Where’s the water? Hydrotropism in plants

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Plants are constantly bombarded with sensory inputs and receive numerous biotic and abiotic signals from their environment. Abiotic signals include gravity, light, water, temperature, oxygen, and carbon dioxide as well as other gases. One way in which plants deal with these inputs is by tropistic growth (or tropism), which is directed growth in response to a stimulus. A tropism is generally termed “positive” if growth is toward the signal and “negative” if it is away from the signal. For example, stems usually exhibit positive phototropism, because they grow toward light. One of the lesser-known tropisms is hydrotropism, directed growth in response to water or moisture gradients. Even though hydrotropism has been studied in plant roots by 19th century German botanists (reviewed in refs. 1 and 2) and by the Darwins (3), the existence of this tropism has been questioned until recent years. The paper by Kobayashi et al. (4) in this issue of PNAS uses an interesting system to study hydrotropism and identifies a novel gene in the hydrotropism pathway in roots.

Tropisms frequently interact between and among each other, and the final growth form of the plant is influenced by such interactions. A good example is the interplay between light and gravity in determining the directional growth of a stem (5). Thus, in shoots, interactions between positive phototropism and negative gravitropism determine the direction of growth in young seedlings (6). Less known is the observation that the orientation of root growth in many plant species is shaped by the interaction between positive gravitropism and negative phototropism (7, 8). In fact, it has been difficult to study phototropism in roots because of the overpowering effects of gravity in modulating the growth of this organ (9, 10). In a similar way, one of the main difficulties of studying hydrotropism in roots has been that roots are strongly gravitropic, which seems to overwhelm any hydrotropic response (1, 11). Thigmotropism, directed growth in response to touch, also may interfere with the expression of hydrotropism (12).

There are several ways to overcome the challenges of studying root hydrotropism, and these methods involve overriding the competing gravitropic response. Some of these techniques include the use of agravitropic mutants, ground-based instruments to mitigate unilateral gravity, and the microgravity environment of spaceflight. One of the first modern papers to convincingly confirm the existence of hydrotropism used a pea mutant lacking both gravitropism and phototropism in roots (12). These authors also suggested that the early-perception phase of hydrotropism occurs in the root cap, because roots grow normally but do not respond to moisture gradients after removal of the cap. Later, Takahashi et al. (13) used a starchless mutant of Arabidopsis, which has a greatly reduced sensitivity to gravity compared with WT (14, 15), to show it is enhanced in root hydrotropism.

Another approach to studying hydrotropism is to use instruments to alter the direction of the gravity vector received by the plants. Although it is not possible to eliminate the effect of gravity on Earth, clinostats rotate plants around an axis or, in some cases, three dimensions in an attempt to neutralize gravity’s effects. The most commonly used varieties are the one-axis slow-rotating (1-4 rpm) clinostat and the two-axis or 3D clinostat, which also is termed the random positioning machine (16, 17). In fact, hydrotropism in roots was more readily apparent when pea and cucumber seedlings were grown on a rotating clinostat (18, 19), and the study by Kobayashi et al. (4) used a clinostat to optimize the hydrotropic response in Arabidopsis seedlings.

An even more interesting approach to studying root hydrotropism is to use the microgravity conditions present during spaceflight. The idea is that, in the absence of significant gravitational forces, the overriding gravitropic responses of roots are effectively negated, so that other root tropisms become more apparent and easier to study. This concept has worked well in terms of phototropism, which can be stronger in microgravity compared with ground conditions (20, 21). In fact, in the only available microgravity study, Takahashi and coworkers (19) found that in their space experiments with cucumber seedlings, lateral roots exhibited a positive hydrotropic response (not apparent on the ground) that was stronger in those roots closer to the wet substrate.

Another obstacle to studying root hydrotropism is the difficulty of setting up a system in which there is a reproducible moisture gradient. The classical methods of the German botanists (reviewed in refs. 1 and 12), also used by the Darwins (3) included placing seeds in a hanging cylinder of wet sawdust, which resulted in roots first growing downward (gravitropism) but then growing back up toward the wet substrate (hydrotropism). Several other approaches were used, but the first to measure moisture gradients was Hooker in 1915 (22), who made a hygrometer to measure relative humidity at two points.

Modern assays to study hydrotropism have been developed by two groups, who also used these methods as screens for isolating novel mutants of hydrotropism in Arabidopsis roots (23, 24). Eapen et al. (24) used a Petri dish with a normal nutrient agar medium at the top of the dish and an agar with a water-stressed medium in the bottom of the dish. The water-stressed medium was made by adding glycerol and alginic acid as osmolytes to the agar. WT seedlings would grow downward (gravitropism) for several days until the roots neared the agar with the lower water potential, and then the roots would grow upward toward the higher water potential. Using this screen, the authors identified a semidominant mutant termed no hydrotropic response (NHR). Although the exact nature of NHR is not known, the results suggest that the plant hormone abscisic acid is involved in water sensing by the root cap.

In another approach, the Takahashi group (13, 23) used a closed acrylic chamber (Fig. 1) with seedlings growing on agar (at the top of the container) and with an open container with a saturated solution of salts (on the bottom).
Thus, a moisture gradient was formed between the agar and the saturated salt solution in the container. The gradient was measured with a hygrometer, and the greater the distance from the container, the greater the relative humidity (i.e., moisture). At the beginning of the experiment, roots of seedlings on the agar grew toward the bottom (gravitropism), but then, after they reached the edge of the agar, grew upward toward the higher level of moisture (hydrotropism; Fig. 1A). An explicit discussion of the differences between the two methods to assay for hydrotropism and the potential advantages/disadvantages of each system is provided in the review by Eapen et al. (2).

In the latest paper by the Takahashi group (4), the authors describe a novel mutant miz1, which is impaired in hydrotropism (Fig. 1B). The term miz comes from “mizu-kusatsu,” which means “water tropism” in Japanese. The miz1 mutant has normal gravitropism and a normal growth rate but reduced root phototropism and a modified wave growth response (described in ref. 25). Thus, in addition to its role in hydrotropism, MIZ1 could have a role in different steps in the phototropism and wavy growth response pathways.

Nevertheless, the isolation of miz1 is an advance, because it is the first mutant in the hydrotropism pathway in Arabidopsis that has normal gravitropism and elongation growth. The other report of a hydrotropic mutant in Arabidopsis is that of the semidominant nhr1 discussed above, but this mutant, in contrast to miz1, has altered growth, gravitropism, and modified root cap cells (24). In fact, the homozygous nhr1 mutant seedlings were dwarfs, and plants never reached the reproductive stage. Therefore, the miz1 mutant is novel and much easier to work with compared with the previously isolated nhr1 mutant strain.

The miz1 mutant was generated by treating plants with ethylmethanesulfonate, and then map-based cloning was used to identify the mutated gene. MIZ1 encodes a novel protein with a region termed the MIZ domain that is highly conserved among terrestrial plants from mosses to monocots to dicots. Interestingly, the MIZ domain is absent in cyanobacteria, algae, and animals. Thus, although they do not know the exact function of this gene, the authors (4) propose that MIZ1 played an important role in the adaptation to terrestrial life for plants.

The Darwins first hypothesized that the root cap was the brain of the growing root (3). More modern studies have confirmed that the root cap is specifically involved in the sensing of gravity (26), touch (27), and light (10, 28). Interestingly, Kobayashi et al. (4) report that the ultrastructure of root caps of miz1 mutants is identical to those of WT plants and that the MIZ1-GUS fusion gene was strongly expressed in the columella cells of the root cap. These results suggest that the root cap plays a key role in sorting out the signals received from the environment (gravity, touch, light, and water) and in determining the final direction of root growth.

This research has improved our understanding of hydrotropism, a phenomenon whose existence was doubted by some plant biologists only a few years ago. Experiments with mutants and spaceflight studies, as well as the development of assays and screens, have demonstrated that hydrotropism plays a role in orienting the growth of plant roots. Understanding the mechanisms of hydrotropic responses may have important implications for agriculture, because lack of water is a key abiotic factor in growing crop plants (2). In fact, Kobayashi et al. (4) may have put hydrotropism at center stage by suggesting that this response is important in understanding the evolution of land plants.