

# Hormonal interactions during root tropic growth: hydrotropism versus gravitropism

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**Abstract** Terrestrial plants have evolved remarkable morphological plasticity that enables them to adapt to their surroundings. One of the most important traits that plants have acquired is the ability to sense environmental cues and use them as a basis for governing their growth orientation. The directional growth of plant organs relative to the direction of environmental stimuli is a tropism. The Cholodny–Went theory proposes that auxin plays a key role in several tropisms. Recent molecular genetic studies have strongly supported this hypothesis for gravitropism. However, the molecular mechanisms of other tropisms are far less clear. Hydrotropism is the response of roots to a moisture gradient. Since its re-discovery in 1985, root hydrotropism has been shown to be common among higher plant species. Additionally, in some species, gravitropism interferes with hydrotropism, suggesting that both shared and divergent mechanisms mediating the two tropisms exist. This hypothesis has been supported by recent studies, which provide an understanding of how roots sense multiple environmental cues and exhibit different tropic responses. In this review, we focus on the overlapping and unique mechanisms of the hormonal regulation underlying gravitropism and hydrotropism in roots.

**Keywords** *Arabidopsis thaliana* · Auxin ·  
*Cucumis sativus* · Gravitropism · Hydrotropism ·  
MIZ1 · *Pisum sativum*

## Introduction

Survival of all living organisms depends on water availability. To survive in terrestrial conditions, and given their sessile nature, plants establish a root system that obtains water from the soil. Plant roots display tropisms in response to the direction or gradient of environmental cues, and these tropisms control the orientation of root growth. Roots respond to gravity, light, water (moisture gradient), touch (mechanical stimuli), temperature, electric fields, magnetic fields, and chemicals by gravitropism, phototropism, hydrotropism, thigmotropism, thermotropism, electrotropism, magnetotropism and chemotropism, respectively (Hart 1990; edited in Gilroy and Masson 2008). In addition, roots exhibit additional growth movements, including circumnutation and the wavy response, which depend on the plant species, as well as growth conditions (Okada and Shimura 1990; Johnsson 1997; Migliaccio and Piconese 2001). Among these important tropisms and movements, gravitropism is considered to exert a major influence in the directional growth of roots. Primary roots usually show orthogravitropism (i.e., growing down), and lateral roots diagravitropism or plagiotropism (i.e., growing sideways). The gravitropism of primary roots, in particular, has been extensively studied (Kiss 2000; Blancaflor and Masson 2003; Morita and Tasaka 2004; Perrin et al. 2005). Among root tropisms other than gravitropism, the phototropic response has been relatively well studied. The isolation of a phototropic mutant (*nph1*) and the identification of the photoreceptor (NPH1) have further facilitated these studies (Liscum and Briggs 1995; Christie et al. 1998; Kagawa et al. 2001; Christie 2007; Mullen and Kiss 2008). In addition, there have been recent advances in understanding the touch response in plants, including root thigmotropism, and cytoplasmic  $Ca^{2+}$  and

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mechanosensitive ion channels have been implicated in touch responses (Legué et al. 1997; Massa and Gilroy 2003; Nakagawa et al. 2007; Monshausen et al. 2008). A great deal of knowledge regarding hydrotropism, the directed growth of roots towards higher water potential or moisture, has accumulated in the last two decades (Takahashi 1997; Eapen et al. 2005). Some studies have also investigated the interactions among tropisms in roots (Kiss et al. 2003; Iino 2006). The question thus arises as to how roots sense multiple environmental cues and exert different responses. In this review, we focus on the hydrotropic response and its interaction with gravitropism in roots. We will briefly review the mechanism for root gravitropism and compare the mechanisms, shared or divergent, of the two tropisms, while emphasizing the role of auxin in, and the unique mechanism of, root hydrotropism.

### **Mechanisms of root gravitropism: the Cholodny–Went theory revisited through molecular and genetic studies**

Auxin is a plant hormone that regulates many aspects of growth and development in plants, including cell elongation, cell division, vascular differentiation, and pattern formation of organs. The Cholodny–Went theory proposes that auxin is involved in mediating plant tropisms, including gravitropism and phototropism (Went and Thimann 1937; Trewavas 1992; Muday 2001; Yamamoto 2003). In gravitropism, auxin is asymmetrically redistributed when plants grown in a vertical position are gravistimulated by reorienting the plant to a horizontal position. Auxin accumulates preferentially in the lower regions of the gravistimulated organs, which ultimately causes the organs to bend. The Cholodny–Went theory includes the hypothesis that auxin has opposite effects on roots and aerial organs; that is, the accumulation of auxin inhibits cell elongation in roots and facilitates cell elongation in hypocotyls and shoots. This then results in downward bending of roots and upward bending of aerial organs. Indole-3-acetic acid (IAA) is a major endogenous auxin that is asymmetrically distributed in gravistimulated organs (Trewavas 1992; Lomax et al. 1995; Muday 2001; Muday and Rahman 2008). Transport of  $^{14}\text{C}$ -labeled IAA occurs in both basipetal and acropetal directions in *Vicia faba* roots: the labeled auxin is transported to the root tip through the central cylinder, and from the root tip to the elongation zone through the outer cells of the root (Ohwaki and Tsurumi 1976). Hasenstein and Evans (1988) proposed that once auxin enters the columella cells via the acropetal transport system, whether auxin is symmetrically or asymmetrically transported basipetally toward the elongation zone of the roots through the cortical and/or epidermal

cells is determined by the presence or absence of gravistimulation. Both influx and efflux carriers of auxin are necessary for this polar transport of auxin, as well as for auxin redistribution in the gravistimulated roots. Inhibitors of auxin influx, including 3-chloro-4-hydroxyphenylacetic acid (CHPAA) and 1-naphthoxyacetic acid (1-NOA), as well as inhibitors of auxin efflux, including 1-naphthylphthamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA), inhibit the gravitropic response of roots (Lomax et al. 1995; Parry et al. 2001; Petrášek et al. 2003, 2006).

AUX1 and PINs are influx and efflux facilitators of auxin, respectively. Immunohistochemical studies indicate that, in roots, the polar localization of PIN1 in the apical plasma membrane mediates the acropetal transport of auxin through the central cylinder (Gälweiler et al. 1998; Blilou et al. 2005). PIN2 is localized to the basal plasma membrane of the epidermal cells in the elongation zone of Arabidopsis roots (Müller et al. 1998). In addition, the necessity of AUX1 localized to the lateral root cap and epidermal cells in root gravitropism underscores the crucial role of AUX1 in the basipetal transport of auxin to the elongation zone (Swarup et al. 2005). The roots of the *aux1* mutant exhibit a defect in root gravitropism (Bennett et al. 1996; Swarup et al. 2005). These results suggest that delivery of auxin from the columella cells to the epidermal cells of the elongation zone in the Arabidopsis roots is mediated by PIN2 and AUX1 (Müller et al. 1998; Swarup et al. 2005). Furthermore, PIN3 is expressed in columella cells where gravity is perceived (Friml et al. 2002). When roots are gravistimulated, PIN3 is rapidly re-localized to the new lower side of the plasma membrane of the columella cells (Friml et al. 2002; Harrison and Masson 2008). These results suggest that a signal generated by graviperception regulates PIN3 localization in the columella cells, which transport auxin to the lower lateral root cap. PIN2 and AUX1 then transport auxin to the lower side of the elongation zone of the gravistimulated roots. The roles played by PIN2 and PIN3 in root gravitropism are supported by analyses of their mutants. The *agr1/agrleir1* mutants, which carry mutations in the *PIN2* auxin efflux facilitator gene, lack root gravitropism (Chen et al. 1998; Utsuno et al. 1998; Luschnig et al. 1998; Müller et al. 1998). The transposon-generated *pin3* null-mutants also exhibit defects in gravitropism (Friml et al. 2002). Because gravitropic defect in the *pin3* null-mutant is subtle, other PIN(s) are probably functionally redundant in the regulation of tropism-related auxin transport (Friml 2003). The mechanisms by which PIN3 proteins change their localization in columella cells in response to gravity remain unknown, but vesicle transport that recycles PIN proteins by endocytosis and exocytosis presumably regulates its localization on the plasma membrane (Geldner et al. 2001; Friml 2003; Muday et al. 2003). Brefeldin A (BFA) is an

inhibitor of vesicle transport that inhibits auxin transport and root gravitropism by altering the auxin efflux facilitator localization (Geldner et al. 2001). BFA acts on a particular type of guanine-nucleotide exchange factor for the ADP-ribosylation factor (ARF-GEF), which regulates vesicle trafficking. GNOM encodes such ARF-GEF and mutation of GNOM disrupts the polarity of auxin transport and causes defects in gravitropism (Geldner et al. 2003). Recently, TIBA was reported to inhibit vesicle transport by stabilizing actin and to inhibit auxin transport as well (Dhonukshe et al. 2008). The localization of AUX1 is also regulated by vesicle transport, but is independent of GNOM (Kleine-Vehn et al. 2006). Thus, auxin redistribution during the gravitropic response in roots is probably triggered by the asymmetric polar transport in columella cells, and not by the lateral movement of auxin across the root proper. In addition to the PIN auxin efflux facilitators, ATP-Binding Cassette (ABC) transporters have been shown to mediate auxin efflux in Arabidopsis (Noh et al. 2001, 2003; Blakeslee et al. 2007; Wu et al. 2007). However, a mutation in gene encoding ABC transporter, MDR4, enhances root gravitropism (Lewis et al. 2007). The role played by ABC transporters in root gravitropism is therefore unclear.

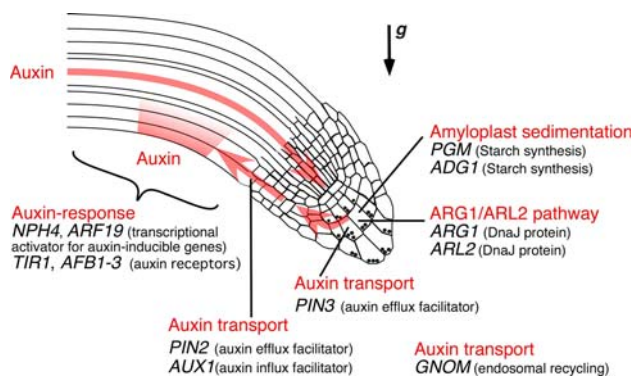
Auxin-inducible genes, including the *Aux/IAA* gene family, *SAUR* (small auxin up-regulated RNAs), and *GH3*, are asymmetrically expressed during the gravitropic response (McClure and Guilfoyle 1989; Conner et al. 1990; Gee et al. 1991; Esmon et al. 2006). The synthetic promoter *DR5* fused with the *uidA* gene which encodes  $\beta$ -glucuronidase (GUS) has been used as a reporter to visualize the endogenous auxin distribution (Ulmasov et al. 1997). The GUS activity in *DR5::uidA* transgenic Arabidopsis roots is maximal in the central columella cells of the root cap and faint in the flanking columella cells (Sabatini et al. 1999). When roots are reoriented from the vertical to the horizontal, more intense *DR5::uidA* expression is observed in the peripheral columella cells on the bottom side of the root than in those on the top (Rashotte et al. 2001; Boonsirichai et al. 2003). Similar observations were reported using *DR5::GFP* transgenic plants. The GFP signal of the *DR5::GFP* transgenic plants was observed in the quiescent center, columella initials and columella cells, and upon gravistimulation the signal increased in the lateral root cap of the lower side (Ottenschläger et al. 2003). This asymmetry in the GFP signal was more pronounced and expanded to the elongation zone of the gravistimulated roots when exogenous auxins such as 1-NAA or IAA were applied. These studies demonstrate that expression of several *Aux/IAA* genes is activated on the lower side of horizontally placed organs, although it is still matter of debate whether these reporter constructs reveal the entire auxin response (Yamamoto 2003). The

degradation of Aux/IAA proteins is necessary for auxin action. Aux/IAA proteins not only self-interact but also interact with auxin response factors (ARFs) and function as repressors of the transcription of auxin-inducible genes (Kim et al. 1997; Tiwari et al. 2001). Dominant mutations of the *Aux/IAA* gene, such as *AXR2/IAA7*, *AXR3/IAA17*, *SHY2/IAA3*, and *SLR/IAA14*, increase the stability of the gene products and repress the expression of auxin-inducible genes, thus conferring a defect in root gravitropism (Rouse et al. 1998; Tian and Reed 1999; Nagpal et al. 2000; Fukaki et al. 2002). The *nph4/arf7* and *arf19* double mutant reduces the gravitropic response in roots, although the *nph4/arf7* mutation alone does not affect root gravitropism (Harper et al. 2000; Okushima et al. 2005). The TIR1 and AFB proteins are auxin receptors mediating the auxin-dependent proteolytic degradation of Aux/IAA proteins (Gray et al. 2001; Dharmasiri et al. 2005a and 2005b; Kepinski and Leyser 2005), and a triple mutant of the *tir1*, *afb2*, and *afb3* is defective in root gravitropism (Dharmasiri et al. 2005b). The compound *p*-chlorophenoxyisobutyric acid (PCIB) acts as an anti-auxin and inhibits auxin-inducible gene expression, probably by blocking the auxin-dependent degradation of Aux/IAA proteins (Oono et al. 2003). PCIB-treated seedlings show a substantially reduced gravitropic response in both shoots and roots. Similarly, terfestatin A, an inhibitor of auxin signaling, inhibits root gravitropism (Yamazoe et al. 2005).

These results provide evidence of the essential role of auxin polar transport and redistribution. These processes result in asymmetric auxin-mediated transcription and differential growth in the elongation zone of the gravistimulated roots (Fig. 1). The basipetal transport of auxin, which requires auxin influx and efflux facilitators and occurs peripherally from the columella to the elongation zone, plays a major role in the redistribution of auxin (Fig. 1). An increased amount of auxin is transported to the lower side of the elongation zone in gravistimulated roots, as auxin efflux facilitators are re-localized to the bottom side of the plasma membrane of the gravisensing columella cells (Fig. 1). Thus, the Cholodny–Went theory is, in principle, applicable to gravitropism in roots.

### Hydrotropism research: development of the experimental system

In classical studies, plant physiologists examined the hydrotropic response of roots using several different systems developed to expose the roots to a moisture gradient (Takahashi 1997). However, little attention has been paid to this phenomenon for much of the last century, due to the difficulty in separating hydrotropism from gravitropism. In 1985, Jaffe et al. discovered that roots of the agravitropic



**Fig. 1** A schematic model for root gravitropism. Genes required for root gravitropism are shown. The molecular functions or characteristics of the gene products are indicated. The black arrow ( $g$ ) indicates the direction of gravitational force, and the red arrows indicate the direction of auxin flow. See texts for details

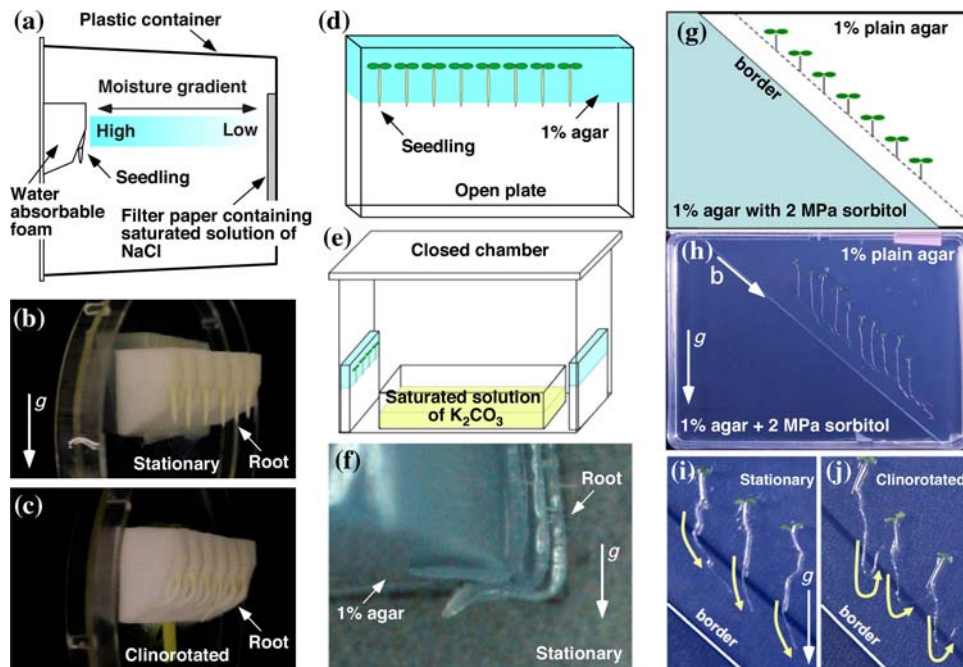
pea mutant *ageotropum* display a distinct hydrotropic response. In this experimental system, humidity-regulated air was introduced into chambers where a specific moisture gradient had been established between the soil, or wet cheesecloth, and the air (Jaffe et al. 1985; Takahashi and Suge 1991). Takahashi and Scott (1993) exposed roots to different moisture gradients that were established by placing wet cheesecloth and aqueous solutions saturated with various salts in a closed chamber. This latter approach yielded relatively stable moisture gradients. This method was modified to induce hydrotropic responses in cucumber roots by clinorotation (Mizuno et al. 2002; Fig. 2a–c). Moreover, Takano et al. (1995) revealed that roots curved hydrotropically in response to a water potential gradient when an agar block containing 0.5–1.5 MPa of sorbitol was applied to one side of the root tip of the *ageotropum* pea. When roots are placed between wet cheesecloth and salt-saturated aqueous solutions in a closed chamber, the moisture gradients at the root tip ranged from approximately 0.03 to 1.84% relative humidity (RH)  $\text{mm}^{-1}$ , depending on the salt introduced into the chamber (Oyanagi et al. 1995). Roots show positive hydrotropism in response to 0.67% RH  $\text{mm}^{-1}$  and this response increases as the moisture gradient increases (Takahashi and Scott 1993; Oyanagi et al. 1995).

To study hydrotropism in *Arabidopsis*, whose roots are thin and fragile, the experimental system was modified as follows (Takahashi et al. 2002; Kaneyasu et al. 2007). *Arabidopsis* roots were exposed to a moisture gradient established between 1% (w/v) agar plates and a saturated salt solution ( $\text{K}_2\text{CO}_3$  or KCl) in a closed acrylic chamber (Fig. 2d–f). Wild-type Columbia (WT) plants responded by hydrotropic bending of roots towards the agar. When water was placed, instead of saturated salt solution, the plants did not exhibit this hydrotropic response in a humidity-saturated chamber. The hydrotropic curvature commenced

within 30 min of exposure and continued to develop thereafter. These results indicate that *Arabidopsis* roots overcome gravitropism and rapidly deviate from their downward growth after exposure to a moisture gradient perpendicular to the direction of gravity. In an alternative approach, Takahashi et al. (2002) established water potential gradients by placing a plate of 1% (w/v) plain agar and agar containing sorbitol side-by-side in a plastic container (Fig. 2g, h). Relatively stable and smooth water potential gradients were thus achieved and maintained for several days. When *Arabidopsis* seedlings were placed vertically on the border between the plain agar and the sorbitol agar, the roots bent away from the sorbitol agar (toward the higher water potential side). *Arabidopsis* roots responded to a sorbitol-agar-based water potential gradient as small as 0.5 MPa. Eapen et al. (2003) also succeeded in inducing a hydrotropic response in *Arabidopsis* roots grown on agar plates in which a water potential gradient was formed by pouring glycerol and alginate into the lower half of the plate.

#### Gravitropism interferes with hydrotropism: species-specific differences

As discussed above, the presence of root hydrotropism in the agravitropic pea mutant *ageotropum* suggests that the hydrotropic response of normal roots is suppressed by gravitropism under normal gravity (1  $g$ ) on Earth (Jaffe et al. 1985; Takahashi and Suge 1991; Takahashi et al. 1992a; Takahashi et al. 1996). The roots of the wild-type pea (cv. Alaska) show a strong response to gravity and only a very weak response to a moisture gradient (Takahashi and Suge 1991; Takahashi et al. 1992a), but the clinorotated wild-type roots exhibit an obvious hydrotropic response that resembles that of the *ageotropum* roots (Takahashi et al. 1996). Cucumber seedlings grown in microgravity develop a number of lateral roots that grow towards the water-containing substrate in the culture vessel, whereas in normal gravity the lateral roots orient perpendicular to the primary root (Takahashi et al. 1999). The response of the lateral roots under microgravity conditions was successfully mimicked by clinorotation of cucumber seedlings on a three-dimensional clinostat. The response of the lateral roots in microgravity and on the clinostat appears to be a positive hydrotropism, which is easily suppressed by gravitropism under normal gravity (Takahashi et al. 1999). Similarly, the primary roots of cucumber seedlings show only a weak hydrotropic response in normal gravity, but the response is marked when clinorotation nullifies the gravitropic response (Mizuno et al. 2002; Fig. 2a–c). The opposing responses of hydrotropism and gravitropism have also been observed in



**Fig. 2** Examples of experimental systems for the induction of hydrotropic responses in cucumber and Arabidopsis roots. **a–c** Experimental system for cucumber seedlings. **d–j** Experimental systems for Arabidopsis seedlings. A moisture gradient was established between water-soaked foam and filter paper containing a saturated NaCl solution in a closed plastic container (**a**). Cucumber seeds were inserted into the foam so that the roots could grow down perpendicular to the direction of the moisture gradient. In the presence of the moisture gradient, the roots of the cucumber seedlings grew down under stationary conditions (**b**), whereas the clinorotated roots bent toward the water-soaked foam (**c**). **d–f** Arabidopsis seedlings were aligned vertically on a 1% (w/v) agar plate placed in an open plastic container, with their root tips (0.2–0.3 mm in length) suspended freely from the edge of the agar (**d**). The container with seedlings was placed vertically such that a moisture gradient was established between the agar and a saturated solution of  $K_2CO_3$

placed on the floor of a closed acrylic chamber (**e**). Arabidopsis roots deviated from the plumb and grew toward the agar in the presence of the moisture gradient (**f**). **g–j** A water potential gradient was established by placing a plain agar plate and an agar plate containing 2 MPa sorbitol side-by-side, and Arabidopsis seedlings were placed vertically with the root tips aligned 1 cm away from the border between the two agar plates in a closed plastic container (**g**). The roots of the Arabidopsis seedlings bent toward the high-water potential side (**h**). The arrow (**b**) indicates the border between the plain agar plate and agar plate containing 2 MPa sorbitol. In the presence of the water-potential gradient, Arabidopsis roots bent toward the high-water potential side under stationary conditions (**i**). The hydrotropic response was further enhanced by clinorotation (**j**). Arrows (**g**) indicate the direction of gravitational force. **a–c**, **d–f**, and **g–j** were modified after Mizuno et al. (2002), Kobayashi et al. (2007), and Kaneyasu et al. (2007), respectively

corn and wheat seedling roots (Takahashi and Scott 1991; Oyanagi et al. 1995). In these seedling roots, however, the hydrotropic response occurs under stationary conditions. These observations strongly suggest that the hydrotropic and gravitropic responses can influence one another depending on the intensities of the stimuli. The interaction between the two tropisms is further supported by the finding that the responsiveness to a moisture gradient varies according to the angle of the gravitropic stimulus. Thus, the hydrotropic response becomes weaker as the roots deviate from the vertical (Takahashi and Scott 1991). The responsiveness of cucumber and pea roots to gravity versus a moisture gradient could be stronger than that of wheat and corn roots, which leads to a distinct hydrotropic curvature in the latter species, even in the presence of normal gravity.

In contrast, as described above, recent studies have shown that Arabidopsis roots display a hydrotropic response by overcoming the gravitropic response (Takahashi et al. 2002; Eapen et al. 2003; Fig. 2d–f). Even in Arabidopsis roots, however, gravitropism interferes with hydrotropism (Kobayashi et al. 2007). The hydrotropic response of the roots of Arabidopsis seedlings clinorotated to nullify the gravitropic response is significantly accelerated, when compared with that of stationary seedlings (Fig. 2g–j). These results imply that the gravitropic response in Arabidopsis roots is relatively weaker, or that the responsiveness to hydrotropic stimulation is greater, than other plant species, including cucumber and pea. Thus, these observations suggest that the relationship between gravitropism and hydrotropism in roots differs among plant species.

### Perception of the moisture or water potential gradient in hydrotropism: Are the root cap cells the site of the interaction between gravitropism and hydrotropism?

The gravity-sensing cells mediating gravitropism in roots are hypothesized to reside in the root cap, and, specifically, in the columella cells, which contain amyloplasts. Although some studies suggest that gravity-sensing cells also exist outside the root cap, both classical and recent studies support this starch-statolith hypothesis. For example, roots of the starch deficient mutants *pgm1* and *adg1* show a substantial reduction in gravitropic responses when compared to wild-type plants (Kiss et al. 1989; Kiss and Sack 1989; Vitha et al. 2000; Kiss 2000). Columella cells of Arabidopsis roots are classified into three layers: story 1 (S1) is closest to the quiescent center of the root cap, whereas story 2 (S2) and story 3 (S3) are located farther from the quiescent center (Kiss and Sack 1989; Blancaflor et al. 1998). Experiments using laser ablation to kill specific cell layers in the root cap revealed that removal of the central S1 and S2 columella cells does not affect growth rates but causes significant inhibition of the gravitropic response, while ablation of both tip cells and S3 columella cells caused only a slight reduction in gravitropic curvature (Blancaflor et al. 1998). These results suggest that the columella cells in the root cap play a major role in gravity sensing and in the induction of the gravitropic response in roots. The *arg1* and *arl2* mutants have been isolated as gravitropism defective mutants (Sedbrook et al. 1999; Guan et al. 2003). Genetic analysis has suggested that the *ARG1* and *ARL2* genes function in the same gravity sensing signal transduction pathway (Guan et al. 2003). *ARG1* and *ARL2* encode membrane-associated DnaJ proteins whose molecular functions have not been elucidated (Sedbrook et al. 1999; Boonsirichai et al. 2003; Guan et al. 2003; Harrison and Masson 2008). Because *ARG1* and *ARL2* are required for PIN3 re-localization and asymmetric distribution of auxin in roots upon gravistimulation (Harrison and Masson 2008), these genes may play a role in inducing auxin transport following graviperception.

Decapped roots of corn or detipped roots of the *ageotropum* pea are unable to respond to hydrotropic stimuli (Jaffe et al. 1985; Takahashi and Suge 1991; Takahashi and Scott 1993). Takano et al. (1995) induced a hydrotropic response in *ageotropum* pea roots by applying a 0.5–1 mm<sup>3</sup> agar block containing 1 MPa sorbitol to one side of the root cap and plain agar to the other side. In contrast, the establishment of a water potential gradient in the elongation zone of the roots using a similar approach failed to induce hydrotropic curvature. Similarly, when 1.5–2.0 mm of the root tips are covered with lanolin, the roots no longer respond hydrotropically in the presence of a moisture

gradient applied to the root above the apical tip (Hirasawa et al. 1997). However, hydrotropic curvature occurs in roots whose elongation and mature regions are coated with lanolin prior to application of a moisture gradient. These results suggest that hydrotropism in roots occurs via perception by the root cap of a water potential gradient. One way to identify the tissue(s) responsible for root hydrotropism is to ablate or inactivate particular cells at the position of interest, as described for the gravitropism studies. Miyazawa et al. (2008) used a laser microbeam to ablate cells in Arabidopsis roots and examined the effects on the hydrotropic response. Similar to the gravitropic response, the hydrotropic response is severely reduced by ablation of the columella cells. These results imply that the moisture gradient sensing cells reside in the root cap. Interestingly, the root tips thus appear to act as sensors for both gravity and moisture gradients. The question then arises, as posed by Darwin and Darwin (1880), as to how the root tips perceive different stimuli and integrate multiple tropic responses vital for root navigation.

In Arabidopsis, the hydrotropic response is accompanied by starch degradation in the root cap cells. Takahashi et al. (2003) found that amyloplast starch in the columella cells is rapidly degraded upon exposure to a moisture gradient. Because starch-containing amyloplasts are required for full gravitropic response, this starch degradation may play a role in reducing gravisensing, and may be involved in the mechanism underlying hydrotropism-dependent growth. Among the Arabidopsis mutants that exhibit a reduced response to gravity and auxin, the *axr1* and *axr2* mutants show an increased hydrotropic response when compared to wild-type plants (Takahashi et al. 2002); Sabatini et al. (1999) reported that the columella cell amyloplasts in the roots of *axr1* and *axr3* mutants have severely reduced starch levels, which suggests that auxin participates in the development of the statoliths. The reduced gravitropism of these roots may be, in part, responsible for their increased responsiveness to moisture gradients. Roots of the Arabidopsis mutant *no hydrotropic response1* (*nhr1*) are tolerant to hydrostimulus-mediated starch degradation, but the amount of starch does not appear to affect the gravitropic response in the roots (Ponce et al. 2008). Ponce et al. (2008) also reported that Arabidopsis roots treated with auxin or ethylene exhibited an increase in the starch content in the root tip but did not show changes in their gravitropic or hydrotropic responses. These authors therefore postulate that starch degradation in the wild-type plant enhances hydrotropism by osmotic regulation, instead of reducing the responsiveness to gravity. However, this conclusion warrants careful examination because the starch-deficiency affects both gravitropic and hydrotropic responses (Kiss et al. 1989; Kiss and Sack 1989; Vitha

et al. 2000; Takahashi et al. 2003). The starch degradation in columella cells could modify certain tropic responses, but it may not be essential for the hydrotropic response in *Arabidopsis* roots due to the following observations. Roots of the starch-deficient *Arabidopsis* mutant show a unequivocal hydrotropic response (Takahashi et al. 2003); starch degradation occurs not only in response to moisture gradients but also to water deficit itself (Takahashi et al. 2003; Sun et al. 2008); and the rapid degradation of starch does not occur in hydrotropic responses of roots of other plant species, including pea and cucumber (Takahashi et al., unpublished data). Thus, how amyloplast dynamics contribute to the interactions between the mechanisms mediating hydrotropism and gravitropism in roots remains unclear.

At present, we know virtually nothing about the mechanism of moisture sensing by the root cap. In bacteria, however, it has been reported that a mechanosensitive ion channel is responsive to osmotic forces acting on the lipid bilayer (Sukharev et al. 1994; Levina et al. 1999; reviewed in Kung 2005). Recent advancements in our understanding of plant mechanosensors (Haswell and Meyerowitz 2006; Nakagawa et al. 2007; Haswell et al. 2008) led us to hypothesize that such channels may be involved in the perception of hydrotropic stimuli. Indeed, the  $\text{Ca}^{2+}$  channel blocker lanthanum inhibits hydrotropism in *ageotropum* roots (Takano et al. 1997). Additionally, application of the calcium chelator, ethyleneglycol-bis-( $\beta$ -aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA), inhibits hydrotropism in *ageotropum* pea roots, but the hydrotropic response of the EGTA-treated roots is restored when EGTA is replaced by a  $\text{Ca}^{2+}$  solution prior to hydrostimulation (Takano et al. 1997). Furthermore, application of  $\text{Ca}^{2+}$  to one side of the root cap in pea and corn roots causes root curvature away from the  $\text{Ca}^{2+}$  source (Takahashi et al. 1992b). These results suggest an important role for  $\text{Ca}^{2+}$  in the induction of root hydrotropism. In *Arabidopsis*, EGTA is inhibitory to both hydrotropism and gravitropism of roots, but roots treated with lanthanum or the  $\text{Ca}^{2+}$  channel blocker gadolinium exhibit a hydrotropic response identical to that of non-treated roots (Kaneyasu et al., unpublished data). A number of studies suggest that  $\text{Ca}^{2+}$  is involved in the gravitropic response of roots, but recent studies have indicated that  $\text{Ca}^{2+}$  signaling is important for the thigmotropic response but not the gravitropic response in *Arabidopsis* roots (Legué et al. 1997; Monshausen et al. 2008). A moisture gradient could cause directional movement of water in the root cap and thereby a change in turgor pressure, which could regulate a mechanosensitive channel. This and other hypotheses need to be examined to clarify the identity of the sensor of hydrotropic stimulation.

## Differential growth in hydrotropically responding roots

Root tropisms occur via differential growth of the stimulated and non-stimulated sides of the elongating tissues. The differential growth of hydrotropically stimulated roots of the *ageotropum* pea has been attributed to greater inhibition of growth on the side exposed to a higher water potential (Takahashi and Suge 1991). The tissue on the side exposed to a lower moisture maintains a greater degree of cell wall extension, whereas the extension of cells on the side exposed to higher moisture significantly decreases to generate the hydrotropic curvature (Takahashi and Suge 1991; Hirasawa et al. 1997). Moreover, the hydraulic conductivity ( $L_p$ ) is significantly increased on the side exposed to the lower water potential, which may underlie the difference in the rate of water uptake during the differential cell growth of hydrotropically responding roots (Miyamoto et al. 2002). In fact, the level of expression of the endoxyloglucan transferase gene in the elongation zone differs according to the water potential to which the cells are exposed (Takano et al. 1999). As discussed above, the perception of moisture or water potential gradients by the root tips and the subsequent curvature response in the elongation zone in hydrotropically responding roots appear similar to those of gravitropically responding roots. Stimulus perception and the bending response in both root gravitropism and hydrotropism are spatially and temporally distinct; therefore, the signal transduction and transmission mechanisms that underlie these two phenomena may have shared components.

## Auxin involvement in hydrotropism: Is the Cholodny–Went theory applicable to hydrotropism?

The scarcity of reports on the mechanisms of hormonal regulation of root hydrotropism reveals the pressing need to elucidate this phenomenon and to compare it to gravitropism. TIBA is an inhibitor of auxin transport that blocks hydrotropism in the agravitropic roots of the *ageotropum* pea mutant (Takahashi and Suge 1991). A recent study conducted by our group confirmed the inhibitory effect of auxin efflux inhibitors on the hydrotropic response in cucumber seedlings. The hydrotropic response of cucumber roots is clear when the gravitropic response is nullified by clinorotation (Takahashi et al. 1999; Mizuno et al. 2002), and TIBA severely inhibits the hydrotropic response of the clinorotated roots, as well as the gravitropic response of stationary roots (Morohashi et al., unpublished data). Application of the auxin transport inhibitor 9-hydroxyfluorene-9-carboxylic acid (HFCA) also inhibits both hydrotropic and gravitropic responses in cucumber

(Morohashi et al., unpublished data). In addition, application of PCIB, an auxin antagonist, substantially reduces hydrotropic and gravitropic curvature in cucumber roots (Morohashi et al., unpublished data). In hydrotropically responding pea roots, auxin is redistributed to and accumulates in the concave side of the elongation zone (Takano 1999). When [ $^3\text{H}$ ]IAA is administered to the root tips of the *ageotropum* pea mutant, the high water potential side of the elongation zone in hydrotropically responding roots retains more labeled IAA. Analysis of the expression of an auxin-inducible gene, *PS-IAA4/5*, revealed that accumulation of transcripts was enhanced in the high water potential side. This asymmetric accumulation of labeled IAA or mRNA is detected within one hour after stimulation by water potential gradient and prior to the commencement of hydrotropic bending. Similar results were obtained in hydrotropically responding roots of cucumber (Mizuno et al. 2003). Expression of another auxin-inducible gene, *CsIAA1*, is greater on the concave side of curving roots. The difference in expression was detected 30 min after stimulation by a moisture gradient and prior to hydrotropic bending. These observations suggest that the role played by auxin in hydrotropism is similar to its role in gravitropism in the roots of these plants. Polar auxin transport is hypothesized to be required for auxin redistribution, which leads to differential growth in the elongation zone of hydrotropically responding roots. The regulation of the auxin transport system in response to gravity or moisture gradients may be regulated by similar or distinct mechanisms for each tropism. Therefore, the interference or interaction between hydrotropism and gravitropism in roots could be due to competitive auxin dynamics. An important question thus arises as to how auxin transport is altered in response to moisture gradient in roots. Are the components in the auxin-mediated hydrotropic pathways the same as those involved in the gravitropic response in roots? To fully understand the role of auxin in root hydrotropism, this question needs to be answered.

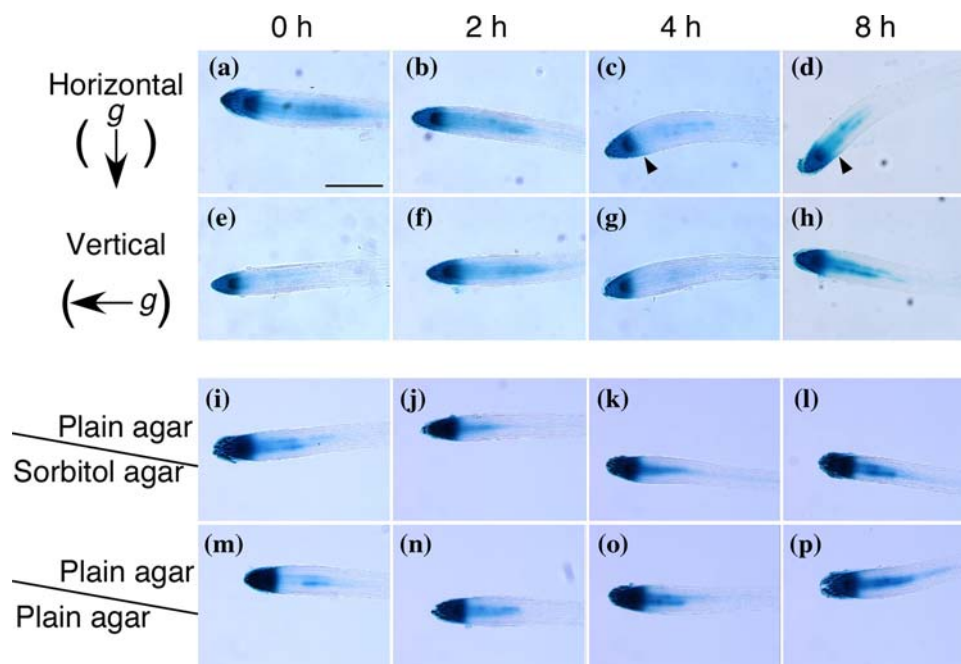
Auxin plays a role in the hydrotropic response of Arabidopsis roots, since the hydrotropic curvature is severely reduced in roots treated with PCIB (Kaneyasu et al. 2007). However, treatment of Arabidopsis roots with TIBA or NPA at various concentrations significantly inhibits the gravitropic response, but has no effect on the hydrotropic response (Kaneyasu et al. 2007). Similarly, Arabidopsis roots treated with an inhibitor of auxin influx, CHPAA, show a reduced gravitropic response, whereas the hydrotropic response was unaffected. These results suggest that auxin is indispensable for both hydrotropism and gravitropism, but that the regulatory mechanisms of auxin dynamics may differ between the two tropisms (Kaneyasu et al. 2007). These results imply that the polar transport of auxin is required for gravitropism but not for hydrotropism, or that the role of auxin is different in the two tropisms.

Similarly, a previous report demonstrated that the roots of the *pin2/agr1/eir1/wav6* mutant exhibited impaired gravitropism but normal hydrotropism (Takahashi et al. 2002). The roots of the *aux1* mutant also exhibit a hydrotropic response similar to that of the wild-type (Takahashi et al. 2002). The asymmetric redistribution of auxin after hydrotropic stimulation has not been reported in Arabidopsis roots. We compared the expression of *DR5::uidA* in gravitropically and hydrotropically responding roots of Arabidopsis (Fig. 3). Asymmetric expression across the root tip was observed in gravitropically responding roots 4–8 h after gravistimulation. In contrast, asymmetric expression was not observed in the hydrotropically responding roots, up to 48 h following hydrotropic stimulation. Because the *GUS* signal is usually observed in the root tip but not along the entire bending region of the root, auxin redistribution due to hydrotropic stimulation cannot be excluded.

Accordingly, the applicability of the Cholodny–Went theory to the differential growth in hydrotropically responding roots has yet to be determined. Nonetheless, it is probable that auxin plays an essential role in both hydrotropism and gravitropism in roots, with mechanistic differences depending on the particular species (Fig. 4). At present, we cannot explain why some plant species require polar auxin transport for the induction of hydrotropism, while others do not. There may be an unidentified mechanism that regulates auxin redistribution or action in a species-specific manner.

#### **Mutants with impaired or altered hydrotropism: involvement of molecular mechanisms unique to hydrotropism and independent of gravitropism**

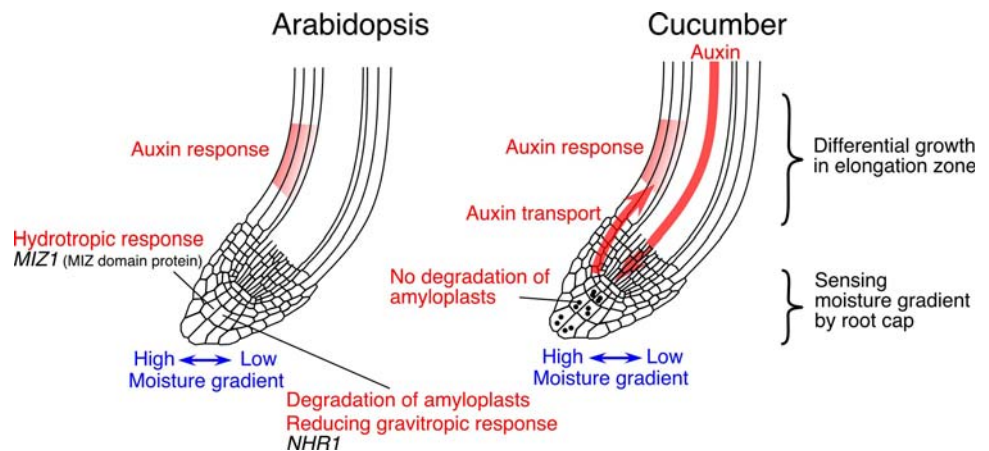
The enhanced hydrotropic responses observed in the roots of agravitropic mutants, clinorotated seedlings, and seedlings grown in microgravity imply that hydrotropism and gravitropism are genetically or physiologically distinct. To identify molecular factors common to, or divergent in, the two tropisms, it is therefore important to dissect and compare the specific mechanisms underlying hydrotropism and gravitropism. The isolation of gravitropic mutants of Arabidopsis and the identification of genes responsible for these mutations have contributed tremendously to our understanding of the molecules or pathways involved in root gravitropism. This approach has been adopted for the study of phototropism and the wavy response in Arabidopsis roots. Because experimental systems for the study of hydrotropism in Arabidopsis roots had been previously established, the search for hydrotropic mutants that can be used as tools for the molecular dissection of root hydrotropism is likely to be successful. Hydrotropic mutants may be expected to exhibit reduced or enhanced hydrotropism,



**Fig. 3** GUS staining pattern of gravitropically and hydrotropically responding roots of *DR5::uidA* Arabidopsis seedlings. Top panel. GUS staining patterns of gravistimulated (horizontal; **a–d**) and control (vertical; **e–h**) seedlings. Seedlings were sampled at the indicated times after stimulation and stained on the agar plate in order to maintain the direction of the stimulus. Arrowheads indicate the differential staining patterns. Bottom panel. GUS staining patterns

of hydrostimulated (sorbitol agar/plain agar; **i–l**) and control (plain agar/plain agar; **m–p**) seedlings. Seedlings were sampled at the indicated times after stimulation and stained on the agar plate in order to maintain the direction of the stimulus. Seedlings sampled at 12, 24, 36, and 48 h after hydrostimulation were stained similarly to those sampled at 2, 4, and 8 h (data not shown). Scale bar; 200  $\mu$ m

**Fig. 4** A schematic comparison of root hydrotropism between Arabidopsis and cucumber. Genes required for root hydrotropism are shown. The putative functions, as well as physiological and molecular characteristics of the gene products are indicated. See texts for details



or to lack a hydrotropic response altogether. These mutations could be accompanied by alteration of other tropic responses as well.

To isolate hydrotropic mutants, Kobayashi et al. (2007) adopted the experimental system described by Takahashi et al. (2002). A moisture gradient was formed between an agar plate and a  $K_2CO_3$  saturated solution in a closed chamber, and Arabidopsis seedlings were placed vertically on the agar plate so that approximately 0.2–0.3 mm of the root tips were suspended in air (Fig. 2d–f). Kobayashi

et al. (2007) screened approximately 20,000 Arabidopsis  $M_2$  plants (Columbia ecotype) mutagenized by treatment with ethylmethanesulfonate (EMS). They subsequently performed an additional screen of  $M_3$  plants to look for putative variants and successfully isolated several Arabidopsis mutants whose roots exhibited impaired hydrotropism. The mutants were named *mizu-kussei* (*miz*; ‘mizu’ and ‘kussei’ stand for water and tropism, respectively, in Japanese). Roots of the *miz1* mutant are impaired in hydrotropism but show normal gravitropism and

elongation growth (Kobayashi et al. 2007). There are no distinct differences in morphology or root structure between *miz1* and wild-type plants. These results suggest that the pathways that mediate hydrotropism are independent of the pathways mediating the gravitropic response. The *miz1* phenotype resulted from a mutation in *MIZ1* (At2g41660), which encodes a novel protein containing a domain (the MIZ domain) that is highly conserved among terrestrial plants, including rice and moss. The MIZ domain is not found in known genomes of organisms such as green algae, red algae, cyanobacteria, or animals. Therefore, *MIZ1* is hypothesized to have evolved as an important adaptation to terrestrial life, as hydrotropism could contribute to drought avoidance in higher plants. Because the T-DNA insertion mutants of *MIZ1*, SALK\_076560 and SALK\_126928, both lack hydrotropic responses, we propose that they be called *miz1-2* and *miz1-3*, respectively, and the original EMS-derived mutant be called *miz1-1*. This gene is the first to be identified as an essential gene for hydrotropism and demonstrates the existence of a molecular pathway for hydrotropism that is independent of gravitropism. The analysis of the *pMIZ1::GUS* fusion gene revealed expression predominantly in the root cap, which is thought to play a role in the perception of hydrotropic stimuli. Therefore, *MIZ1* may participate in early events of the hydrotropic response. A hydrotropic stimulus does not change the expression pattern of the *MIZ1* gene or the level of *MIZ1* protein (Kobayashi et al., unpublished data). Other *miz* mutants have also been characterized in our laboratory (unpublished data). Although the phenotype of *miz2* is similar to that of *miz1*, these mutants are not allelic. The roots of *miz3* mutants completely lack hydrotropic responses and exhibit reduced gravitropism. Additionally, the negative phototropism of roots is reduced, to some extent, in all *miz* mutants. The *miz1* and *miz2* mutants carry recessive mutations.

Eapen et al. (2003) reported an *Arabidopsis* mutant named *no hydrotropic response1* (*nhr1*) whose seedling roots lack hydrotropic responses. Seedlings of *nhr1* show reduced elongation growth, an increased amount of starch in the columella cells, and abnormal morphogenesis of the root cap. Interestingly, *nhr1* roots exhibit an enhanced gravitropic response compared to wild-type roots. The *nhr1* is a semi-dominant mutant, but the causative gene has not been identified. Cassab (2008) recently described a hydrotropism-enhanced mutant, *super hydrotropic response1* (*suh1*). The identification of the causative genes in these mutants, as well as in the *miz2* and *miz3* mutants, and the functional analysis of their gene products will further our understanding of the molecular basis of hydrotropism and its relationship to other root tropisms. In addition, it is noteworthy that both the hydrotropic and wavy responses are enhanced in some wavy mutants. The

roots of *wav2* and *wav3* show increased sensitivity to moisture gradient-induced hydrotropism (Takahashi et al. 2002). Because these mutants show hyper-waving with a short pitch (Okada and Shimura 1990), hydrotropism and the wavy response may share, in part, a common molecular pathway that controls the directional growth of roots (Takahashi et al. 2002). The *WAV2* protein contains a transmembrane domain at the amino-terminus and an  $\alpha/\beta$ -hydrolase domain in the carboxy-terminal region (Mochizuki et al. 2005). A *WAV2::GFP* fusion protein was localized to the plasma membrane and intracellular membranes, but its molecular function in the directional growth of roots is unknown (Mochizuki et al. 2005). Interestingly, the roots of the *miz1* mutant show a reduced wavy response. In contrast to *miz1* roots, *nhr1* roots show a pronounced wavy response. These results suggest that the hydrotropic pathway somehow affects the wavy response, although there is no correlation between the intensity of the hydrotropic and wavy responses.

### Concluding remarks

Hydrotropism is thought to be a response evolved by plants to promote growth in a terrestrial environment by enhancing the ability to obtain water; however, the molecular mechanisms mediating hydrotropism have not yet been fully elucidated. As described in this review, investigators have established several experimental systems to study hydrotropism in seedling roots and have demonstrated unequivocally the existence of the hydrotropic response. These studies have revealed important physiological aspects of hydrotropism and its universality in higher plant species. However, the interactions between hydrotropism and gravitropism in roots differ across species. *Arabidopsis thaliana* has been found to be most suitable for the study of root hydrotropism and provides a potent working tool to dissect the molecular mechanisms underlying hydrotropism. The successful isolation of hydrotropism-impaired mutants and the identification of the corresponding causative genes led to the elucidation of a *MIZ1*-mediated mechanism which is essential for hydrotropism, but is independent of gravitropism. Although the function of *MIZ1* remains unknown, the identification of this gene is a promising milestone in the study of hydrotropism in roots. Studies using the hydrotropism-impaired mutants *miz2*, *miz3*, and *nhr1*, as well as the hydrotropism-enhanced mutant *suh1*, also represent promising discoveries of unique mechanisms underlying the hydrotropic response and its interaction with other tropisms. We are now able to differentiate hydrotropism from gravitropism at both the physiological and molecular levels. Auxin plays a crucial role in both hydrotropism and

gravitropism in roots, but the regulatory mechanisms governing auxin behavior may vary in a species-specific manner. When compared with the significant body of knowledge available regarding gravitropism, many questions remain unanswered regarding the mechanisms mediating hydrotropism. Specific issues include: the identification of sensors of hydrotropic stimuli (moisture or water potential gradients), the clarification of the molecular basis of auxin dynamics and action, and the integration of the regulatory molecules into a network that allows the establishment of a working model for hydrotropism. Abscisic acid (ABA) has been implicated in both gravitropism and hydrotropism of seedling roots (Takahashi et al. 2002; Ponce et al. 2008). Interaction of these regulators with auxin in root hydrotropism needs to be analyzed and related to the function of molecules such as MIZ1. The outcome of such studies will provide new insights into the mechanisms that regulate the navigation of roots in response to environmental cues, and will further our understanding of the highly sensitive mechanisms for detecting multiple stimuli, including moisture gradients and gravity, by the root cap.

Root hydrotropism is an important subject of study because limited precipitation and water availability are the most important factors affecting plant growth and survival in a terrestrial environment. The ability of plant roots to display hydrotropism has been demonstrated, but evidence of its precise role in nature is still lacking. Positive root hydrotropism occurs when a steep and stable water potential gradient was applied across roots of an agravitropic mutant grown in vermiculite (Tsuda et al. 2003). A model simulation based on growth experiments using soybeans predicts that hydrotropism plays a major role in orienting plant roots in a suitable direction (Tsusumi et al. 2003). In contrast, a simulation experiment using coastal dune shrubs failed to find an ecological significance for hydrotropism (Cole and Mahall 2006). Therefore, well-designed experiments are needed to answer these pressing questions. The various ecotypes of plant species available in nature together with the hydrotropism-impaired or hydrotropism-enhanced mutants represent invaluable tools for future experiments.

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