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Robust root growth in *altered hydrotropic response1* (*ahr1*) mutant of *Arabidopsis* is maintained by high rate of cell production at low water potential gradient

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ABSTRACT

Hydrotropism is the directional root growth response determined by water stimulus. In a water potential gradient system (WPGS) the roots of the Arabidopsis wild type have a diminished root growth compared to normal medium (NM). In contrast, the *altered hydrotropic response1* (*ahr1*) mutant roots maintain their robust growth in the same WPGS. The aims of this work were to ascertain how *ahr1* roots could sustain growth in the WPGS, with a special focus on the integration of cellular processes involved in the signaling that determines root growth during abiotic stress and their relation to hydrotropism. Cellular analysis of the root apical meristem of *ahr1* mutant contrary to the wild type showed an absence of changes in the meristem length, the elongation zone length, the length of fully elongated cells, and the cell cycle duration. The robust and steady root growth of *ahr1* seedlings in the WPGS is explained by the mutant capacity to maintain cell production and cell elongation at the same level as in the NM. Analysis of auxin response at a transcriptional level showed that roots of the *ahr1* mutant had a lower auxin response when grown in the WPGS, compared to wild type, indicating that auxin signaling participates in attenuation of root growth under water stress conditions. Also, wild type plants exhibited a high increase in proline content while *ahr1* mutants showed minimum changes in the Normal Medium → Water Stress Medium (NM → WSM), a lower water potential gradient system than the WPGS. Accordingly, in this condition, gene expression of *Δ1-6 Pyrroline-5-Carboxylate Synthetase1* (*P5CS1*) involved in proline synthesis strongly increased in wild type but not in *ahr1* seedlings. The *ahr1* phenotype shows unique features since the mutant root cells continue to proliferate and grow in the presence of a progressively negative water potential gradient at a level comparable to wild type growing in the NM. As such, it represents an exceptional resource for understanding hydrotropism.

1. Introduction

Plants depend on environmental cues to coordinate their growth and development to a greater extent than animals. One of the most important traits that plants have evolved is the power to sense environmental changes in order to direct their growth orientation to cope with stress. The directional growth of plant organs related to an environmental stimulus constitutes a tropism. Gravitropism and phototropism are the most studied tropisms, and many genes involved have been identified thus far (Morita, 2010). Hydrotropism (tropism towards water) has been less examined although it is essential for drought resistance (Cassab et al., 2013; Moriwaki et al., 2013; Bao et al., 2014). Hydrotropism implies the perception of a water gradient, the transduction of hydrotropic signal(s), and subsequent alteration of root growth directionality. The perception and absorption of water during hydrotropism has implications on plant survival in the presence of water-limited

Abbreviations: ABA, abscisic acid; dpg, days post germination; GUS, β, glucuronidase; MS, Murashige and Skoog medium; NM, normal medium; P5CS1, Δ1-6 pyrroline-5-carboxylate synthetase1; PRODH1, proline dehydrogenase1; QC, quiescent center; RAM, root apical meristem; WPGS, water potential gradient system; WSM, water stress medium.

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conditions. Nonetheless, the molecular mechanism controlling this tropism only started to be elucidated. The genes responsible for altered hydrotropic phenotype of two mutants, *miz1* and *miz2*, have been cloned (Kobayashi et al., 2007; Miyazawa et al., 2009). *MIZ1* encodes a protein of unknown function, which is expressed in the root cap and in the stele (Kobayashi et al., 2007). The expression analysis of this gene in the root has not revealed whether *MIZ1* is spatially regulated by moisture or dryness, and it is not clear in which root tissue *MIZ1* acts to regulate hydrotropism. The *miz2* phenotype results from the mutation in *GNOM* gene, which encodes an ADP-ribosylation factor-type G (ARF-GEF) protein regulating vesicle trafficking (Miyazawa et al., 2009). The best-characterized GNOM function is the polar targeting of PIN proteins to the plasma membrane (Geldner et al., 2003). Because the subcellular polarity of PIN proteins determines the direction of auxin flow, allelic *gnom* mutants show defects in auxin-dependent developmental processes such as embryogenesis, lateral root formation, and gravitropism (Shevell et al., 1994; Geldner et al., 2004). In partial loss-of-function of *gnom*, both gravitropism and hydrotropism are disrupted; however, the $gnom^{miz2}$ mutant showed defects in hydrotropic, but not in the gravitropic response. Current data indicate that hydrotropism utilizes a different GNOM-mediated vesicle trafficking compared to those involved in gravitropism (Miyazawa et al., 2009).

Two other hydrotropic mutants, *no hydrotropic response1* (*nhr1*) and *altered hydrotropic response1* (*ahr1*), have been characterized, but the genes responsible for the mutant phenotype have not yet been identified (Eapen et al., 2003; Saucedo et al., 2012). For the isolation of the *ahr1* mutant, we used a water potential gradient system (WSM → NM system) that consists of a vertically oriented square Petri dish where the Water Stress Medium (WSM, containing 2.5% w/v glycerol) was located at the top and the Normal Medium (NM) was at the bottom. Contrary to the wild type, primary roots of *ahr1* grow towards the source of higher water availability (NM) and developed an extensive root system over time (Saucedo et al., 2012). Roots of the *ahr1* mutant also maintain their growth towards the lower water potential gradient in the NM → WSM system (NM at the top and WSM at the bottom of square Petri dish) (Eapen et al., 2003). These reports suggest that the gene responsible for the *ahr1* mutation plays an important role in the perception of water deficiency.

In higher plants, proline accumulation is one of the main responses to abiotic stress (Sharma et al., 2011). Drought stress induces proline accumulation at high levels, for example in maize (Ober and Sharp, 1994) and Arabidopsis (Szekely et al., 2008) among other species. Proline is considered an important osmolyte that acts as a molecular chaperone stabilizing the structure of proteins, as well as a regulator of cellular redox potential and an antioxidant, controlling free radical levels (Hare et al., 1999; Hong et al., 2000). During stress responses, Δ1-6 Pyrroline-5-Carboxylate Synthetase1 (P5CS1) and Proline Dehydrogenase1 (PRODH1) synthesize and degrade proline, respectively, and P5CS1 is the rate-limiting enzyme (Delauney and Verma, 1993; Nanjo et al., 2003; Roosens et al., 1998). Proline synthesis and catabolism are required for optimal growth at low water potential since mutants in both genes show severe growth deficiencies under water stress conditions (Sharma et al., 2011), suggesting that proline biosynthesis and catabolism in Arabidopsis are required for growth at low water potential.

The roles of hormones ABA, auxin, and cytokinin in hydrotropism have been reported (Antoni et al., 2013; Eapen et al., 2003; Kaneyasu et al., 2007; Ponce et al., 2008; Saucedo et al., 2012). ABA increases the high root growth elongation phenotype of *ahr1* in the WSM → NM system since it significantly enhances the development of a long and highly branched root system. Cytokinin, however, completely inhibits this development, since *ahr1* roots develop a hydrotropic curvature in the oblique NM → WSM system similar to the wild type roots. It has been reported that auxin signaling plays an important role in hydrotropism in Arabidopsis roots, taking into account that a specific inhibitor of the auxin response (*p*-chlorophenoxyisobutylacetic acid) reduces hydrotropism, whereas inhibitors of auxin influx or efflux have no effect (Kaneyasu et al., 2007). Henceforth, we hypothesized that contrary to the wild type plant, *ahr1* mutant does not perceive low water potential gradients and the processes involved in cell division and expansion are preserved, allowing efficient root growth.

To address whether a greater root performance in the mutant under water stress conditions results from impact on cell proliferation, cell elongation, or both, we analyzed cell division and cell elongation in the primary root of the *ahr1* mutant. Additionally, we also examined the possible role of auxin in *ahr1* root phenotype when plants grew in water stress conditions. We evaluated the root growth rates and the sensitivity of *ahr1* roots to auxin under a milder water potential gradient system (WPGS), which consists of square Petri dish with the NM at the top and the WSM containing 0.4% (w/v) glycerol at the bottom.

Our results provide a novel understanding of how roots perceive the changes in water potential and point out the role of the root apical meristem (RAM) and cell production in this process. In addition, the fact that the *ahr1* roots' response to auxin at a transcriptional level is lower compared to those of wild type in the WPGS, opens new possibilities for deciphering the role of auxin in the integration of the signaling that controls abiotic stress responses and its relation to hydrotropism. We also analyzed proline content in roots of wild type and *ahr1* mutant in response to water stress. Contrary to wild type, proline was not accumulated in roots of *ahr1* seedlings grown under lower water potential. Finally, these results imply that the *ahr1* mutant represents a valuable genetic resource for the development of crops better adapted to drought.

2. Materials and methods

2.1. Plant materials, growth media and mutant screen

Wild type *Arabidopsis thaliana* (L.) Heynh. Columbia-0 (Col-0) seeds were provided by the Arabidopsis Biological Resource Center (Ohio State University). $CYCB1;1_{DB}$:*GUS* and *DR5:GUS* lines were in Col-0 background and have previously been described by Colon-Carmona et al. (1999) and Ulmasov et al. (1997). For studies of root growth dynamics presented in this work the WSM media supplemented with different amounts of glycerol were used. For analysis of the cellular bases of root growth of *ahr1* growing in the WPGS, the WSM was supplemented with 0.4% (w/v) glycerol and 0.2% (w/v) alginic acid. For preparing the WPGS, 46 mL of the NM were poured into the upper sector of a 10-cm square Petri dish containing an acrylic slab (90 mm × 10 mm × 4 mm) for separating the two media. After solidification of the NM, the slab was removed and 4 mL of the WSM were poured in the lower sector of the Petri dish. Once the WSM solidified the water potential in the WPGS was established. Seeds were placed immediately onto the NM sector in a line, 6.5 cm from the border of the WSM. Dishes were sealed with Parafilm (Sigma-Aldrich, MO, USA) and maintained in a vertical position. Plants were grown at 21 °C, 16/8 h light/dark cycle and the light intensity was 105 μmol photons m^{-2} s^{-1}. The hydrotropic response of *ahr1* mutant was also tested in a moisture gradient according to Kobayashi et al. (2007).

2.2. Water potential analysis of the WPGS

The water potential of the WPGS was measured utilizing the Osmometer model Vapro 5520 (Wescor Inc.). A piece of agar