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MIZU-KUSSEI1 plays an essential role in the hydrotropism of lateral roots in *Arabidopsis thaliana*

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ABSTRACT

Root hydrotropism is the directional growth of roots toward a source of moisture in response to a water potential gradient. Root hydrotropism is therefore one of the most important factors for efficient water acquisition under water-limited conditions. However, the mechanisms underlying hydrotropism are not well understood. A previous study of the hydrotropism of primary roots in *Arabidopsis thaliana* identified the *MIZU-KUSSEI (MIZ) 1* and *MIZ2* genes as essential for this process. However, despite the important role of lateral roots in water acquisition under water deficient conditions, the mechanism of hydrotropism in lateral roots has not been investigated to date. The present study established an experimental system for the induction of lateral root hydrotropism in *Arabidopsis* and analyzed its characteristics in comparison with those of primary roots. Lateral roots were found to exhibit hydrotropism irrespective of their length. Expression of a *pMIZ1::GUS* fusion gene at the tip of lateral roots and assessment of lateral root hydrotropism in the ahydrotropic mutant *miz1*, which did not display hydrotropism, demonstrated that the lateral roots of *Arabidopsis* have the capacity to respond to water potential gradients, and that this activity is regulated by MIZ1.

1. Introduction

The root system of terrestrial plants is responsible for the acquisition of water and nutrients. Lateral roots comprise the majority of the root system in mature plants and their development is therefore critical to support the life of a plant. The sessile nature of terrestrial plants has resulted in the evolution of a diversity of mechanisms to respond and adapt to unfavorable environments. Indeed, the development of a root system depends on the conditions of the soil such as the availability of water and nutrients (Lynch, 1995; Zhang and Forde, 2000). Tropism is the directed growth toward or away from an external stimulus and is an adaptive mechanism in roots. Lateral roots display tropisms in response to environmental stimuli such as gravity and light (Hangarter, 1997; Correll and Kiss, 2002; Kiss et al., 2002). In some species, lateral roots show diagravitropism or horizontal growth (Marler and Discekici, 1997) or plagiotropism or oblique growth (Yamashita et al., 1997; Tsusumi et al., 2003; Kuya et al., 2006). Although the lateral roots of *Arabidopsis* also show diagravitropism and plagiotropism, when they reach a length of approximately 10 mm these roots begin to show orthogravitropism (Kiss et al., 2002). In addition, the lateral roots of *Arabidopsis* display negative phototropism in response to white and blue light, and positive phototropism in response to red light (Kiss et al., 2002). Besides these tropisms, roots display hydrotropism in response to water potential gradients, which is thought to play an important role in the efficient uptake of water and nutrients (Eapen et al., 2005; Takahashi et al., 2009). Root hydrotropism therefore plays a role in the regulation of root system development. The root hydrotropism of primary roots has been analyzed in several plant species including pea, wheat, cucumber, radish and *Arabidopsis* (Oyanagi et al., 1995; Takahashi et al., 1996, 1999, 2002, 2003). Recent progress in the design of experimental systems for the investigation of the hydrotropism of the primary root of *Arabidopsis* provided a new approach for the elucidation of the molecular mechanisms underlying root hydrotropism (Takahashi et al., 2002; Eapen et al., 2003). The recent application of this system has enabled a better understanding of the molecular mechanisms unique to the hydrotropism of primary roots (reviewed in Takahashi et al., 2009; Miyazawa et al., 2009a). The ahydrotropic mutant *mizu-kussei1 (miz1)* was isolated and *MIZ1* was identified as a gene essential for root hydrotropism in *Arabidopsis* (Kobayashi et al., 2007). MIZ1 contains an uncharacterized domain (DUF617) that was named the MIZ domain. Because *MIZ1* is expressed at the root cap where the moisture gradient is sensed, it is suggested that MIZ1 acts at the very early stage of root hydrotropism. In addition, a second ahydrotropic mutant, *miz2*, an allele of *gnom*, was identified (Miyazawa et al., 2009b). GNOM encodes a guanine-nucleotide exchange factor for ADP-ribosylation factor-type G proteins (ARF-GEF), which play an essential role in vesicular trafficking, suggesting that the regulation of vesicular trafficking could be involved in the process of root

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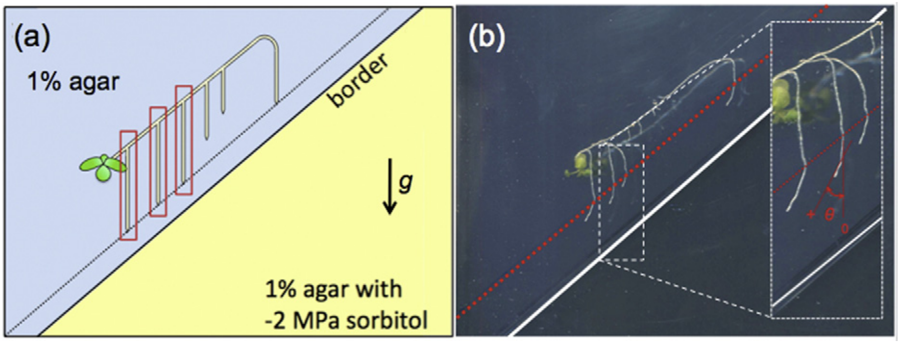

Fig. 1. Experimental system for the study of hydrotropism in lateral roots. (a) A schematic representation of the experimental system used in this study. A water potential gradient was established with a agar with no sorbitol plate and a 14.8% sorbitol agar plate, and the roots were placed vertically with their lateral root tips (red frame) aligned 1 cm away from the border between the two agar plates in a closed plastic container. (b) The roots of 10-day-old *Arabidopsis* seedlings bent toward the high-water potential side at 72 h after hydrotropic stimulation.

hydrotropism. In addition to primary roots, positive hydrotropism has been observed in the lateral roots of an agravitropic pea mutant (Jaffe et al., 1985). However, the molecular mechanisms underlying the hydrotropism of lateral roots are not well understood. Because most of the root system is composed of lateral roots (Dittmer, 1937; Yamauchi et al., 1987), an analysis of the mechanism of lateral root hydrotropism is important to understand the function of the root system as a whole. In the present study, an experimental system for the induction of hydrotropism of lateral roots in *Arabidopsis* was established and applied to investigate the characteristics of lateral root hydrotropism.

2. Materials and methods

2.1. Plant growth conditions

The plant species used in this study were *Arabidopsis thaliana* ecotype Columbia, its ahydrotropic mutant, *miz1* (Kobayashi et al., 2007), and a transgenic plant containing the *uidA* gene under the control of the *MIZ1* promoter (Kobayashi et al., 2007). Seeds were surface sterilized with a solution containing 5% (v/v) sodium hypochlorite, washed with distilled water, and germinated on half-strength Murashige and Skoog medium (Sigma–Aldrich, St. Louis, MO) solidified with 0.8% agar (Wako Pure Chemical Industries,

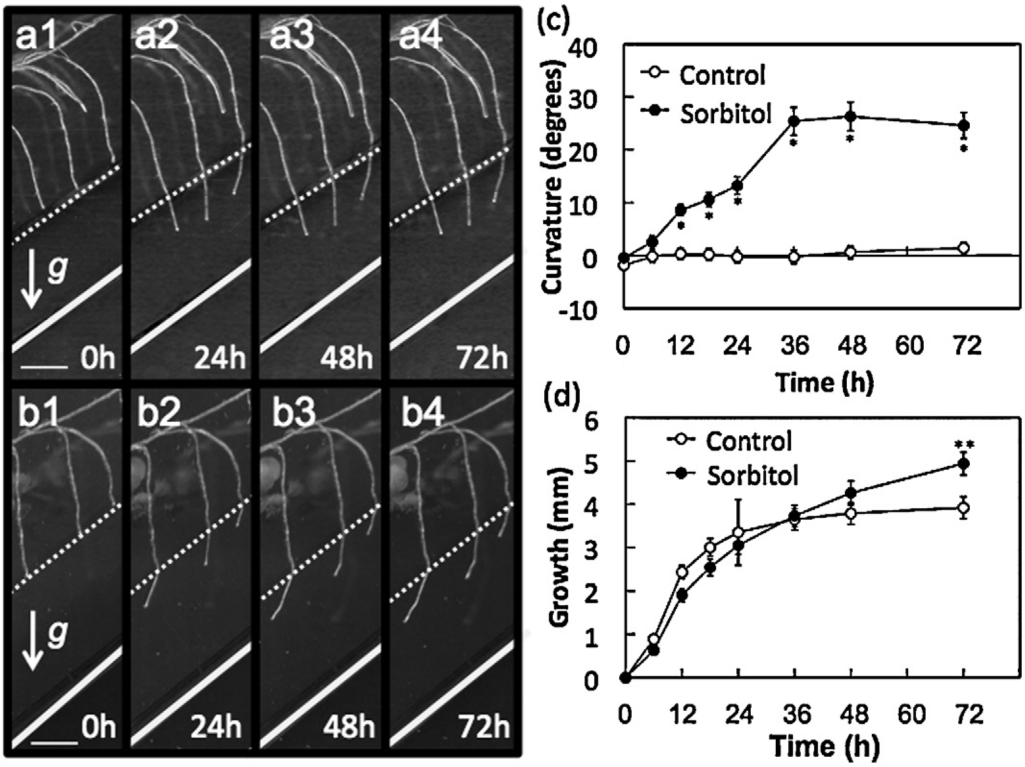

Fig. 2. Hydrotropic response of the lateral roots of *Arabidopsis*. Lateral roots grown under control condition (no hydrostimulation; a1–a4) and those grown in the presence of hydrostimulation (b1–b4) are shown. Lateral roots were scanned at 0 h (a1 and b1), 24 h (a2 and b2), 48 h (a3 and b3), and 72 h (a4 and b4) after the start of the experiment. Bars represent 5 mm. Root curvature (c) and root growth (d) were measured at 0, 6, 12, 18, 24, 36, 48, and 72 h after the start of hydrotropic stimulation. Open circles: control; filled circles: in the presence of hydrostimulation. Data are the means ± S.E. Asterisks indicate statistically significant differences in the means, as determined by Student's *t* test ($^{*}P < 0.01$; $^{**}P < 0.05$) ($n = 34–68$).