A method for rapid analysis of the root hydrotropic response in *Arabidopsis thaliana*

L Noriega-Calixto¹, María E Campos¹ & Gladys I Cassab^{*1}

ABSTRACT

The system for analyzing the hydrotropic curvature with a moisture gradient in wild-type *Arabidopsis* roots was modified. Optimal conditions were determined for detecting a hydrotropic curvature of 90° just after 4 h of stimulation. This system only requires 15 ml of a solution of K₂CO₃ with a density of 1.48 g·ml⁻¹ to generate a rapid moisture gradient inside a square Petri dish without decreasing root growth. In this, the root growth rate observed in hydrostimulated wild-type and *miz1* mutant, utilized as a negative control, increases sixfold compared with those roots examined using the former method.

METHOD SUMMARY

A modified bioassay was established to examine the root hydrotropic response of *Arabidopsis* in a square Petri dish containing 15 ml of a solution of K₂CO₃ with a density of 1.48 g·ml⁻¹. The root curvature was rapidly induced in the presence of a moisture gradient and reached 90° after 4 h. Root elongation and growth rate were maintained in these conditions for 8 h.

KEYWORDS:

Arabidopsis thaliana • hydrotropic curvature • moisture gradient

¹Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Av. Universidad 2001, Col. Chamilpa, Cuernavaca, Mor., 62210, México
*Author for correspondence: gladys@ibt.unam.mx

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INTRODUCTION

In view of the increasing demand for food by growing populations and of the limited water availability in many countries due to global warming, there is an urgent need to understand what root properties facilitate the extraction of water from drying soils [1]. Global warming threatens many aspects of human life [2] by reducing agricultural harvests, resulting in widespread risk of food insecurity and social problems [3–6]. Climate change has already altered the distribution of species and phenotypic variation, and the impact of changing climates is expected to intensify since there are very few attempts to stop CO₂ emissions [7]. By 2040, droughts and other extreme weather will be commonplace [8,9], hence improving tolerance to drought and heat is a foremost target of crop improvement programs. However, tolerance can come with trade-offs; for example, it has long been known that stress-tolerant plants have lower growth rates and productivity [10]. Plants have evolved a variety of mechanisms to adapt to drought conditions [11]. In recent decades, researchers have identified a number of traits that might help plant roots to acquire water efficiently and tolerate desiccation, as well as a

smaller number of root traits that may assist soil water acquisition [12]. Importantly, when roots encounter changes in environmental conditions they will change growth direction in order to optimize plant survival. Such directional changes in response to several stimuli (tropisms) include roots sensing soil water content and growing towards water to avoid dry soil, by either changing direction or halting growth. Hydrotropism is a physiological trait that most probably evolved to avoid drought [13–15]. Root growth direction is strongly directed by gravity and also by other stimuli such as light, obstacles and nutrients. However, root gravitropic response influences all the other root tropic responses, since gravity is ubiquitous in Earth. This makes it problematic to observe hydrotropism in a short period of time since the early tropic response of roots is to follow the direction of the gravity vector. Despite water sensing being the subject of very early plant physiology studies, until recently the mechanisms of this growth response were essentially unknown. Phenotyping and screening procedures in model plant and crop species are needed for genetic dissection of complex biological processes such as hydrotropism [16,17]. Therefore, ►

Benchmarks

► an easier protocol that allows the analysis of the hydrotropic response in *Arabidopsis* seedlings would help to isolate new genes implicated in this response. Takahashi *et al.* [18] and Eapen *et al.* [19] described two different protocols to assess the hydrotropic response in root of *Arabidopsis* seedlings. The basis of both protocols is the stimulation of roots with water potential or moisture gradients, with it expected that it will cause root curvature toward the water source and not to the gravity vector. The utilization of these protocols has allowed the isolation of *Arabidopsis* mutants that show an absence of a normal hydrotropic response such as *miz1*, *miz2/gnom*, *ahr1* and *nhr1* [19–22]. Some genes required for hydrotropism, such as *MIZ1* and *MIZ2/GNOM* [20,21] have been thoroughly characterized and it has been demonstrated that *MIZ1* participates in the generation of a systemic Ca^{2+} signal by its direct binding to and inhibition of ECA1, an endoplasmic reticulum Ca^{2+} pump, which is essential for water tracking [22]. Moreover, the site of water perception and differential growth control was localized to the root cortex [23]. On the other hand, some plant growth regulators such as ABA, cytokinin and brassinosteroids have been implicated in hydrotropism regulation [14,24–28]. In particular, ABA signaling through PYR/PYL/RCAR receptors is required for hydrotropism in *Arabidopsis* [26]. Recently, it was reported that the apoplastic proton extrusion in root tips is influenced by brassinosteroids (BRs) receptor BR-INSENSITIVE1 (BRI1), which is also crucial for the hydrotropic root response in *Arabidopsis* [27,28]. Whereas

loss-of-function mutations in the ubiquitously expressed BRI1 receptor leads to drought resistance at the expense of growth, overexpression of the BRL3 receptor confers drought tolerance without penalizing overall growth [29]. Hence, BRs signaling links the hydrotropic root response with drought resistance. In addition, it has been previously suggested that the genes involved in hydrotropism could be important targets for crop improvement by enhancing drought avoidance [13,30]. A recent demonstration that a robust hydrotropic response leads to better growth under drought and partial lateral irrigation in different maize cultivars strongly supports this notion [31]. Here, we describe a system for examining the root hydrotropic response with a moisture gradient in *Arabidopsis*. The previous system that utilized moisture gradients for inducing a hydrotropic curvature in *Arabidopsis* reported by Kobayashi *et al.* [20] was modified for optimizing the conditions for detecting the curvature of 90° just after 4 h of stimulation. Moreover, our system requires only 15 ml of K_2CO_3 solution with a density of $1.48 \text{ g}\cdot\text{ml}^{-1}$ to generate the moisture gradient, compared with the 500 ml described earlier. Finally, root growth of *Arabidopsis* seedlings increased approximately sixfold compared with the aforementioned system.

We modified the system reported by Takahashi *et al.* [18] and determined the optimal conditions for detecting the hydrotropic curvature in *Arabidopsis* in just 4 h (Figure 1A & B). We first analyzed the effect of exposing seedling roots of wild-type (Col-0) and *miz1* mutants to two K_2CO_3 solutions

with different densities, $0.74 \text{ g}\cdot\text{ml}^{-1}$ and $1.48 \text{ g}\cdot\text{ml}^{-1}$, respectively, and H_2O as a control condition. Wild-type and *miz1* seedling roots exposed for 12 h to the K_2CO_3 solution with a density of $0.74 \text{ g}\cdot\text{ml}^{-1}$ showed vertical growth following the direction of the gravity vector as did those subjected to H_2O (data not shown). By contrast, roots of wild-type seedlings exposed to the K_2CO_3 solution with a density of $1.48 \text{ g}\cdot\text{ml}^{-1}$ developed a hydrotropic curvature toward the H_2O source (agar block), while roots of the *miz1* mutant displayed their characteristic absence of hydrotropic response [20]. Hence, we utilized the K_2CO_3 solution with a density of $1.48 \text{ g}\cdot\text{ml}^{-1}$ for our further analyses.

We measured the relative humidity (RH) at three different positions in the square Petri dish + K_2CO_3 solution (Figure 2A & B). Measurements of RH were also made in the control system (+ H_2O) (Figure 2C). After 1 h of sealing the plate of the control system, the RH at the three different positions was close to 90%, which was nearly sustained for 23 h. Thus, no substantial moisture gradient was observed under this condition (Figure 2C). However, the RH in the experimental system + K_2CO_3 solution decreased to 83% in the lower and central part of the plate, differing to the RH of 89% detected in the upper part (Figure 2B). Afterwards, the RH measured in the lower and central part of the plate showed a very mild increase for the next 4 h, reaching about 88% after 23 h. The RH at the upper part of the plate of the experimental system + K_2CO_3 solution was around 88% at all time points examined (Figure 2B).

Once the conditions for the development of a moisture gradient were established in the experimental system + K_2CO_3 solution, we analyzed the hydrotropic root curvature at 3, 4 and 8 h after hydrostimulation (Figures 3 & 4). Roots of wild-type seedlings showed a hydrotropic curvature of nearly 76° after merely 3 h of stimulation, while roots of the *miz1* mutant did not display a hydrotropic response (Figures 3 & 4A). After 4 h, roots of the wild-type developed a curvature of 90° (Figures 3 & 4A). The rapid development of the hydrotropic curvature up to 90° in wild-type roots might be related to the formation of a moisture gradient in 1 h close to their tips (2 cm) (Figure 2A & B). In the experimental system + K_2CO_3 solution, root elongation and growth rate of both wild-type and *miz1* roots was equivalent ►

Figure 1. Schematic representation of the experimental system to analyze the root hydrotropic response of *Arabidopsis*. (A) Experimental system containing the solution of K_2CO_3 with a density of $1.48 \text{ g}\cdot\text{ml}^{-1}$. (B) Control system containing H_2O .

Figure 2. Generation of a moisture gradient in the experimental system. (A) The diagram indicates the places in the plate where the relative humidity (RH) was measured. The RH was measured at 1.5 cm above (upper part, open circles), 2.0 cm (central part, filled diamonds) and 5.5 cm (lower part, open squares) below the agar edge placed in the square Petri dish containing K₂CO₃ solution (1.48 g·ml⁻¹) or H₂O. (B & C) Graphs indicate the RH detected in the experimental system plus the K₂CO₃ solution (B) and in the control system plus H₂O (C) at 0, 1, 2, 3, 4, 5, 6, 7 and 23 h after sealing the Petri dish. Each measurement was repeated three times for every time point in a time-course experiment.

► after 3 and 4 h of stimulation (Figure 4B). The growth rate of wild-type and *miz1* roots was nearly 0.35 mm·h⁻¹ at these time points (Figure 4C). Thus, the conditions of the experimental system + K₂CO₃ solution did not impair growth in both wild-type and *miz1* seedling roots for 4 h (Figure 4C). However, growth rate of *miz1* roots decreased after 8 h compared with the wild-type (Figure 4C).

In the absence of moisture gradient, no hydrotropic curvature was elicited in either wild-type or *miz1* roots (Figures 3 & 4D). In the control system, growth rate of the wild-type roots was comparable to those of the *miz1* mutant (Figure 4F).

Takahashi *et al.* [18] and Eapen *et al.* [19] designed experimental systems for the induction of the hydrotropic response in

Figure 3. Root hydrotropic curvature of wild-type (Col-0) and *miz1* mutant seedlings in the experimental system containing the K₂CO₃ solution or H₂O. Three representative images show roots of Col-0 and *miz1* mutant seedlings at 0, 3, 4 and 8 h after the start of hydrotropic stimulation with K₂CO₃ solution. The roots of wild-type (Col-0) and *miz1* mutant seedlings showed an orthogravitropic growth in the control system containing H₂O at 0, 3, 4 and 8 h after sealing the square Petri dish. Bar = 1 cm.

Arabidopsis roots. These systems generated a water potential gradient in agar by including an osmolyte (sorbitol or glycerol) in square Petri plates. Antoni *et al.* [17] and Shkolnik *et al.* [22] modified the split-agar/sorbitol system of Takahashi *et al.* [18] by placing *Arabidopsis* root tips closer to the border between the two media, and the method requires at least 6 h for observing a hydrotropic curvature of 40° [26]. Takahashi *et al.* [18] and Kobayashi *et al.* [20] described another experimental system that generated a moisture gradient with a saturated solution of K₂CO₃ in an acrylic box in which *Arabidopsis* roots developed a curvature close to 90° after 8 h of stimulation.

In the previous experimental system reported by Takahashi *et al.* [18] and Kobayashi *et al.* [20], the authors used 4-day-old seedlings for analyzing the hydrotropic response. After 4 h of stimulation, the authors reported that wild-type seedling roots showed a hydrotropic curvature near 50°, and the root growth rate of wild-type and *miz1* mutant seedlings was 0.05 mm·h⁻¹. In our experimental system, we used 5–6-day-old seedlings and the wild-type roots reached a hydrotropic curvature of 90° after 4 h of hydrostimulation displaying a root growth rate of 0.3 mm·h⁻¹, which was similar to those observed in *miz1* mutant roots. Therefore, in this system wild-type roots showed an increase of sixfold in their growth rate compared with those roots incubated in the previous system.

The changes observed between these two experimental systems might be caused by the difference in the age of the seedlings, the close contact of root tips to a moisture gradient generated after 1 h of sealing the plate, and also by the fact that we did not use a saturated solution of K₂CO₃. Previous analysis of hydrotropism in maize demonstrated that a solution of K₂CO₃ with a density of 1.48 g·ml⁻¹ did not cause dehydration of roots after 24 h of the start of hydrotropic stimulation [28]. In our experimental system, the root growth rate of the *miz1* mutant seedlings decreased after 8 h of the hydrostimulation, as reported by Kobayashi [20] (Figure 4B & C). This decrease might be related to the fact that *miz1*-mutant roots did not bend to the agar block and were exposed longer to the low RH conditions generated in the experimental system. ►

Figure 4. Time course of root hydrotropic curvature, elongation and growth rate of wild-type and *miz1* mutant seedlings. The root curvature (A) and the increment of root length (B) were measured at 0, 3, 4, and 8 h after the start of hydrotropic stimulation. The root growth rate (C) was calculated by dividing root length (mm) by time (h). Seedling roots of the *miz1* mutant were used as negative control. In the control system, the lack of root hydrotropic response of both Col-0 and *miz1* mutant is shown in (D), as well as their elongation (E) and growth rate (F). Each data point is the average of measurements of 30 individuals from four independent experiments. Error bars represent SD. Asterisks indicate statistically significant differences in the means as determined by Student's t-test.

**p = 0.01.

Filled circles, wild-type Col-0; filled squares, *miz1* mutant seedlings.

► In summary, we developed a simple and low-cost experimental system for analyzing hydrotropism in *Arabidopsis* roots, in which roots developed a hydrotropic curvature of 90° in just 4 h after the start of the hydrostimulation. Likewise, this experimental system required only agar and a small volume of K_2CO_3 solution within a square Petri plate. We propose that this system represents a useful tool for identifying new elements implicated in the early signaling of the hydrotropic response and for isolating new hydrotropic mutants.

AUTHOR CONTRIBUTIONS

LNC and MEC designed the experimental system and performed the experiments. LNC and GIC wrote the manuscript.

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SUPPLEMENTARY DATA

A supplementary protocol accompanies this paper. Reference 32 is in the supplementary data. To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/suppl/10.2144/btn-2018-0173

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