auxin-inducible gene expression (Dharmasiri et al., 2005; Kepinski and Leyser, 2005).

Auxins regulate the expression of genes that participate in cell cycle, directly affecting plant morphogenesis such as primary root growth and lateral root formation (De Veylder et al., 2007; Francis and Sorrell, 2001; Himanen et al., 2002). Himanen et al. (2002) showed that during early lateral root initiation NAA increased *HISTONE H4*, *E2Fa* and *CYCD3;1* expression at 4 h, the B-type cyclin dependent kinases (CDKs) *CDKB1;1* and *CDKB2;1* followed the same pattern but showed clear peaks at 8 h. *CYCD1;1*, *CYCA2;1*, *CYCB1;1*, *CYCB2;1*, *CDKB1;1* and *CDKB2;2* were induced at 6 h, while *CDKA;1* transcripts were constitutive. Another important component in regulating the cell cycle machinery in plants is the CYCLIN-DEPENDENT KINASE REGULATORY SUBUNIT (CKS1). In *Arabidopsis*, AtCKS1 associates with the A- and B-type CDKs (De Veylder et al., 2001a). Jacquemard et al. (1999) showed that *AtCKS1* expression is particularly important in tissues with proliferative potential such as root and shoot apical meristems. Over-expression of *AtCKS1* in *Arabidopsis* strongly inhibits plant growth by reducing leaf size and root growth (De Veylder et al., 2001b). In maize, cell cycle genes have been examined during germination. *CYCD4;2* and *CYCD2;2* expression did not apparently change in the presence of IAA, the cytokinin benzyladenine (BA) or abscisic acid (ABA) (Buendía-Monreal et al., 2011; Gutiérrez et al., 2005; Lara-Núñez et al., 2008). In contrast, *CYCD5;3b* and DNA polymerase  $\alpha$ , increased their levels in response to IAA but *CYCA1* or *PCNA* were not affected (Arellano et al., 2008; Buendía-Monreal et al., 2011). This suggests that particular cyclins can be induced by auxins and may play an important role in maize seed germination.

The expression of the heat shock protein of 101 kDa (HSP101) is developmentally regulated in several plant species including rice, wheat, *Arabidopsis* and maize and is apparently induced by heat, drought, dehydration and ABA (Campbell et al., 2001; Gulli et al., 2007; Nieto-Sotelo et al., 2002; Queitsch et al., 2000; Wu et al., 2009; Young et al., 2001). López-Frías et al. (2011) described the role of HSP101 protein in crown root formation in maize seedlings. The authors showed that HSP101 is expressed mainly in crown root primordia, thus associating this protein with the crown root organogenesis program. Currently, there are no further data correlating HSP101 expression with auxins as modulators of maize root development.

Since auxins manifest differences in transport and activity, understanding their particular roles in root development in crops may open new possible biotechnological applications. To gain insight into the root developmental processes that determine the maize root architecture, we studied the maize root morphogenesis in two experimental systems by analyzing the timing of formation of the different types of adventitious roots as well as lateral roots that are formed during the first two weeks of growth. The effect of auxins on maize growth and root architecture was assessed by comparing the effects of IAA, NAA, IBA and 2,4-D on shoot growth, primary root growth, and crown root formation and levels of HSP101, CKS1, CYCA1 and CDKA proteins in root and shoot tissues. Our data support the role of HSP101 in mediating auxin responses in roots and show that cell cycle-related proteins are differentially regulated in response to auxins.

## Materials and methods

### Plant material and growth conditions

Seeds and embryonic axes of *Zea mays* L. cv. Chalqueño were used for all experiments. Seeds were surface sterilized with 50% (v/v) sodium hypochlorite (NaClO, Chloralex, 6% active Cl) for 15 min and washed three times with sterilized deionized water. Subsequently, the seeds were exposed to 70% (v/v) ethanol for 3 min and then washed three times with sterilized deionized water. Finally, the seeds were placed in sterile water-embedded cotton

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