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ROOT HYDROTROPISM: AN UPDATE[1]

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While water shortage remains the single-most important factor influencing world agriculture, there are very few studies on how plants grow in response to water potential, i.e., hydrotropism. Terrestrial plant roots dwell in the soil, and their ability to grow and explore underground requires many sensors for stimuli such as gravity, humidity gradients, light, mechanical stimulations, temperature, and oxygen. To date, extremely limited information is available on the components of such sensors; however, all of these stimuli are sensed in the root cap. Directional growth of roots is controlled by gravity, which is fixed in direction and intensity. However, other environmental factors, such as water potential gradients, which fluctuate in time, space, direction, and intensity, can act as a signal for modifying the direction of root growth accordingly. Hydrotropism may help roots to obtain water from the soil and at the same time may participate in the establishment of the root system. Current genetic analysis of hydrotropism in *Arabidopsis* has offered new players, mainly *AHR1, NHR1, MIZ1*, and *MIZ2*, which seem to modulate how root caps sense and choose to respond hydrotropically as opposed to other tropic responses. Here we review the mechanism(s) by which these genes and the plant hormones abscisic acid and cytokinins coordinate hydrotropism to counteract the tropic responses to gravitational field, light or touch stimuli. The biological consequence of hydrotropism is also discussed in relation to water stress avoidance.

Key words: ABA; abscisic acid; hydrotropism; gravitropism; phototropism; wavy growth response; water stress; cytokinins.

In contrast to animals that can move around to meet their necessities, plants display differential growth movements to position their organs to meet their needs. Growth is fundamental to the life of plants. When they build a new leaf, a prop root, or a flower, they modify their growth. Underground plant parts such as stolons, corms, stems, and roots grow either toward or away from various environmental stimuli to place themselves at their "physiological depth", adequately deep to be protected from threats at the soil–air interface, but not so deep that they fail to contact air and microorganisms growing at the top of the soil (Koller and Van Volkenburgh, 2011). Roots are destined to perform two fundamental tasks: anchoring plants into the soil and providing them with water and inorganic nutrients by spreading their tips into new territories for gathering nutrients and water. Since water and nutrients are often not evenly allocated in soil, plants must optimize soil exploration with their root system. For soil exploration, plants evolved a series of tropic responses, or tropisms, which condition the direction and extent of root directional growth toward environmental clues such as gravity, light, water gradient, pathogens, or temperature. When plants colonized the land, they had to give up unlimited access to water and therefore had to acquire developmental programs and/ or specialized organs that allow them to obtain water and nutrients and anchored them to the ground. Hence, the root deployed a variety of responses that allowed not only anchoring the plant with the benefit of gravitropism, but also obtaining water by hydrotropism. Root anchoring is achieved by progressive penetration of the soil environment, led by the root tip (of the primary, adventitious, or lateral roots). The root tips are driven through their particular environment via a growth-mediated tissue, known as the elongation zone located a short distance behind the apex. This growth movement comes about by metabolically driven elongation resulting from anisotropic growth of the cells in this zone, to the direction along the axis of the root. The tissues composing the elongation zones are in a constant state of flux. New cells, produced in the apical meristems in the root tip, are constantly being added to the elongation zone, while maturing cells, at the other end lose their capacity for elongation and join the precursors in the maturation zone (Baluska et al., 2010). In contrast to root elongation during root exploration, tropic root growth occurs when actively growing root tips change their spatial orientation by growth-mediated curvature of the elongation zone subtending their apex. Differential rates of growth in opposite sectors of this elongation zone modify the spatial orientation of the roots; acceleration or inhibition of cell elongation in one segment gives rise to the curvature of this organ.

Plant growth and development result from the responses to internal and external signals, such as tropic bending, de-etiolation, nastic movements, and flower induction. Then, plant success can likewise be regarded as adaptively variable growth and development during the lifetime of the individual. The goal of studies on root hydrotropism, or any other plant differential growth response, is therefore to critically approach how plants sense, discriminate, and record images of their environment, which finally yield an effect at the whole organismic level.

Although the deficiency of abundant water is the single-most significant factor influencing world agriculture, there are amazingly few studies on root traits that could improve crop yield in drought-tolerant crop plants. Drought is a global social and economic problem, which affects several regions on Earth

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(Wilhite, 2005). Low water availability is one of the major causes for crop yield reductions, affecting the majority of the agricultural fields around the world. Agriculture accounts for a 92% of the global water footprint (rainwater and volumes of water use for producing imported products and water use within the country for making export products [Hoekstra and Mekonnen, 2012]). Not only are crops naturally water intensive, but also livestock are getting more than a third of the global grain harvest to satisfy our desires for meat and other animal products. As water resources for agronomic uses become a limiting factor, the development of drought-avoidance and drought-tolerant lines becomes increasingly significant. Grain yield under water-stressed conditions is frequently the prime trait for selection (Rosales et al., 2012). Root systems clearly play a crucial role in water acquisition for plants and are a substantial component of tolerance to water deficit stress. However, there are few studies that consider root traits that meet the criteria of being genetically associated with grain yield under drought, highly heritable, and not associated with yield loss under ideal growing conditions. Hence, studies that define traits of early roots of seedlings that correlate with stress tolerance would benefit the selection process and time to develop new germplasm. Research on hydrotropism is unfortunately not very popular, although there are some hints that a robust hydrotropic response is correlated with an improvement to cope with drought (Bolaños and Edmeades 1993; D. Eapen et al., unpublished data). Herein, there is a promising future on studying root hydrotropism for the development of robust hydrotropic and stress avoidance lines. Most recent hydrotropism studies have been made in the model plant *Arabidopsis* (Eapen et al., 2005; Cassab, 2008; Takahashi et al., 2009). *Arabidopsis* is native to a wide range of environments with varying drought constraints, which suggests large variation in the response of these natural populations to water deficit. *Arabidopsis* is highly responsive to hydrotropic stimuli (Takahashi et al., 2002; Eapen et al., 2003). Understanding key genes and alleles important for this trait in *Arabidopsis* will help to find new levers for crop breeding. It is known that similar genetic networks in different species control complex phenotypes (Paran and Zamir, 2003), so we can expect that studying hydrotropism in *Arabidopsis* will help to understand similar mechanisms in crops. In this review, we discuss that hydrotropism might be a stress avoidance strategy of plants to cope with drought, and it is worth consideration in crop improvement programs for global conditions of the near future. For instance, the global agricultural sector will need 19% more water by 2050 to meet a 70% increase in demand for food (Hoekstra and Mekonnen, 2012); hence, it should be a priority of plant scientists to use their creativity and resources for the implementation of sustainable agriculture on Earth.

HISTORY

Studies on hydrotropism have been limited since Giammattista Della Porta (1515–1616), Knight (1811) and Sachs (1887) showed that roots move in the direction of a water gradient (Porta, 1658; Eamon, 1996; Takahashi, 1997). Sachs (1887) showed that in seedlings grown in a freely hanging sieve basket, the emergent roots become diverted from the gravity vector and grow along the wet substrate. Near that time, Darwin, Molisch, Hooker, Pfeffer and Weisner (who introduced the term hydrotropism) were certain that moisture gradients influenced root directional growth in many species (Hart, 1990). Curiously, the notion that plant roots penetrate the soil in search of water was first considered as the explanation for the downward orientation of roots (Dodart, around 1700, reviewed by Hart, 1990). However, studies on hydrotropism have been remarkably limited in comparison with investigations on gravitropism and phototropism (Eapen et al., 2005; Cassab, 2008). Indeed, isolation of the first hydrotropic mutants in *Arabidopsis thaliana* lagged 19 yr and 23 yr, respectively, behind the first accounts of gravitropic and phototropic response mutants (Eapen et al., 2003; Koorneef et al., 1980; Olsen et al., 1984).

Hydrotropism studies have always been hard to interpret because both thigmotropism and gravitropism interact with hydrotropism. Mechanical stimuli can generally be avoided but gravity is ubiquitous on Earth. Consequently, several tools, such as those including agravitropic mutants, clinorotation, or microgravity in space have been used to differentiate the hydrotropic from the gravitropic response (Takahashi, 1997). Notably, experiments with the pea mutant *ageotropum,* which has agravitropic roots and responds positively to hydrotropism, showed that there are independent sensing and signaling pathways for these two tropisms (Jaffe et al., 1985).

Darwin (1881) was captivated by "plant movements" and was the first to locate the sensory system for root tropisms. He covered the root apex from different species with a hydrophobic mixture of olive oil and lampblack and found that they no longer responded to a moisture gradient, gravity vector, or obstacles. When root caps from the pea mutant *ageotropum* or corn roots were dissected, they failed to curve hydrotropically, but their growth was not affected (Jaffe et al., 1985; Takahashi and Scott, 1993). These results indicate that the sensory cells for a moisture gradient reside in the root cap. Root cap cells perceive and respond to a gradient in water potential as small as 0.5 MPa (Takano et al., 1995). Herein, a gradient in water potential sufficient to induce hydrotropism could develop between the soil surface and air and between the surface and deeper regions in the soil. Interestingly, the water potential threshold for inducing a hydrotropic response varies not only among plant species (Takano et al., 1995), but also among different land races and lines of maize and beans (G. I. Cassab et al., unpublished data), indicating the complexity of understanding the mechanism that controls this array of sensibilities to moisture gradients.

HYDROTROPISM PLAYERS

Several players have been connected with hydrotropism. Cytoplasmic Ca^{2+} has been postulated to be a transducer for the gravitropic (Plieth and Trewavas, 2002) and hydrotropic signals (Takano et al., 1997). Auxin is another important participant in gravitropism (Ottenschläger et al., 2003) but not as much in hydrotropism (Takahashi et al., 2002; Kaneyasu et al., 2007; Ponce et al., 2008b). Cholodny (1927) and Went (1926) hypothesized over 80 yr ago that plants bend in response to a gravity stimulus by generating a lateral gradient of a growth regulator at an organ's apex and later found it to be auxin. Reorientation in *Arabidopsis* seedlings has been shown to generate the asymmetric release of auxin from gravity-sensing columella cells at the root cap. The resulting lateral auxin gradient is thought to drive a differential growth response, in which cell expansion on the lower side of the elongation zone is reduced relative to the upper side, causing the root to bend downward (Boonsirichai et al., 2002). Apparently, auxin asymmetry is lost as bending root tips attained an angle of 40° to the horizontal, a mechanism that seems to reverse this asymmetric flow at the midpoint of root bending (Band et al., 2012). On the contrary, neither genetic nor pharmacological inhibition of auxin transport inhibited hydrotropic response in *Arabidopsis* (Takahashi et al., 2002; Kaneyasu et al., 2007). Furthermore, the asymmetric redistribution of auxin after hydrotropic stimulation expression was not observed in hydrotropically responding roots (Ponce et al., 2008b; Takahashi et al., 2009), so it remains to be established whether auxin affects hydrotropism differently compared to gravitropism. Light, is another major player in hydrotropism (Moriwaki et al., 2012a), which also influences root gravitropism (Hangarter, 1997). Other significant players in hydrotropism such as abscisic acid and cytokinins have a modest influence on gravitropism (Feldman et al., 1985; Aloni et al., 2004). Ponce et al. (2008a, b) reported that the application of ABA disrupted the hydrotropic response in roots of wild-type *Arabidopsis* seedlings. Cytokinins also participate in hydrotropism since the hydrotropic response of the *altered hydrotropic response1* (*ahr1)* mutant is strongly modulated by this class of hormones. Furthermore, the lack of hydrotropism of the *no hydrotropic response1 (nhr1)* roots was eliminated in the presence of kinetin (Saucedo et al., 2012).

Mutant analysis in *Arabidopsis* has also brought in new players of hydrotropism (Eapen et al., 2003; Kobayashi et al., 2007; Miyazawa et al., 2009; Saucedo et al., 2012). Two genes, *MIZ1* and *MIZ2*, that participate in hydrotropism were revealed recently (Kobayashi et al., 2007; Miyazawa et al., 2009). *MIZ1* encodes an unknown protein, which contains a DUF617 domain present in several proteins from different plant species. Interestingly, MIZ1 domain homologues were not found in the genomes of animals or in microbes and thus exist only in terrestrial plants. *MIZ1* is expressed in root tips and hydathodes (Kobayashi et al., 2007). Recent biochemical and microscopic analyses indicate that MIZ1 is a soluble protein, which is primarily associated with the endoplasmic reticulum membrane at the cytoplasmic side in root cells (Yamazaki et al., 2012). However, MIZ1 was recently shown to be a participant in hormonally mediated lateral root development (Moriwaki et al., 2012b). Numerous lines of evidence support auxin and cytokinin as positive and negative regulators of lateral root development (Benkova and Bielach, 2010; Laplaze et al., 2007). Overexpression of *MIZ1* (*MIZ1OE*) in *Arabidopsis* roots reduced the number of lateral roots. This effect was reversed by the application of auxin, implicating that the reduction in lateral roots *MIZ1OE* was most probably caused by the lower level of auxin. In contrast, *miz1* roots had increased levels of auxin compared with those of the wild type, indicating that *MIZ1* negatively modulated auxin concentration. Cytokinins inhibited lateral root formation in wild type and in seedlings overexpressing *MIZ1* (*MIZ1OE*) under optimal and osmotic stress conditions, but *miz1* was insensitive. Thus, *MIZ1* apparently regulates cytokinin-dependent lateral root formation, which may indicate that genes that take part in root hydrotropism also function in the hormonal regulation of root architecture, that is, the spatial configuration of the entire root system (Lynch, 1995). The second gene associated with hydrotropism, *MIZ2*, is a weak allele of *gnom* (Miyazawa et al., 2009), a guanine nucleotide exchange factor for ADP-ribosylation-type-G proteins. GNOM participates in membrane-trafficking processes, and most *gnom* mutants are affected in auxin transport. The defective gravitropic response phenotype in *gnom* mutants is consistent with the requirement of auxin transport during gravitropic growth (Geldner et al., 2003). Along this same line of thought, the lack of hydrotropism and the manifestation of gravitropism in *miz2* mutants can be explained because auxin transport was not affected by this mutation, implying that GNOM had an unidentified role in root hydrotropism, other than participating in auxin transport. Moriwaki et al. (2012b) proposed that MIZ1 and MIZ1-like proteins participate in the control of root architecture by modulating cytokinin and auxin signaling and/or accumulation. *MIZ1* seems to require GNOM activity since *miz2* mutation repressed auxin levels in *MIZ1OE* roots. It is still unclear whether the participation of these genes in the regulation of root system architecture is dependent or independent of the hydrotropic response.

Two other hydrotropic mutants, *nhr1* and *ahr1*, have been described, although the genes responsible for these mutants have not been revealed (Eapen et al., 2003; Saucedo et al., 2012). Roots of the semidominant *nhr1* mutant did not display hydrotropism in the presence of moisture or substrate water potential gradients but displayed orthogravitropism (Eapen et al., 2003) (Fig. 1A). Physiological studies revealed that *nhr1* mutant roots had a faster rate of gravitropic response than that of the wild type when both seedlings were set horizontally either in air or in agar. The *nhr1* roots also showed enhanced wavy growth pattern on an inclined agar plate as described by Okada and Shimura (1990). Although the mechanism for this particular root growth is not yet clear, it is probably directed by the gravitational field and/or friction sensing and signaling responses (Oliva and Dunand, 2007). Thus far, all hydrotropic mutants exhibited atypical wavy responses on an inclined agar medium (Kobayashi et al., 2007; Miyazawa et al., 2009; Eapen et al., 2003; D. Eapen and G. I. Cassab, unpublished data), while some of the wavy mutants, such as *wav3* and *wav2,* displayed increased hydrotropism (Takahashi et al., 2002). Hence, hydrotropism might share part of its signaling pathway with other root tropisms. Future studies should examine whether responses to moisture, light, and touch directly shape root growth patterns or only modulate them indirectly by affecting the response to gravity.

Hydrotropically stimulated *nhr1* roots maintain starch in amyloplasts of columella cells in contrast to those of the wild type (Fig. 1E). Amyloplast sedimentation had been thought to be essential for sensing the gravitational field (reviewed by Kiss, 2000; Morita and Tasaka, 2004). Two studies proposed that amyloplasts are inhibitory to hydrotropism because of the counteracting effect of the gravitropic modification of growth (Takahashi et al., 2003; Nakayama et al., 2012). According to the authors' proposal, the decreased responsiveness to the gravity field in hydrotropically responsive roots is triggered by amyloplast degradation. However, amyloplasts are also degraded in water-stressed roots treated with mannitol or sorbitol, suggesting that roots perceive and respond to hydrotropic stimulation similarly as to water stress. On the other hand, hydrostimulated *miz1* mutant grew following the gravity vector while showing amyloplast degradation (Kobayashi et al., 2007). Hence, amyloplast degradation seems to be a water stress response triggered during hydrostimulation. Because hydrotropism might be one of the plant mechanisms for avoiding water stress and ABA is traditionally considered as the water stress hormone (Finkelstein, 2006), it was not surprising that the no-hydrotropic phenotype of the *nhr1* mutant (Fig. 1B) was significantly intensified with ABA or inhibited with abamineSG, a specific inhibitor of ABA biosynthesis (Kitahata et al., 2006; Quiroz-Figueroa et al., 2010). Additionally, ABA treatment inhibited hydrotropism of wild-type

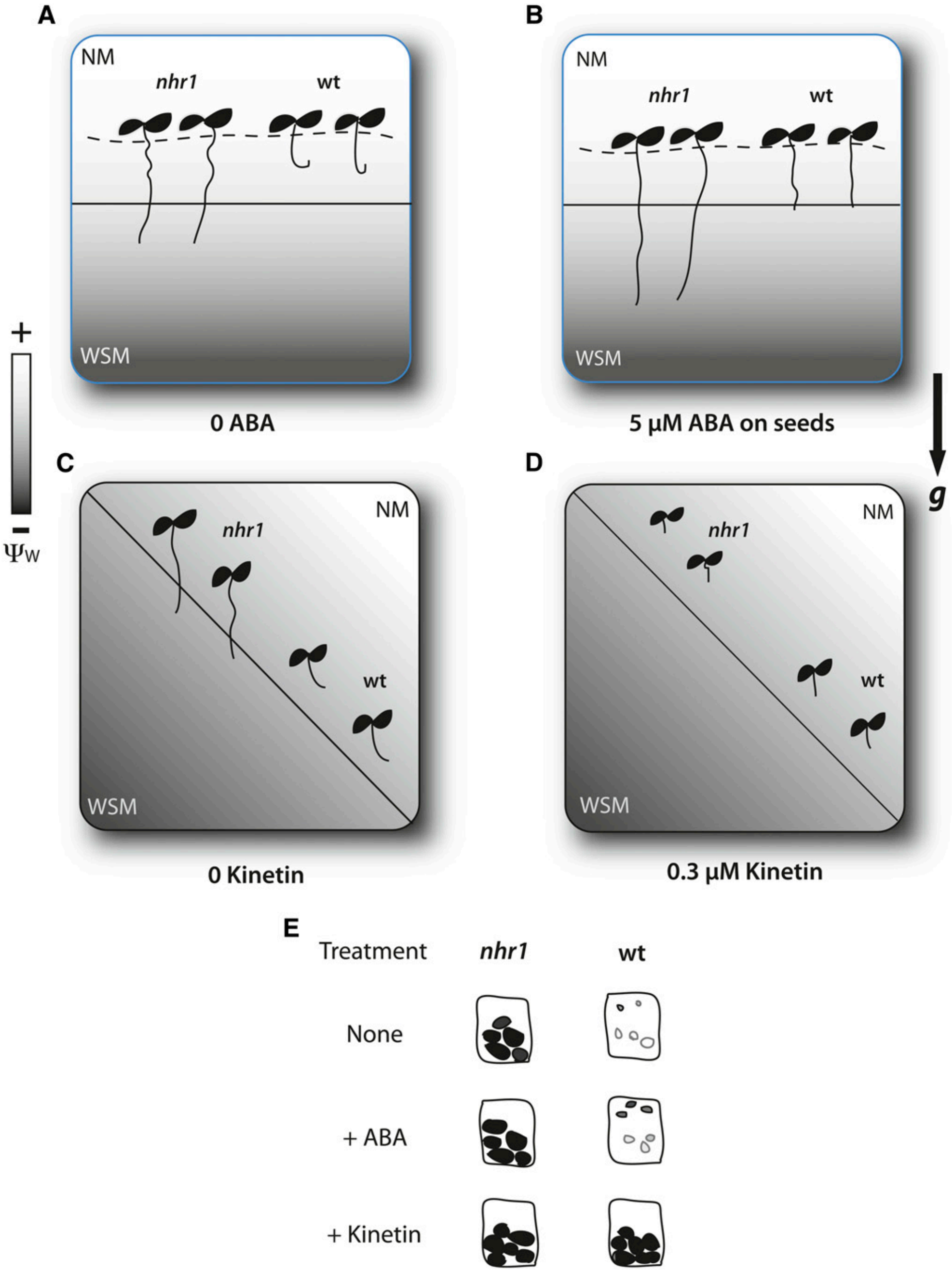

Fig. 1. Diagrams illustrating the hydrotropic growth response of the *no hydropic response1* (*nhr1*) mutant. Water potential gradients were generated with glycerol and seedlings tested in two orientations: (A, B) horizontal normal medium (NM)→ water stress medium (WSM) with or without ABA and (C, D) oblique NM→WSM with or without kinetin. (A) Mutant *nhr1* showed no hydrotropic response compared to those of wild type (wt), which is also affected by (B) ABA and (D) kinetin. (C) Phenotype of *nhr1* mutants in an oblique system. (E) Illustrations of columella cells with starch grains in amyloplasts. Presence or absence of starch in amyloplasts is shown in *nhr1* mutants and wt root tips of seedlings grown in the horizontal NM→WSM with or without ABA and in the NM→WSM oblique system with or without kinetin. *g*, gravity vector; Ψw, water potential; bar indicates the direction of the water potential gradient. White to black gradient in plates indicates lower to higher osmotic gradient of the medium. The schematic representations depicted in (A–D) are based on data published by Eapen et al. (2003), Ponce et al. (2008a), and Saucedo et al. (2012). Illustrations of columella cells with starch grains in amyloplasts depicted in (E) were taken from images of seedlings grown in either the horizontal or the oblique NM→WSM test media for 11 d, whose root tip amyloplasts were visualized with I_2-KI solution (D. Eapen and G. I. Cassab, unpublished data). The size of amyloplasts depicted represents the average perimeter (mm) of these organelles in root cap columella cells from 25 representative roots in each category and from three different replicas.

roots, whose downward growth was independent of the presence of starch granules in the root tip (Fig. 1B) (Ponce et al., 2008a, b). Evidently, ABA signaling has a critical role during the development of a hydrotropic response of *Arabidopsis*. Transcriptome examination revealed that a set of genes responsive to ABA stress and water stress overlap with those triggered by hydrostimulation, but very little with those induced during gravity and light stimulation (Moriwaki et al., 2010). However, these findings cannot explain the physiological antagonism between ABA and hydrotropism. More work is needed to explain at the transcriptomic level the interaction between ABA and hydrotropism. An alternative hypothesis is that a set of genes that responds only to ABA but not to hydrotropism is responsible for the antagonism. Another possible explanation is more methodological, as in the work by Moriwaki et al. (2010), the whole seedling was used for the transcriptomic analysis rather than just the primary root, which is the organ involved in the hydrotropic response.

On the other hand, an assessment of the hydrotropic response in the *ageotropum* mutant of pea showed that hydrotropic signaling is independent of gravitropic signaling and that gravitropism interfered with hydrotropism (Jaffe et al., 1985). Hence, how roots suppress their tropic response to the gravity field and activate their response to moisture gradients still need to be elucidated. *MIZ1* and *MIZ2* genes, which were postulated to have an essential role in hydrotropism, were not detected by a microarray analysis after 1 and 2 h of hydrostimulation (Moriwaki et al., 2010). Thus, these genes might not be induced directly by changes in moisture gradients or by water stress, but most probably ABA, cytokinin, or other stress-signaling components of roots activated their products later in the signaling. In contrast, *NHR1* might be induced directly by hydrostimulation and/or water stress since *nhr1* mutants maintain their starch in amyloplasts (Fig. 1E). It is postulated that the degradation of starch does not influence the extent of gravitropism, but most probably helps the root to resist osmotic stress and respond hydrotropically. The work on *nhr1* challenged the hypothesis of gravity sensing based on sedimenting plastids. However, strictly speaking, it does not refute the evidence that supports the role of amyloplasts in gravity response in the absence of water stress, but rather shows that long-term responses to the gravity field under these conditions differ from the majority of published studies on root gravitropism (Ponce et al., 2008a; Morita, 2010). There are some indications that several types of gravity sensing exist in plants (Barlow, 1995; Sack, 1997; Perbal, 1999), implying a multiplicity of systems for gravity perception. Resolution of the *NHR1* identity will possibly open the way for interpreting the mechanism that suppresses gravitropism and concomitantly activates hydrotropism. Thus far, we do not even know whether only one moisture perception mechanism exists in root caps.

In nature, plants normally receive a heterogeneous water supply and develop their root system accordingly (Canadell et al., 1996; Dorlodot et al., 2007). Until now, hydrotropism has not been considered as an adaptive trait for avoiding drought. Hence, it will be helpful to identify additional genes involved in determining the sensitivity to moisture gradients to dissect how their action affects root growth orientation and branching and how this mechanism may help plants to cope with drought. An in vitro screening system was established for identifying candidate genes that participate in this mechanism (Saucedo et al., 2012) (Fig. 2A). The system selected mutant seedling roots that grow from a medium with low water potential (water stress medium, WSM) toward one with high water potential (normal medium, NM) because wild-type roots do not respond this way. The identified mutants were considered to display an altered hydrotropic response because their roots were strongly directed to the higher water availability medium when the water-rich source was placed downward relative to the root tip (referred to as horizontal WSM→NM system, Fig. 2A); an extensive root system also developed over time. This phenotype was enhanced by ABA (Fig. 2B) and was not observed when *ahr1* seedlings were grown in a water-stressed medium without a gradient in water potential. Additionally, *ahr1* mutants were capable of remaining in the NM-WSM interphase when placed vertically in the NM medium with a WSM medium below that was positioned obliquely relative to the gravity vector (Fig. 2C, referred to as oblique NM→WSM). This response was strongly inhibited when cytokinins were added to the medium (Fig. 2D). In addition, the *nhr1* mutant plants lost their no-hydrotropic response phenotype in the oblique NM→WSM system in the presence of kinetin (Fig. 1D), indicating that both *NHR1* and *AHR1* are directly regulated by cytokinins. Starch was observed in amyloplasts of wild-type roots treated with kinetin (Figs. 1E, 2E), which suggests a direct effect of cytokinins in inhibiting starch degradation when hydrostimulated or water-stressed (D. Eapen and G. I. Cassab, unpublished data). The factors that govern amyloplast development in intact root tip cells are as yet unknown (Barlow and Grundwag, 1974). However, there are reports showing that cytokinin controls chloroplast biogenesis (Stetler and Laetsch, 1965). The chloroplast cytokinin-binding protein CBP70 accumulated in amyloplasts, which indicates that cytokinin is involved in the control of transcription during the biogenesis of these organelles (Brovko et al., 2010). Remarkably, roots of the cytokinin receptor histidine kinase mutant *ahk4/cre1/wol1* displayed a faster hydrotropism than those of the wild type (Saucedo et al., 2012). Cytokinins modulate root elongation under high osmotic conditions (Tran et al., 2007; Wolhlbach et al., 2008; Werner et al., 2003). It was assumed that when roots were hydrostimulated, cytokinins inhibited root elongation on the side opposing the higher water potential, thus enabling root hydrotropic curvature (Saucedo et al., 2012). The connection between hydrotropism and cytokinin is interesting since cytokinin traditionally is the hormone that interacts antagonistically with auxin (Dello Ioio et al., 2008; Müller and Sheen, 2008). Auxin is the principal regulator of gravitropism but scarcely controls hydrotropism. Thereof, the finding that cytokinin, in conjunction with ABA, are critical players of hydrotropism points to new directions in examining this tropic response. Presumably, cytokinins and ABA counteract or antagonize either auxin transport or regulation of root elongation, or both. Consequently, this effect might be sufficient to suppress auxin control of gravitropism. Marhavý et al. (2011) reported that cytokinin affected endocytic recycling of the auxin efflux carrier PIN1 by redirecting it for lysis in the vacuoles. Hence, by controlling auxin distribution, cytokinins might regulate developmental processes such as hydrotropism. Overall, these results indicate that cytokinins play a critical role in root hydrotropism.

The altered hydrotropic response phenotype displayed by the *ahr1* mutant in the two test systems (Fig. 2A, C) might be explained because, unlike the wild type, they preserve the structure of their root caps, when confronted with a water potential gradient (Fig. 2E). According to Sharp et al. (2004), root system development in plants living under drought is usually less inhibited than shoot growth and may even be promoted. The maintenance of root growth under water deficit is not only advantageous for sustaining an adequate water supply for the plant but also is under genetic control (O'Toole and Bland, 1987). Future research on the mechanism sustaining cell elongation and division under water potential gradients must focus on the apical region of *ahr1* roots, as well as in its amyloplast regeneration mechanism, which allow *ahr1* roots to maintain long-term growth in low water potential gradients. These studies may shed light on how different sensibilities

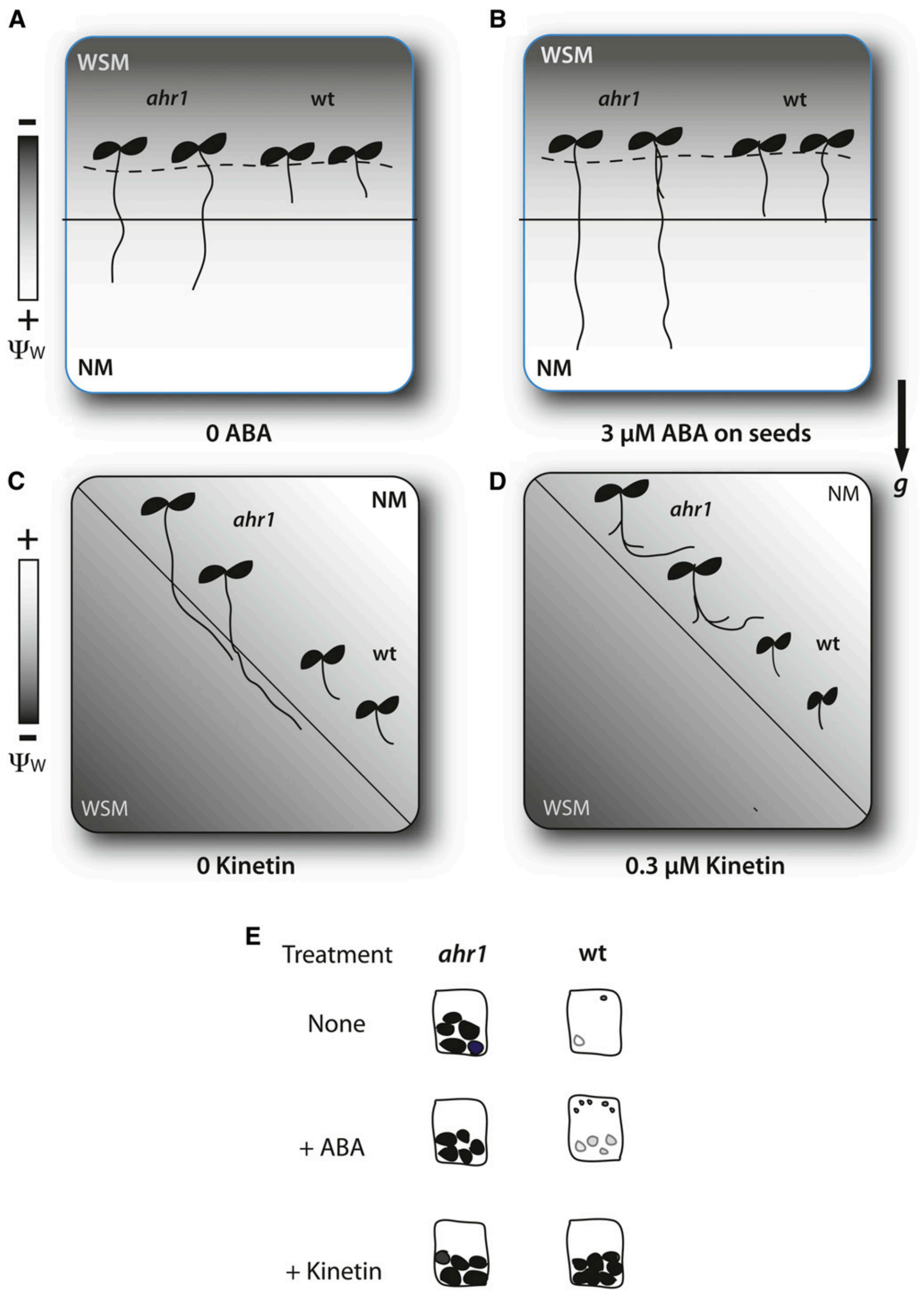

Fig. 2. Diagrams illustrating the growth response of the *altered hydrotropic response1* (*ahr1*) mutant in test media for hydrotropism with water potential gradient generated with glycerol in two positions: (A, B) horizontal water stress medium (WSM)→normal medium (NM) with or without ABA, and (C, D) oblique NM→WSM with or without kinetin. (A) Mutant *ahr1* displayed altered hydrotropic response compared to those of the wild type (wt), which is also affected by (B) ABA and (D) kinetin. (C) Validation of the phenotype of *ahr1* mutants in an oblique system. (E) Illustrations of columella cells with starch grains in amyloplasts. Presence or absence of starch in amyloplasts is shown in *ahr1* mutants and wt root tips of seedlings grown with or without supplemented ABA and kinetin in the two systems, respectively. Note that the lack of starch in amyloplasts is not sufficient for inhibiting orthogravitropism in wt seedlings treated with ABA. Kinetin treatment prevents starch degradation in amyloplasts of wt root cap columella cells. *g*, gravity vector; Ψw, water potential; bar indicates the direction of the water potential gradient. White to black gradient in plates indicates lower to higher osmotic gradient of the medium. The schematic representations depicted in (A–D) are based on data published by Saucedo et al. (2012). The illustrations in (E) represent columella cells with starch grains in amyloplasts and were obtained as described for Fig. 1.

to water potential gradients are perceived and acted upon to result in differential growth.

The molecular mechanisms behind the root hydrotropic response may not be understood if we do not examine other directional environmental cues such as light. Like water and gravity, light also has been a continuous influence and a source of information during the evolution of root tropic responses. Hence, the negative phototropic response of roots also needs to be addressed. Several plant physiologists have considered the positive phototropism of the shoot as an adaptive plant response, which influences the final form of the plant (Darwin, 1881; Iino, 1990; Hangarter, 1997; Holland et al., 2009). However, light has also been shown to modify graviresponses in roots through the action of phytochrome and phototropin photosensory systems. Phytochromes are red-light photoreceptor pigments that mediate plant responses to shade-induced variation in light quality. Phototropins are blue-light photoreceptors that regulate growth responses of stems and roots to directional light (Briggs and Olney, 2001). The best example of phytochrome regulation of root gravitropism is that of maize roots of the Merit variety, which grew perpendicular to the gravity vector in darkness, but upon exposure to red light exhibited positive gravitropism (Feldman and Briggs, 1987). In *Arabidopsis*, root gravitropism is reduced in mutants defective in phytochromes (Correll and Kiss, 2005). Moreover, the photomorphogenetic mutant *hy5* (long hypocotyl phenotype in the light) displayed deficiencies in root gravitropism and hydrotropism, as well as in wavy root growth (Oyama et al., 1997; Moriwaki et al., 2012a). Both *nhr1* and *ahr1* hydrotropic mutants also were defective in photomorphogenesis and had wavy growth responses (D. Eapen and G. I. Cassab, unpublished results), implying a connection among these physiological responses. HY5, a bZIP type transcription factor, regulates several genes downstream of the light signal transduction pathway. Some of these are genes, such as *AUX/IAA*, that actively participate in auxin signaling (Sibout et al., 2006). Light regulation of root development seems to be modulated by changes in the intracellular distribution of auxin efflux carrier PIN2 from the plasma membrane to the vacuole via endocytosis (Laxmi et al., 2008). This regulation enables light to control auxin fluxes and distribution, which might consequently influence root gravitropic responses.

According to Galen et al. (2004, 2007), root phototropism contributes to fitness during seedling establishment and adult plant growth. The intensity of blue-light cues (300–500 nm) in the soil is determined by seed depth and particle grain size, sandy soils with small grain size having the maximum light penetration (Mandoli et al., 1990). Root negative phototropism in *Arabidopsis* is mediated by phototropin 1 and also probably by phytochrome A (Kutschera and Briggs, 2012). Interestingly, fitness of field-grown *Arabidopsis phot1* mutant plants was considerably poorer than those of wild-type plants grown in same soil plots (Canadell et al., 1996). Subsequently, negative root phototropism was demonstrated to augment the ability of the plant to obtain water by directing the orientation of roots to grow deep into the soil just after germination. For instance, in dry, but not in wet soil, phototropin-1 increased root growth and enhanced plant size at maturity (Galen et al., 2007). On the basis of these results, we have depicted root behavior in normal field conditions in Fig. 3A. Roots display orthogravitropism following the gravity vector and/or negative phototropism avoiding the light signal. Both tropic responses help the root not only to orient the plant, but also to avoid drying at the soil/air interphase and in time to obtain water, which moves down through the soil by gravitation. Further development of the root system might be controlled by the soil water flow, that is, roots tend to grow toward the direction of the water supply (Fig. 3B). An increase in the root elongation rate in a favorable environment has been called “compensatory growth” (Tsutsumi et al., 2003). For instance, withholding water experimentally resulted in deeper penetration of maize roots and higher depletion rates of soil water compared with well-watered plants (Sharp and Davies, 1985). In fact, these observations imply that, during seedling establishment, root phototropism and hydrotropism might work in concert to rapidly direct root growth toward water and avoid desiccation.

Root hydrotropism seems to require light signaling since hydrotropic curvature of wild-type *Arabidopsis* roots was reduced in dark-grown seedlings (Moriwaki et al., 2012a). However, a recent report indicates that darkness reduces root growth by influencing the root apical meristem size (Sassi et al., 2012), and thus differential root growth can also be affected. Localization of green fluorescent protein (GFP)-fused MIZ1 in the root cap was regulated reversibly by light stimulation indicating that the light signal modulates genes implicated in hydrotropism. Examination of hydrotropism in several photoreceptor and light signaling mutants revealed that phytochromes A, B (phyA, phyB), and HY5-mediated blue-light signaling participate in light-mediated induction of MIZ1 (Moriwaki et al., 2012a). In particular, *hy5* mutants were considerably reduced in hydrotropic curvature similar to the dark-treated wild-type roots, revealing that light signaling mediated by HY5 participates in hydrotropism. ABA, on the other hand, regulated *MIZ1* expression in wild-type roots and could recover weak hydrotropism in *hy5* mutants, independently of light signaling. However, *miz1* roots did not regain hydrotropism after ABA treatment. Hydrotropic curvature was reduced around 30% in root seedlings of *hy5* mutants treated with abamineSG, and in *aba1* mutant seedlings, which lack ABA synthesis as well (Moriwaki et al., 2012a). Even though this suggested that the abamineSG treatment could mimic an ABA deficient phenotype on hydrotropism, it also implies that hydrotropism is not solely controlled by ABA because the hydrotropic curvature was not completely suppressed. Previously, abamineSG was shown to restore the hydrotropic response to about 70% of treated *nhr1* seedling roots (Quiroz-Figueroa et al., 2010). To a large extent, these results reveal that environmental cues, such as light and water stress, mediated by ABA and cytokinin signaling control the activity of the genes that participate in hydrotropism (Fig. 3B).

Recently, the effect of the lunisolar tidal force on the growth of plant roots has been reviewed (Barlow and Fisahn, 2012; Fisahn et al., 2012). According to the model discussed, control of cell growth and polarity entails the formation of coherent states of water molecules. The coherent state of water molecules is induced upon the interaction of the lunar gravitational field with a defined number or mass of water molecules. Thus, in terms of the coherent state model, water potential gradients might affect the sizes and localization of the coherent water particle assemblies and might be able to direct root elongation in accord with a water potential gradient. However, whether the lunisolar tidal force profile function comes into play during the development of a hydrotropic response when indeed a daily photoperiodic entrainment for growth is present remains to be determined. The tidal force usually becomes apparent whenever light/dark or temperature entrainment signals are weak or absent. Hydrotropism in *Arabidopsis* has been examined in two entrainments. Eapen et al. (2003) and Saucedo et al. (2012)

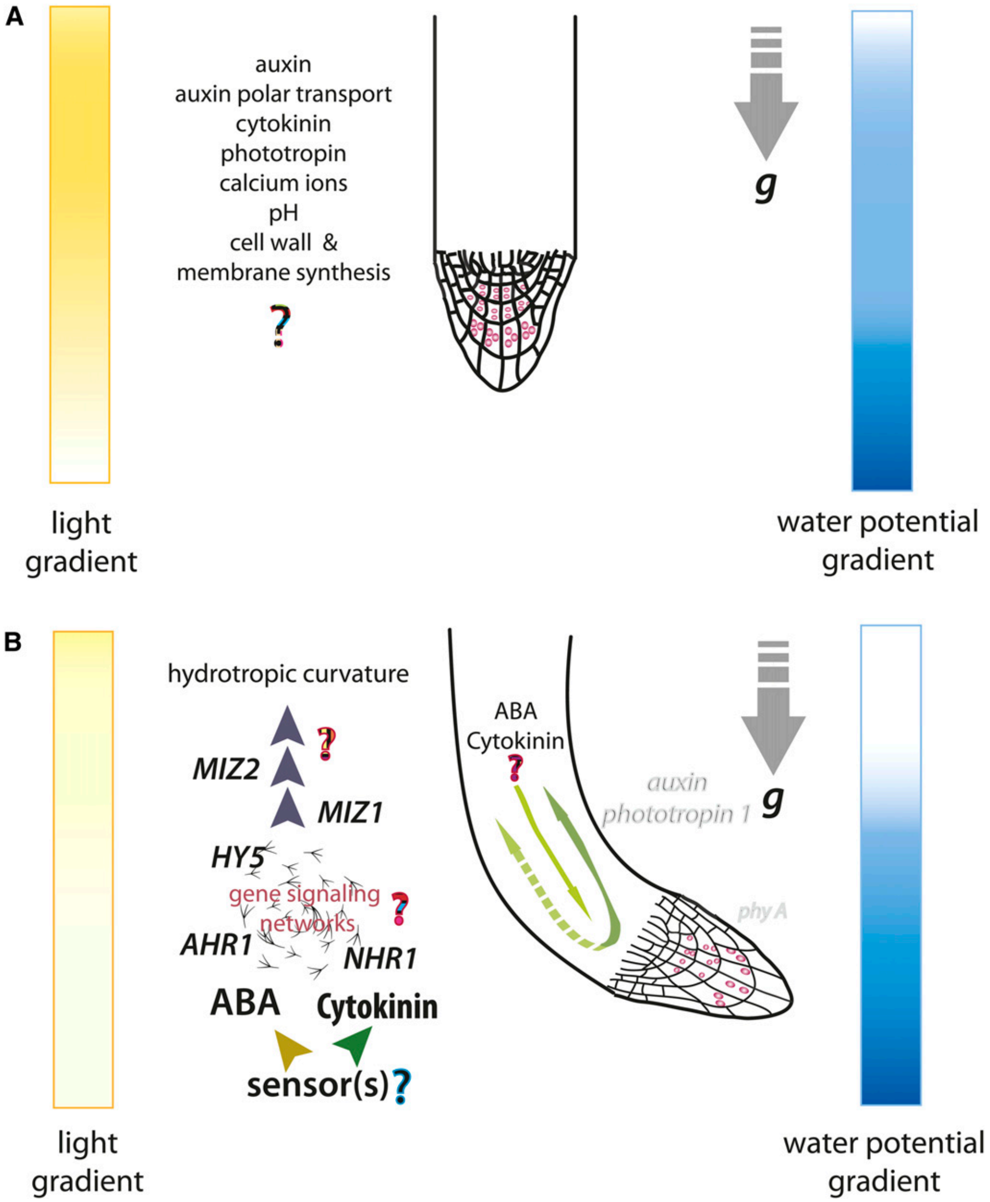

Fig. 3. Current model for the development of a hydrotropic response in *Arabidopsis* root seedlings. (A) Under optimal soil growth conditions *Arabidopsis* roots show downward growth, in which the gravity vector and the moisture gradient are in the same direction, the light gradient is in the opposite direction. Auxin, auxin polar transport, cytokinin, phototropin, calcium ions, pH, cell wall, and membrane synthesis are the known players that participate in orthogravitropism. Amyloplasts are illustrated in the columella cells of the root cap. Question mark denotes other possible unknown players, such as gravity sensor(s) and transducers. (B) Roots display a hydrotropic curvature in response to a moisture gradient. During the hydrotropic response, the stimulus perception starts with the unknown sensor(s) and is transduced through signals such as ABA and cytokinins. Light intensity decreases as the root grows into the soil a few centimeters. Fewer amyloplasts are observed during the development of a hydrotropic curvature. Auxin transport and possibly phototropin 1 signaling might be inhibited by the signal(s) triggered by the sensor(s) of water potential gradients. Water potential gradients and/or moisture sensor(s) likely trigger ABA and cytokinin signaling to modulate hydrotropism gene networks. This network is known so far to comprise *MIZ1 MIZ2, NHR1, AHR1*, and *HY5.* These genes, except *MIZ2*, seem to be modulated by ABA and light. However, *NHR1* and *AHR1* might be directly regulated by ABA and cytokinins because both *ahr1* and *nhr1* mutants retain starch in their amyloplasts upon hydrostimulation and/or water stress, in contrast to *miz1* and *miz2* mutants. Question marks denote the lack of information regarding components of the gene-signaling network of hydrotropism, the molecular nature of the water potential gradient sensor(s), and all the players that regulate the hydrotropic response. Hydrotropism and the compensatory growth of roots might be dominant factors in root system development. *g*: gravity vector.

used a photoperiod of 16 h light and 8 h dark. In contrast, Kobayashi et al. (2007) grew *Arabidopsis* seedlings in continuous light for 4 d and set hydrotropism experiments in continuous darkness. Hence, the effect of lunisolar tidal force was not taken into consideration. Nonetheless, analyses of hydrotropism in the common bean (*Phaseolus vulgaris* L.) and maize (*Zea mays* L.) have been done in either continuous artificial illumination or continuous darkness (D. Eapen et al., unpublished data). Our observation is that in the presence of light, these roots show other stress responses; hence, the modulation of light signals in hydrotropism assays has to be studied in different plant species to confirm these findings, and the effect of the lunisolar tidal force should be considered as well.

In our opinion, the real test for the future will be to comprehend how genes, which apparently regulate hydrotropism in the laboratory, actually operate in plants grown in field conditions. In fact, some authors claim that hydrotropism has not been clearly shown to occur under simulated field conditions (Cole and Mahall, 2006). Nonetheless, partial root drying, a modern irrigation technique recently used to enhance water-use efficiency, presumably works because hydrotropism allows roots to sense the water gradient in the soil. In this technique, water is distributed unevenly to the soil around the plant, with one side receiving water and the opposite side remaining dry (Davies et al., 2005). More such studies are fundamental to clarify whether plants can indeed grow hydrotropically under natural conditions. These studies are required before hydrotropic and phototropic signaling are included in plant breeding programs for the development of cultivars capable of growing in regions experiencing drought that maintain or even increase their yield under these conditions.

CONCLUSIONS AND FUTURE DIRECTIONS

Overall, how directional growth responses evolved, and in particular hydrotropism, is still mysterious and is a fundamental subject that needs to be addressed in terms of how plants developed cross talk between the sensitivity to moisture gradients and hormone signaling and how they relate to other root tropic responses such as gravitropism, thigmotropism, and phototropism (Barlow, 1995; Rensing et al., 2008; Whippo and Hangarter, 2009). Plant roots frequently monitor environmental variables such as light, moister gradients, obstacles, nutrients, and gravity with remarkable sensitivity to explore and thus program their growth and development. These variables most probably act in opposition and to different degrees and affect the perception of one above the other; therefore, the combination of potential environments experienced by any root and to which it must respond is immense. Hence, root cap cells might have modules of perception working together not only for triggering the proper signaling response to each individual stimulus, but also for coordinating hierarchically the proper response to simultaneous stimuli. The question arises as to how root cap cells, which are constantly dividing and differentiating (Hawes et al., 2003; Arnaud et al., 2010) can act as sources of memory of long and short-term responses to environmental cues. Conceivably, these questions will be dissected if plant scientists learn how to examine plant changes in the direction of growth in the plant time scale, which differs significantly from those of animals. Continued growth of plants generates new environments for both roots and shoots, and responses to the environment in both organs change ontogenetically. The long-term growth resistance to water potential gradients displayed by the *ahr1* mutant is a remarkable feature that should be further examined (Saucedo et al., 2012).

Drought avoidance response in plants has been thoroughly described (Kramer, 1980). It is assumed that all the avoidance responses to drought, whether physiological or morphological, must be initiated and transduced by a mechanism that can evaluate the existing supply of water against an estimated optimal supply. How the root evaluates its existing supply of water is a fundamental question. We can speculate that roots constantly evaluate their rhizosphere to minimize further damage to the plant provoked by a lack of water and to respond to these assessments; whether plants use root hydrotropism remains to be proven. Some aspects of water stress avoidance are hypothesized to have evolved by natural selection of root tropic responses. For this, it might be useful to examine water stress responses in mutants of *Arabidopsis* that are defective in hydrotropism, phototropism, thigmotropism, and hormone signaling to perhaps discern whether there is indeed a modular connection between these tropisms and a well-known drought avoidance response(s). Additionally, using crop plants such as maize or beans to examine root hydrotropic responses and to test their growth responses to drought in the field conditions will be increasingly helpful for encouraging agricultural diversity and sustainability. Such programs should be promoted through organized strategies for research and development strategies within each nation.

The wise words of the great poet and scientist Johann Wolfgang von Goethe (1749–1832) are worth remembering when studying plant tropisms: “A plant is like a self-willed man, out of whom we can obtain all which we desire, if we will only treat him his own way” (Goethe, 2009).

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