# Turning up the volume: How root branching adaptive responses aid water foraging

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## Abstract

Access to water is critical for all forms of life. Plants primarily access water through their roots. Root traits such as branching are highly sensitive to water availability, enabling plants to adapt their root architecture to match soil moisture distribution. Lateral root adaptive responses hydropatterning and xero-branching ensure new branches only form when roots are in direct contact with moist soil. Root traits are also strongly influenced by atmospheric humidity, where a rapid drop leads to a promotion of root growth and branching. The plant hormones auxin and/or abscisic acid (ABA) play key roles in regulating these adaptive responses. We discuss how these signals are part of a novel "water-sensing" mechanism that couples hormone movement with hydrodynamics to orchestrate root branching responses.

## Addresses

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## Introduction

Global food demand is projected to increase by 62% as the world population reaches 9.1 billion by 2050 [1]. This places an unprecedented pressure on existing food production systems, particularly given the increased likelihood of altered rainfall patterns and droughts arising as a result of climate change [2]. Solutions such as expanding irrigation to rain-fed croplands are unsustainable given already depleted blue water resources (e.g., water from rivers, reservoirs, and lakes) [3]. Instead, improving water use efficiency in crops provides one promising way forward to deliver food security [4].

Understanding how root traits contribute to plant productivity and water use efficiency under water scarcity is vital [5,6●]. For instance, steeper and deeper roots provide yield stability under drought [7]. Similarly, genotypes with longer and denser root hair possess larger rhizosheaths that improve water status under drought stress [8,9]. Longer tap roots, lateral roots (LRs), higher branching density, and decreased coarse to fine roots also confer drought tolerance [10,11]. Knowledge of such water-responsive root traits can help crop breeders and molecular biologists to select or engineer new varieties with improved drought tolerance.

## Lateral root positioning is regulated by soil water availability

Seasonal variation in precipitation, temperature, and evaporation influences soil moisture levels and leads to the heterogenous distribution of water in the soil profile [12,13]. In addition, edaphic factors such as soil properties (soil type, structure, texture, and hardness) and tillage practices also account for uneven soil moisture distribution [14,15●,16]. Among root classes, laterals constitute most of the root length and massively increase the root surface area while foraging for water (and nutrients). LRs are also the most active sites of water absorption [17]. To maximize plants' access to heterogeneously distributed soil water resources, LRs exhibit developmental plasticity [15●]. For example, roots growing down a soil macropore undergo a hydropatterning response, where the one-sided availability of water results in the preferential positioning of LRs toward regions of high-water availability [18●●]. In contrast, when roots temporarily lose contact with water from all sides (e.g. crossing air spaces), LR initiation is completely suppressed until roots reconnect with moist soil (Figure 1). This novel adaptive response, termed xerobranching, describes the branching behavior of roots in "dry" (Greek xēros) air spaces [19●●].

**Figure 1**
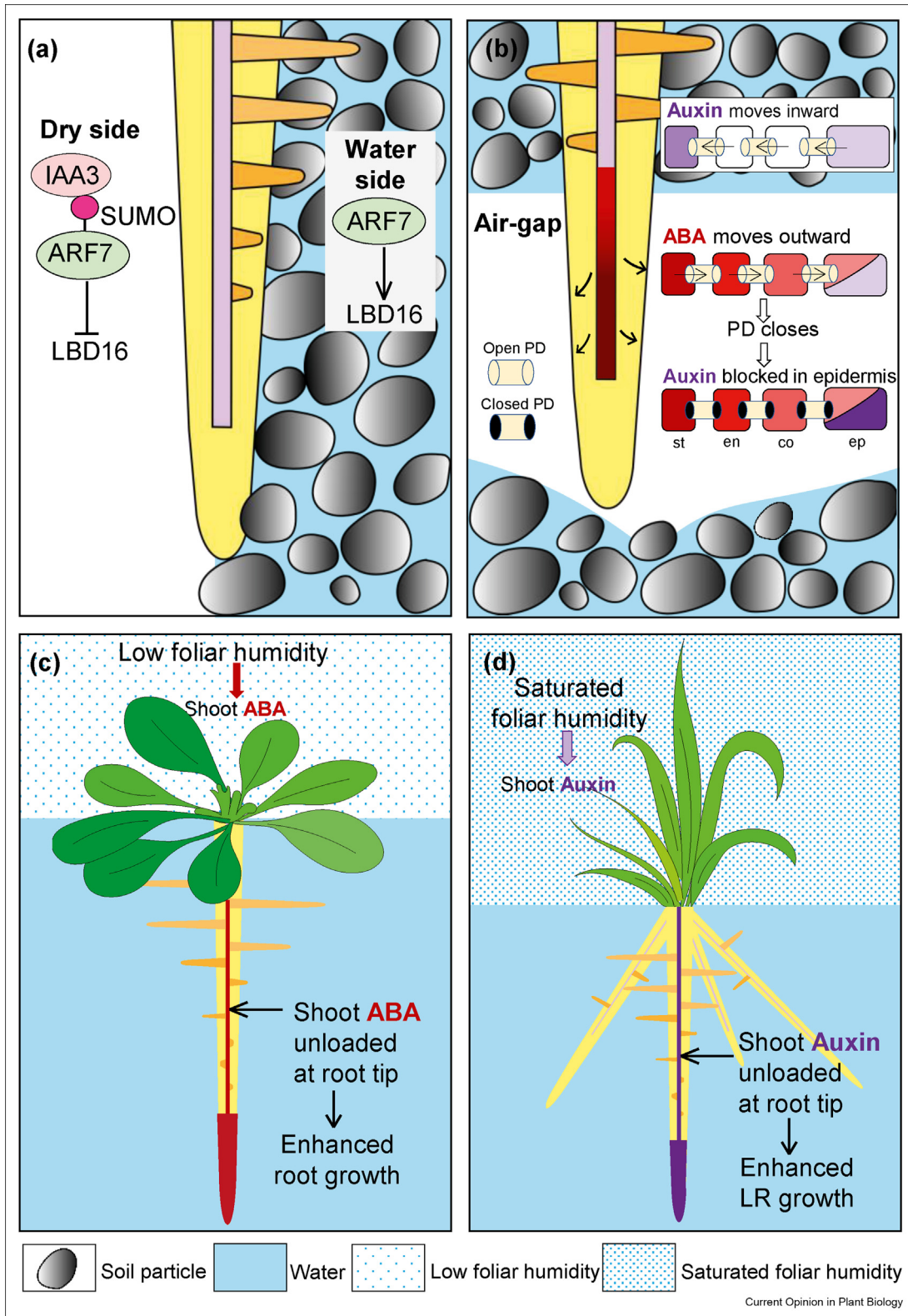


Water availability in soil and atmospheric humidity regulate lateral root (LR) development. (**a**) Model showing molecular mechanisms underlying LR positioning during hydropatterning. On the water side of the root, ARF7 induces LR development through LBD16 activation. On the air side of the root, SUMOylated ARF7 recruits IAA3 to repress downstream auxin signaling. (**b**) Roots exhibit xerobranching (no branching) response while growing through air gaps in soil. When roots are in contact with water, auxin (LR-promoting signal) from epidermis flows inward to pericycle through open plasmodesmata (PD). In the air gap, phloem water co-mobilizes ABA radially outward leading to PD closure. Blocking inward symplastic auxin flow triggers xerobranching. (**c**) Low foliar humidity induces ABA biosynthesis in shoot. ABA is subsequently transported to roots through phloem to promote root growth. (**d**) Saturated foliar humidity enhances phloem unloading of shoot-derived auxin at root tip promoting LR growth.

Both hydropatterning and xerobranching represent LR adaptative responses regulated by the microscale availability of water in naturally structured soils. Considering the magnitude of water stress and branching response, xerobranching could be inadvertently interpreted as an extreme version of hydropatterning. However, genetic studies have demonstrated these adaptive responses employ distinct sets of signals and molecular mechanisms, demonstrating hydropatterning and xerobranching function independently of each other.

Hydropatterning requires asymmetric biosynthesis and transport of the LR-promoting signal auxin toward the side of roots exposed to water rather than the air [18••,20••]. Higher water availability induces auxin biosynthesis via TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1 (TAA1) and PIN-FORMED 3 (PIN3)-dependent auxin transport, promoting LR initiation on the water exposed root side [18••]. Asymmetric water availability also triggers an auxin response gradient mediated by post-translational modification of transcriptional activator, AUXIN RESPONSE FACTOR 7 (ARF7) [20••]. On the air side, ARF7 is post-translationally modified by small ubiquitin-like modifier (SUMO). SUMOylated ARF7 preferentially recruits auxin signaling repressor IAA3 which contains a SUMO interacting motif (SIM). IAA3 binding blocks ARF7-dependent expression of transcription factor *LATERAL ORGAN BOUNDARIES-DOMAIN 16 (LBD16)*, which is required to trigger LR initiation. Conversely, on the water-exposed side, non-SUMOylated ARF7 continues to trigger LR initiation via *LBD16*. Mutants defective for ARF7 SUMOylation disrupt hydropatterning, demonstrating the functional importance of this post-translational modification and auxin for this root water adaptive response [20••]. Surprisingly, genetic studies have revealed that hydropatterning is independent of the classical water stress signal phytohormone abscisic acid (ABA) [18••].

In contrast to hydropatterning, ABA represents a key regulator of xerobranching [19••,21••]. Transient ABA treatment temporarily suppresses LR formation and thus phenocopies xerobranching [19••]. In a recent study, Mehra et al. [21••] designed an agar-based air-gap bioassay that mimics the transient loss of water when roots grow through air spaces in soil. This novel bioassay, when combined with the highly sensitive FRET-based ABA biosensor nlsABACUS2 [22••], revealed a xerobranching stimulus triggers dynamic ABA redistribution in root tissues. As root tips transit an air gap, phloem-derived ABA gradually moves radially outward, accumulating in root elongation zone cells. Hydraulic modeling revealed that, in the absence of an external source of water, elongating root cells rely on phloem-derived water to grow [21••,23,24]. During xerobranching, this phloem source of water acts to co-mobilize ABA radially outward. Most ABA biosynthesis

enzymes are specifically expressed in phloem companion cells (PCC) [25]. Hence, PCC expression of the ABA biosynthetic machinery functions to couple unloading of water and ABA in the absence of an external water source. Elevated ABA levels in outer root tissues trigger the reversible closure of plasmodesmata (PD) [26]. This serves to block the inward symplastic movement of the branching signal, auxin (that activates LR "stem cells" in the pericycle), triggering a xero-branching response. Once root tips reconnect with an external water source, ABA levels are rapidly reduced in the outermost tissues, leading to the restoration of symplastic auxin transport via re-opened PD [21••,27,28]. It is also important to note here that ABACUS biosensors detected ∼300−400 nM of physiological ABA during xerobranching [21••]. This finding is particularly intriguing as it reveals that the estimated ABA levels required for LR suppression are considerably lower than those reported in earlier studies that utilized exogenous ABA treatments [19••]. This highlights the significance of physiological doses of ABA in regulating LR growth, providing valuable insights for further research in the field.

## Identifying *when*, *where*, and *how* changes in external water availability are sensed during hydropatterning and xerobranching

LR development is a multi-stage process that includes: (i) pericycle priming; (ii) pericycle founder cell (FC) specification; (iii) first divisions in pericycle FCs (LR initiation); (iv) lateral root primordium (LRP) formation; (v) LR emergence; (vi) LR meristem activation; and (vii) LR growth [29]. LR formation progresses through these successive stages as cells transit different developmental zones (apical meristem, basal meristem, elongation zone, and differentiation zone) in the primary root [30]. Environmental cues regulate a number of these LR developmental stages [31−33•]. Several studies report that a root's decision to branch (or not) in response to external water availability is made at a very early stage of LR development [34••]. Xerobranching blocks LRP formation before initiation [19••]. Similarly, hydropatterning also occurs before LR initiation [34••]. Imaging of LR initiation marker *gLBD16:GFP* exposed to a hydropatterning stimulus revealed differential expression on the side of the root in contact with moisture which was dependent on ARF7 and its SUMOylation status [20••]. The *gLBD16:GFP* reporter's asymmetric expression pattern was detected in root elongation zone pericycle cells as they undergo priming or FC specification. Despite these advances, the precise stage of LR development that pericycle cells exhibit maximum responsiveness to fluctuating external water availability remains to be established.

An alternate approach is to pinpoint the "water-sensing zone" in root tips. The concept of a "*competence zone*" for

hydropatterning was first proposed by Robbins and Dinneny [34••]. Experimental evidence in maize revealed that "the competence zone" showing maximum water responsiveness coincides with the actively growing root zone (between ∼4.0 and 5.5 mm from root tip). Although transcriptomic analysis of the root "competence zone" did not pinpoint specific water-sensing candidates, it is highly likely that key regulators involved in water-sensing such as hormones like ABA are active in the "competence zone." With the advent of highly sensitive hormone biosensors, it has become increasingly feasible to monitor *in vivo* hormone distribution patterns in real time and at cellular resolution [22••,35]. Recently, Mehra et al. [21••] employed the biosensor ABACUS2 to visualize ABA unloading following a xerobranching stimulus. Time course analysis of ABA distribution in different root zones revealed ABA unloading from protophloem in the basal meristem and early elongation zone. Interestingly, this root region contains the oscillation zone, the site for auxin-regulated LR priming [36,37]. This suggests that ABA targets the earliest stage of LR development (i.e. pericycle priming) during xerobranching. In agreement with these results, genetic complementation of loss-of-function *snrk2.2* mutant (disrupted in xerobranching) with functional *SnRK2.2* expressed under zone-specific promoters also demonstrated that basal meristem is a key zone for branching decisions during xerobranching [21••]. Thus, ABA signaling in the basal meristem and early elongation zone regulates xerobranching and most likely delineates the "water-sensing niche" in *Arabidopsis* root tips.

Until recently, little was known about how water availability is perceived by root tip tissues. A few studies suggest that root hairs may act as water stress sensors in plants [38]. Interestingly, during hydropatterning, root hairs are produced more profusely on the air side [18••]. Although root hairs may enhance water uptake [39,40•], there is no direct evidence to support their role in water-sensing. Robbins and Dinneny [34••] proposed that root cells in the "competence zone" are highly sensitive to water potential gradients. The authors observed that slight differences in root growth rate led to different water potential gradients in the "competence zone" and therefore proposed a "*sense-by-growth*" theory. Roots with higher growth rate establish strong water potential gradients at the root tip which leads to strongly hydro-patterned branching. Conversely, slower growing roots developed weaker water potential gradients and hence exhibited weaker hydropatterning. These observations suggest that differences in water potential gradients are sufficient to alter LR branching. However, how these differences in water potential gradients are initially perceived and ultimately translated into root branching responses remained elusive until very recently. Mehra et al. [21••] reported that in moist soil, water flows from the root surface into vascular tissues, but when this external water source is removed during xerobranching, water flows from the phloem out into growing root tissues. This reversal in water direction mobilizes phloem-derived ABA, which transiently closes PD, blocking the ability of auxin to initiate LR branching (see discussion above). Once root tips reconnect with an external water source, ABA levels rapidly reduce in the outermost tissues, leading to the restoration of symplastic auxin transport and LR formation. These dynamic regulatory events, coupling changes in hydraulic fluxes with hormone redistribution are termed "hydrosignaling," enabling plant roots to calibrate spatial root branching responses in soil environments featuring heterogeneous water availability. This hydraulic flux-based water-sensing mechanism relies on the ability of these hormone "hydrosignals" to be co-mobilized with water, acting as proxies for the direction of radial hydraulic flux and hence external water availability. By coupling hydraulic fluxes with redistribution of these hydrosignals, roots fine-tune their lateral branching pattern with external water availability.

## Lateral root formation in relation to humidity in air

As roots are primarily responsible for meeting a plants' water requirements, soil water availability (rather than above-ground humidity) is often considered more critical for shaping root architecture. However, an increasing body of evidence supports that shoot tissues actively control root architecture when experiencing water stress. Controlled humidity experiments revealed that a slight drop in foliar humidity can be sensed by plants irrespective of root water status [22••]. Genetic evidence coupled with ABACUS2 biosensor studies revealed that a drop in foliar humidity leads to significant rise in root ABA levels. Interestingly, shoot-derived ABA is transported down to actively growing root tissues in the elongation zone via phloem unloading. This shoot-derived ABA promotes primary root growth, enhancing foraging for soil water [22••,41]. Thus, shoot-derived ABA acts as a long-distance signal that regulates root responses to water stress. Reciprocal graft experiments, using wild-type and ABA-deficient mutants, have provided strong evidence for the critical role of shoot-derived ABA in regulating leaf responses to soil drying, such as stomatal closure. However, emerging evidence suggests that root-to-shoot ABA transport can also affect stomatal responses [42]. It is important to note that these responses are strongly dependent on environmental factors. For example, low humidity or salinity stress can enhance the effects of ABA transport from the roots to the shoots, resulting in decreased stomatal conductance, whereas these effects are diminished under control conditions [43,44].

Long-distance ABA transport through phloem also regulates LR development such as LR emergence [45]. In

rice, saturated foliar humidity (compared to normal air humidity) has been shown to enhance long-distance auxin transport which promotes LR number, density, and length [46••]. Notably, shoot-to-root auxin transport in this case was largely phloem dependent as treatment with N-1-naphthylphthalamic acid(NPA) (inhibitor of polar auxin transport) failed to produce any significant difference in shoot-to-root auxin transport [46••]. Phloem-mediated auxin transport offers an important advantage in terms of velocity (five times faster) as compared to polar auxin transport [46••]. Thus, phloem provides an instantaneous means of root-shoot systemic communication for locally experienced water stresses. Besides auxin and ABA, many other phytohormones such as gibberellins, jasmonates, cytokinin, and methyl salicylate are also transported through the phloem [47−51]. It would be interesting to explore whether these other hormones respond to foliar humidity to regulate root architecture.
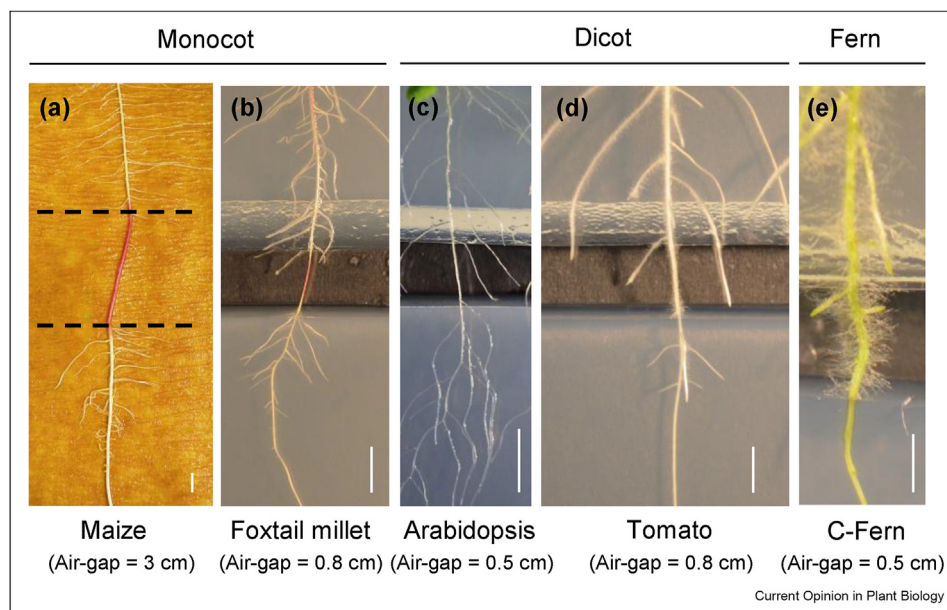
## Conclusions and perspectives

Water is a critical resource that often limits crop yields. At present, agricultural activities predominately consumes 70% of total abstracted freshwater resources [52]. Climate change and erratic rainfall patterns are predicted to worsen agricultural water scarcity, affecting 84% of croplands worldwide [53]. One promising approach for reducing the impact of water stress on food production is to engineer roots to be more efficient at water foraging. This could involve the development of root systems with specific traits that enable them to better access and utilize available water resources. For instance, a root system with fewer but longer LRs could be better equipped to forage for water across a wider area of soil [54]. Other desirable traits may include increased root depth, root hair density, and improved capacity for hydraulic redistribution [5]. By leveraging these specific root traits, breeders may be able to develop more resilient crops that can better withstand water stress and support sustainable food production in regions with limited water resources.

Further, it is crucial to gain a more nuanced understanding of the underlying genetic mechanisms that govern water-sensing and moisture-driven root adaptive responses. In this context, hydropatterning and xerobranching provide model systems to discover novel water-sensing mechanisms and water-deficit responses.

Root water adaptive responses such as hydropatterning and xerobranching are highly conserved across flowering plant (angiosperms) species [18••,19••,21••]. For example, monocots (such as maize, barley, and foxtail millet) and eudicots (e.g. tomato and *Arabidopsis*) exhibit xerobranching (Figure 2). Air-gap assays with ABA-deficient mutant roots in Arabidopsis, tomato, and maize have provided compelling evidence that ABA acts

**Figure 2**



| Monocot | | Dicot | | Fern |
|---|---|---|---|---|
| (a) | (b) | (c) | (d) | (e) |
| Maize | Foxtail millet | Arabidopsis | Tomato | C-Fern |
| (Air-gap = 3 cm) | (Air-gap = 0.8 cm) | (Air-gap = 0.5 cm) | (Air-gap = 0.8 cm) | (Air-gap = 0.5 cm) |

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Xerobranching is widely conserved root adaptive response. Monocots such as (**a**) maize and (**b**) foxtail millet exhibit xerobranching in paper-based and agar-based air-gap assays, respectively [21]. Eudicots such as (**c**) *Arabidopsis* and (**d**) tomato show xerobranching in agar-based air-gap assays. (**e**) Intriguingly, non-flowering vascular plants such as C-fern (*Ceratopteris richardii*) do not show xerobranching suggesting the absence of ABA-regulated root branching mechanisms in early land plants. Scale bar = 5 mm. Please note that in (**a**) root section between two dashed lines represents the region of root grown in air gap. Brown paper has been simply used for the purpose of background during imaging.

as a key hydrosignal, mediating the xerobranching response across a range of eudicot and monocot species [21••]. While these findings clearly highlight the functional importance of ABA during xerobranching in these model species, it is still necessary to investigate the conservation of the regulatory mechanisms acting downstream of ABA across different plant species. Interestingly, non-flowering vascular plants such as ferns do not appear to exhibit xerobranching (Figure 2), consistent with ABA signaling being recruited for water-stress-related adaptive roles in seed plants [55]. These observations suggest that angiosperms are better adapted to forage in heterogenous soil environments. In the future, it would be interesting to explore exactly when hydropatterning and xerobranching responses were developed during plant evolution.

While the co-transport of water and hormones in roots is an intriguing concept, direct evidence supporting this mechanism is still missing. One of the key challenges in studying this phenomenon is the difficulty in tracking the movement of water and hormones in live plant cells, particularly in roots. To overcome this challenge, researchers need to embrace new technologies that can visualize water fluxes at sub-cellular and sub-second resolutions. Techniques such as neutron tomography and Raman microspectroscopy hold great promise [56,57•]. When combined with hormone biosensors, such non-invasive imaging techniques can image water and hormone fluxes in root tissues simultaneously, which is vital to probe the hydrosignaling water-sensing mechanism. Furthermore, it is an intriguing idea to examine whether the concept of hydrosignaling extends to other ABA-related abiotic stresses such as heat, drought, and soil compaction. Given that these stresses also trigger soil water deficit and elicit comparable root adaptations [33•,58,59], it would not be surprising if they share a core fundamental signaling mechanism for regulating root responses. Hence, comprehending the genetic basis of water-sensing would significantly advance our efforts in creating crops that are resilient to a wide range of environmental stress in the future.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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