

Diurnal changes in shoot water dynamics are synchronized with hypocotyl elongation in *Arabidopsis thaliana*

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We recently demonstrated the circadian clock modulated water dynamics in the roots of a small model plant, *Arabidopsis thaliana*, by the Nuclear Magnetic Resonance (NMR) microimaging technique. Our developed technique was able to visualize the water distribution that depended on differences in the ¹H signal among region in the shoot, such as the shoot apex, the hypocotyl and the root shoot junction. Water content in the shoot increased during periods of light in comparison with dark periods, and continued through the early stage of seedling growth until the dark period. When the water content changed, elongation and/or movement occurred in the hypocotyl, and these events were synchronized. The water dynamics of the shoot also displayed an opposite phase with the root water dynamics.

Nuclear magnetic resonance (NMR) microimaging nondestructively detects water in tissues, and creates anatomical images of the tissue.^{1,2} *Arabidopsis thaliana* is one of the best candidates for systematic analysis of root water relations, and we recently demonstrated a system for NMR microimaging of plant growth.³ The water dynamics of *Arabidopsis* roots were altered by diurnal oscillation under 12 h light, 12 h dark conditions. Moreover, an autonomous rhythm of water dynamics was observed in continuous light or dark. Our observations suggested that the change in water dynamics of the root is caused by circadian clock modulation. The water dynamics of the root should be closely related to water dynamics of the shoot. Here, we demonstrate that diurnal changes in water content of the *Arabidopsis* shoot are phased with hypocotyl elongation and are different from root water content.

The Water Dynamics of the Shoot are Synchronized with Hypocotyl Elongation, and Display a Different Phase from Root Water Dynamics

NMR images were acquired as described in our previous report.³ Images at various times of seedling growth are shown in **Figure 1A**. Our technique allowed visualization of the water distribution, which depends on differences in the ¹H signal among

regions in the shoot, such as the shoot apex, the hypocotyl and the root-shoot junction. The relative ¹H signal intensity from the whole shoot per pixel oscillated rhythmically under LD conditions (light: dark = 12 h: 12 h) and generally increased with hypocotyl elongation (**Fig. 1B**). Water content in the shoot increased more during periods of light than dark, and continued through the early stage of seedling growth (100–113 h). In the late stage of seedling growth (140–156 h), the change in the water content decreased. When the water content increased, elongation or movement, such as circumnutation, occurs in the hypocotyl, and these events are synchronized. On the other hand, water content in the root decreases during light periods and increases in the dark.³ Therefore, the water dynamics of the shoot are synchronized with hypocotyl elongation, and display an opposite phase with the root water dynamics. Two alternatives can explain the water dynamics of the shoot: the influence of hypocotyl elongation and the influence of transpiration from the leaves.

Hypocotyl growth occurs by cell elongation with almost no contribution from cell division.⁴ The growth of the hypocotyl is modulated by environmental stimuli such as light,^{5,6} temperature⁷ and nutrition,^{8,9} and by internal growth factors.¹⁰ A large quantity of water is used for elongation of the hypocotyl,¹¹ and our technique enables observation of the dynamics of water

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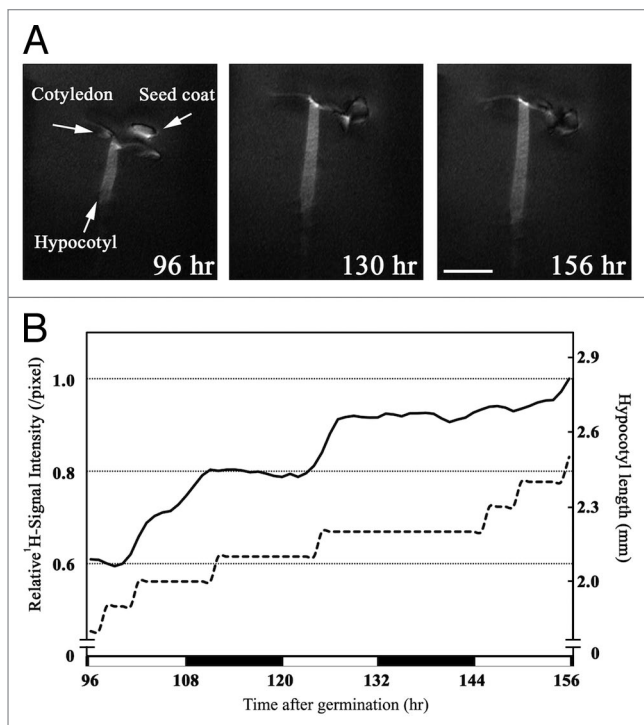


Figure 1. Diurnal oscillation of water content in the shoot during diurnal light oscillation. **(A)** Water distribution in the *Arabidopsis* shoot 96, 130 and 156 h after germination as determined by NMR imaging. More intense white indicates a region containing a large amount of water. Bar = 1 mm. **(B)** The change in water content in a shoot during LD conditions (light: dark = 12 h: 12 h). Solid line indicates shoot water dynamics; dotted line indicates hypocotyl length (in mm). White and black boxes indicate light and dark periods, respectively.

in the intact plant. When the hypocotyl elongates, the ¹H signal intensity in the shoot increases in parallel. Thus, the signal intensity may reflect the inflow of water necessary for the volume increase of the cells undergoing elongation in the hypocotyl. On the other hand, the signal intensity of the root decreases during

the light period.³ These observations suggest that the supply of water from the root to the shoot is promoted during this period.

Shoot water dynamics are also influenced by transpiration. Transpiration and the velocity of water absorption and transport fluctuate diurnally; active water transport in the daytime is mostly driven by the pressure difference between root and leaves, which is generated by transpiration.¹² However, the influence of transpiration on water transport from the root might be minimal under the conditions we used, because the seedlings in our previous study were grown in a sealed tube with the relative humidity maintained close to 100%.³ Rather than transpiration, therefore, water may enter cells due to a change in their osmotic pressure in response to some signal. In the daytime, some metabolic product might accumulate in cells and raise osmotic pressure, thus accelerating water uptake. An increase in the velocity of water flow in the root might also explain the decrease in ¹H signal intensity in the root.^{3,13,14} These assumptions are consistent with the hypocotyl elongation occurring during the light periods (Fig. 1B), and can explain the water movement through the root and the shoot in the absence of transpiration.

We have developed a technique for visualization of intact *Arabidopsis* seedlings by NMR imaging and demonstrated the water balance in supply and demand of both shoots and roots. Our technique may enable noninvasive study of cell expansion, such as measurements of water flow to the vacuoles.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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