Abscisic acid controls root hydrotropism via a cortex-specific growth regulatory mechanism

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Plants can acclimate by using tropisms to link the direction of growth to environmental conditions. Hydrotropism allows roots to forage for water, a process known to depend on abscisic acid (ABA) but whose molecular and cellular basis remains unclear. Here, we show that hydrotropism still occurs in roots after laser ablation removed the meristem and root cap. Additionally, targeted expression studies revealed hydrotropism depends on the ABA signalling kinase, SnRK2.2, and the hydrotropism-specific MIZ1 both acting in elongation zone cortical cells. Conversely, hydrotropism is inhibited by preventing differential cell-length increases in the cortex, but not in other cell types. The cortex surrounds the endodermis, the major site of ABA accumulation in roots, and might function like a 'perimeter fence', sensing lateral movement of ABA. We hypothesize that differential water movement could induce a hormone response gradient that reorients root growth direction towards a water source.

Tropic responses represent important differential growth mechanisms roots employ to explore their surrounding soil environment efficiently. In general, a tropic response can be divided into several steps, comprising perception, signal transduction, and differential growth. All of these steps have been well characterized for gravitropism, where gravity sensing cells in the columella of the root cap generate a lateral auxin gradient, whilst adjacent lateral root cap cells transport auxin to epidermal cells in the elongation zone, thereby triggering the differential growth that drives bending[1-4]. In gravi-stimulated roots, the lateral auxin gradient is transported principally by AUX1 and PIN carriers[3-5].

Compared with gravitropism, the tropic response to asymmetric water availability, i.e., hydrotropism, has been far less studied. Previously, it was reported that surgical removal and ablation of the root cap reduces hydrotropic bending in pea[6-8] and *A. thaliana* roots[9], suggesting that the machinery for sensing moisture gradients resides in the root cap. It has also been reported that hydrotropic bending occurs due to differential growth in the elongation zone[7,10]. However unlike gravitropism, hydrotropism in *A. thaliana* is independent of AUX1 and PIN-mediated auxin transport[11,12]. Indeed, roots bend hydrotropically in the absence of any redistribution of auxin detectable by auxin-responsive reporters[13,14]. Instead, root hydrotropism requires signalling by the hormone abscisic acid (ABA)[12]. These findings imply that, compared to gravitropism, hydrotropism requires a distinct signalling mechanism.

The involvement of ABA in hydrotropism was initially suggested by aberrant responses in *A. thaliana* mutants deficient for ABA synthesis or response[12]. More recently, loss-of-function ABA receptor and response mutants that are insensitive or hypersensitive to ABA have been shown to be insensitive and hypersensitive to a hydrotropic stimulus, respectively[15]. In addition, hydrotropism in *A. thaliana* roots requires a gene called *MIZU-KUSSEI1* (*MIZ1*)[16], which is upregulated by application of 10 μM ABA[17]. Despite *miz1* roots being oblivious to water potential gradients, they nevertheless bend like wild type in response to gravity[16]. The *MIZ1* sequence contains a DUF617 domain that is conserved among the genomes of terrestrial plants, but absent in algae and animals, suggesting a role for hydrotropism in the

evolution of land plants[16]. A functional *MIZ1:MIZ1-GFP* fusion protein is expressed in lateral root cap cells as well as cortex and epidermis cells in the meristem and elongation zone[17,18]. However it is unclear whether this broad expression pattern is necessary for MIZ1's function in hydrotropism or whether ABA signal transduction components have to be expressed in specific root tip tissues for a hydrotropic response. The present study describes a series of experiments in *A. thaliana* designed to identify the root tissues essential for a hydrotropic response. We report that MIZ1 and a key ABA signal-transduction component SnRK2.2 expressed specifically in the root cortex are sufficient to drive hydrotropism, and conversely that hydrotropism is blocked by inhibiting the ability of specifically the cortex to execute a differential growth response. Our results support a re-evaluation of hydrotropic signalling, revealing the importance of the cortex and the elongation zone for signal perception as well as bending.

Results

The root meristem and columella are dispensable for hydrotropism

To uncover which root cell types and zones are required during a hydrotropic response in *A. thaliana* roots, we ablated cells using a femtosecond laser. Successful ablation of the columella cells was confirmed by propidium iodide staining of root tissues (Fig. 1a, b) and hydro- and gravitropism assays performed as described previously[11,16] (for details on hydrotropism assays, see also Supplementary Fig. 1). Whilst columella ablation successfully inhibited the gravitropic response as previously reported[1], it did not inhibit the hydrotropic response (Fig. 1c, e). Furthermore, the ablated roots elongated at an equivalent rate as the intact roots throughout both gravitropism and hydrotropism assays (Fig. 1d, f). Even when the ablation encompassed essentially the entire meristem, hydrotropism was scarcely affected (Supplementary Fig. 2). When seedlings with ablated root cap or meristem were placed in an assay system that lacked the moisture gradient, ablated roots responded in the

same way as intact roots, with only minimal bending, which showed that laser ablation does not lead to unspecific, wounding associated bending responses (Supplementary Fig. 2). Because previous experiments with laser ablation implicated the columella[9], the experiments were repeated in the split agar-sorbitol system, with manual excision of the distal region of the root tip (~250 µm). In this system, control root tips bend 30° or more by 10 hours after transfer, but do not bend detectably when the lower medium lacks sorbitol (Fig. 2b). As with laser ablation, hydrotropic bending, though more variable, was unaffected on average in roots without a columella and meristem and only happened in response to a water potential gradient (Supplementary Fig. 2). We suggest the discrepancy arises from the earlier experiments having been run under adverse conditions as indicated by the roots in the previous report growing more than an order of magnitude more slowly than those used here. We conclude that water potential gradients are sensed as well as responded to within the elongation zone.

Root hydrotropism depends on the ABA signalling component SnRK2.2ABA represents a critical signal for numerous plant abiotic stress responses[19] including root hydrotropism. ABA responses are mediated by a negative regulatory signalling module involving soluble receptors of the START-domain superfamily (PYR/PYL/RCARs), clade A type 2C protein phosphatases (PP2Cs) and subclass III Snf1-related kinases (SnRK2s)[19]. ABA binds to PYR1/PYL/RCAR, which induces a conformational change that allows the receptor proteins to bind to, and thereby inhibit, PP2Cs[20,21]. PP2Cs dephosphorylate SnRK2s, suppressing their activity; thus SnRK2 activity increases in the presence of ABA due to PP2Cs being bound to the PYR1/PYL/RCAR ABA receptors[22]. When active, the SnRK2s phosphorylate transcription factors and other downstream targets[19,22].

To investigate how ABA controls hydrotropism, we characterised a double mutant lacking the ABA signalling kinases SnRK2.2 and SnRK2.3[23]. Although retaining some ABA responsiveness, this double mutant was selected for experiments because, in contrast to most mutants in ABA perception, it is neither dwarfed nor wilty. We initially assayed

hydrotropism in a split agar-based system. Hydrotropism in the *snrk2.2 snrk2.3* double mutant was strongly attenuated, but was restored in the *snrk2.2 snrk2.3* double mutant expressing the *SnRK2.2* gene under the control of its own promoter (Fig. 2b). Identical results were obtained using a moisture gradient in air hydrotropic assay (Supplementary Fig. 5). Hence, the SnRK2.2 kinase appears to be required for hydrotropism.

As the *snrk2.2 snrk2.3* double mutant had slightly shorter roots and a reduced growth rate compared to wild type (Supplementary Fig. 3), we compared the growth rates of the double mutant on half strength Murashige and Skoog (MS) medium and hydrotropism plates and found them to be comparable (Supplementary Fig. 3), ruling out hypersensitivity of the *snrk2.2 snrk2.3* double mutant to sorbitol. In addition, we performed split agar hydrotropism assays with younger wild type seedlings to assess whether a reduction in tip angle was caused simply by a reduced root growth rate. Roots bent with similar kinetics despite differences in length and growth rate, indicating that hydrotropic bending is not proportional to root growth rate (Supplementary Fig. 3).

Hydrotropism requires SnRK2.2 signalling only in the root cortex

To gain insight into the tissue specificity of hydrotropism, we created a translational GFP fusion to the *SnRK2.2* genomic sequence and expressed the reporter in the *snrk2.2 snrk2.3* double mutant background. In the resulting lines, roots regained wild type sensitivity to 10 µM ABA (Supplementary Fig. 4) and bent hydrotropically in the moisture gradient in air assay (but not the split agar assay) (Supplementary Fig. 4). We assume that the differences in hydrotropic response obtained using the different assays could be due to the moisture in air gradient providing a steeper water potential gradient than the split agar assays. Hence, the translational reporter appeared partially functional. Using confocal imaging SnRK2.2-GFP signal was detected in nuclear and cytoplasmic compartments, consistent with the sub-cellular localisation of its known regulatory targets[24,25]. Moreover, at the tissue scale, *SnRK2.2:SnRK2.2-GFP* was ubiquitously expressed throughout the root apex, including root cap and elongation zone tissues (Fig. 2c).

To pinpoint the root tissue where SnRK2.2 is required during a hydrotropic response, we expressed the *SnRK2.2* genomic sequence in the *snrk2.2 snrk2.3* double mutant background using a suite of tissue- and zone-specific promoters. *SnRK2.2* expressed under the control of the meristem and transition zone-specific *RCH1* promoter[26] complemented the *snrk2.2 snrk2.3* hydrotropic defect (Fig. 2d). Surprisingly, rescue failed when *SnRK2.2* was expressed specifically in the root cap (SOMBRERO[27], *SMB:SnRK2.2*), epidermis and lateral root cap (WEREWOLF[28], *WER:SnRK2.2*), or endodermis (SCARECROW[29], *SCR:SnRK2.2*) (Fig. 2d). In contrast, double mutant roots bent hydrotropically as the wild type when expressing *SnRK2.2* in just the cortex (Co2[30], *Co2:SnRK2.2*) (Fig. 2d). *SnRK2.2* expression levels in the *Co2:SnRK2.2* line were low in comparison to non-rescuing epidermal, lateral root cap or endodermal driven lines, demonstrating that mutant rescue is not simply a dose effect (Supplementary Fig. 5). In addition, we confirmed the hydrotropism response of the *Co2:SnRK2.2* line using the moisture in air gradient assay (Supplementary Fig. 5). Hence, root hydrotropism appears to require the ABA response machinery specifically in the cortex.

Cortex-specific MIZ1 expression rescues the miz1 hydrotropic defect

To independently assess tissue specificity for the hydrotropic response, we determined which tissues require MIZ1, a protein previously identified as essential for hydrotropism and localized to cortex, epidermis, and lateral root cap[18]. We used various promoters to express MIZ1-GFP in specific tissues in the *miz1* background (Supplementary Fig.6). When constructs that included the *MIZ1* terminator were used, MIZ1-GFP expression driven by *RCH1* was detected in the meristem, by *SMB* in the root cap, by *SCR* in the endodermis, and by *COR* and by *Co2* in the cortex as expected[26,27,29,31] (Supplementary Fig. 6). Compared to *SCR* or *Co2*, the *COR* promoter drove MIZ1-GFP expression farther into the elongation zone. In contrast, the *WER* promoter drove MIZ1-GFP expression not only in the epidermis and lateral root cap, as expected[28], but also in the cortex. Like *COR*, expression from *WER* continued well into the elongation zone. Note that none of these constructs altered root growth rate appreciably (Supplementary Fig. 6).

192 Using the tissue-specific MIZ1-GFP constructs, we assayed hydrotropism using the
193 moisture gradient in air method that gave approximately 80° bending after 12 hours. As
194 expected, hydrotropic bending was fully rescued by expressing MIZ1-GFP under the *MIZ1*
195 promoter (Supplementary Fig. 6). In contrast, little or no hydrotropic curvature resulted when
196 MIZ1-GFP was expressed in root cap (*SMB*), in endodermis (*SCR*), or in the meristem
197 (*RCH1*). Mutant complementation was only partial using *Co2* to drive MIZ1-GFP expression,
198 but rescue was complete employing either *WER* or *COR* promoters, revealing a requirement
199 for MIZ1 in the elongation zone (Supplementary Fig. 6). Mutant rescue was also complete
200 when MIZ1-GFP expression was driven by the *PIN2* promoter, which like *WER*, drives
201 expression in lateral root cap, epidermis and cortex, which for the latter tissues continues
202 well into the elongation zone (Fig. 2 e-g). Finally, when *WER*-driven expression was
203 removed from the cortex, which happened if the native MIZ1 terminator was replaced by a
204 terminator from a heat-shock protein (HSP), *miz1* rescue essentially failed (Fig. 2e,g).
205 Identical responses for *WER*- and *PIN2*-driven MIZ1-GFP expression were obtained using
206 the split agar assay (Supplementary Fig. 5). Taken together, these results show that
207 hydrotropic bending requires MIZ1 expression specifically in the root cortex and that the
208 expression domain must span at least part of the elongation zone. This conclusion is
209 consistent with laser ablation and SnRK2.2 expression experiments that, when taken
210 collectively, questions the functional importance of root cap and meristem tissues during a
211 hydrotropic response.

212

213 ***Low levels of ABA promote root cell elongation***

214 Root cortical cells abut the endodermis (Fig. 2a), the recently reported site of ABA
215 accumulation in roots[32]. Hence, ABA response machinery in the cortex would be ideally
216 positioned to function like a 'perimeter fence', sensing any lateral movement of ABA from the
217 endodermis into outer root tissues, and presumably triggering growth responses. Whilst high
218 ABA levels inhibit root growth[23], low levels of this hormone are required to sustain root

219 elongation at low water potential[33-35]. To understand the ABA-dependent growth mechanism
220 underlying hydrotropism, we next investigated the effect of low doses of ABA on root growth.
221 Transferring seedlings onto 100 nM ABA stimulated root growth rate in the wild type but had
222 minimal effect on *snrk2.2 snrk2.3* (Fig. 3a, Supplementary Fig. 7). Comparing meristem and
223 elongation zone of those roots, 100 nM ABA appeared to change neither the length nor cell
224 number within the meristem but significantly increased elongation-zone length in wild type
225 and *Co2:SnRK2.2* complementation lines (Fig. 3c, Supplementary Fig. 7). The increased
226 root growth rate was accompanied by both an increased rate of cell production and an
227 increased mature cell length (Supplementary Fig. 7, Fig. 3b). Taken together, these data
228 suggest that low doses of ABA in these non-stressed plants stimulate rates of cell division
229 and elemental elongation.
230 As root hydrotropism represents an ABA-dependent differential growth response
231 (Fig. 2), we next addressed what size a hypothetical ABA gradient would have to be to drive
232 root bending. To calculate this, we first used image analysis to determine growth kinetics
233 during a hydrotropic response (Fig. 4a). Root tips bending in response to a water-potential
234 gradient initiated and maintained organ curvature in transition and elongation zones (Fig.
235 4b). Based on the observed growth promotion during the hydrotropic response, as well as on
236 the above results with 100 nM ABA, we calculated the minimum size of an ABA
237 concentration difference between cortical cells on either side of the root required for root
238 bending to be 4 nM (see Methods and Supplementary Note 1 for further details). Such a
239 small difference is effective because of the thinness of the root combined with the large size
240 of its elemental elongation rate. Detecting such a hypothetical ABA concentration difference
241 is currently beyond the limits of the hormone responsive reporters[36], helping to explain our
242 inability to detect changes in the hormone's tissue distribution following a hydrotropic
243 stimulus (Supplementary Fig. 8).
244
245 ***Hydrotropism is driven by differential cortical cell expansion***

Cells in the elongation zone, along with expanding anisotropically, also undergo a process termed endoreplication (i.e. rounds of DNA replication without cell division)[37]. To examine tissue specificity in the promotion of root growth by ABA, we analysed nuclear ploidy of specific tissues by performing cell sorting and DNA-content measurements. Significantly, 100 nM ABA stimulated endoreplication specifically in root cortical cells, as evidenced by the increased fraction of 8C nuclei at the expense of 4C (Fig. 3e). In contrast, 100 nM ABA had little if any effect on endoreplication in either atrichoblast or endodermal cells (Fig. 3d, f). Hence, ABA appears to specifically trigger changes in cell cycle machinery in just the cortex, consistent with our 'perimeter-fence' model.

One might question whether an asymmetry of growth-promoting mechanisms within a single tissue could provide sufficient mechanical leverage to trigger root curvature. To explore whether such changes in the dynamics of cortical cells are sufficient to drive root bending during hydrotropism, we developed a mathematical model (see Methods and Supplementary Note 1), taking advantage of recent theoretical work that successfully recapitulates the root's growth rate profile by ascribing distinct mechanical contributions to the various tissues[38]. For a short period following exposure to the water potential gradient, a small group of cortical cells on the dry side of the root were treated as undergoing early entry into rapid elongation, changing their mechanical properties to be the same as cells in the elongation zone. This differential elongation, coupled with the cell adhesion typical for plant cells, caused the root midline to bend in this region. The predicted evolution of root tip angle, and the curvature of the root midline, simulated bending at a similar rate to that observed experimentally (Fig. 4c). Hence, asymmetric elongation in the root cortex appears to be a plausible mechanism to drive hydrotropic bending. Taken together, these experimental data and model simulations support our hypothesis that hydrotropism is driven by differential cortical cell expansion.

If hydrotropic bending is driven by an asymmetric expansion of cortical cells in the elongation zone, we reasoned that hydrotropism could be blocked by interfering with the orderly progression of cells through the growth zone. To test this, we took advantage of the

overexpression phenotype of the cyclin-dependent kinase inhibitor SIAMESE (SIM), in which cell division is inhibited and endoreplication is stimulated[39]. We used a GAL4-VP16 driven transactivation system to co-express SIM and a nuclear-localised GFP marker specifically in either epidermis, cortex, or endodermis. In each case, root meristem cells overexpressing SIM were enlarged (Fig. 5a-c) but cells in adjacent tissues were not detectably affected and were of similar length to cells of roots expressing only the *GFP* marker (Fig. 5d-f). Next, we tested each tissue-specific *SIM* over-expressing line for hydrotropism. Roots over-expressing *SIM* in root epidermis or endodermis bent indistinguishably from the parental lines, whereas *SIM* overexpression in the cortex blocked root hydrotropic bending (Fig. 5g). In contrast, roots of every *SIM* overexpression line retained a wild-type response to gravity (Fig. 5h), revealing that *SIM* overexpression in the cortex did not simply prevent all differential root growth processes.

Discussion

We report that root tropic responses to gravity and water are driven by distinct molecular and tissue-based mechanisms. In the case of gravity, root re-orientation is sensed by columella cells at the root tip[1], triggering the formation of a lateral auxin gradient across the root with higher concentrations on the lower side of the root[40,41]. This auxin gradient is then transported via the lateral root cap to the elongation zone[3] where it elicits downward root bending by stimulating elongation on the upper side and inhibiting elongation on the lower-side[42]. In contrast, laser ablation experiments demonstrate here that neither meristem, lateral root cap nor columella cells are required to perceive a water potential gradient (Fig. 1). Hence, unlike its role in root gravitropism, the elongation zone performs a dual function during a hydrotropic response both to sense a water potential gradient and subsequently undergo differential growth.

We also confirm that root hydrotropism employs the hormone ABA and that the ABA signal transduction components SnRK2.2 and SnRK2.3 play a key role regulating root re-

orientation. Surprisingly, targeted *SnRK2.2* expression studies in *snrk2.2 snrk2.3* (Fig. 2) revealed the critical importance during hydrotropism of ABA response machinery just in the cortex. The importance of this specific root tissue for hydrotropism was further supported by the response depending on cortical expression of *MIZ1* (Fig. 2). Taken together, our results demonstrate that ABA and *MIZ1* responses in the cortex of the root elongation zone play a central role in hydrotropic response of *A. thaliana* roots (Fig. 6). Hence, root gravitropic and hydrotropic responses are driven by distinct signals and tissue-based mechanisms. Consistent with our conclusion, Krieger *et al.*[43] recently described the opposing effect of ROS on these tropic responses and the distinct positions at which roots bend during gravitropic and hydrotropic responses.

A key question for hydrotropic research is to understand how a modest gradient in water potential across the root is perceived (and presumably amplified) into a growth response. Mechanosensing, differential movement of water, ions or signalling molecules all represent likely candidates[44]. In the case of ABA, localisation of its biosynthesis genes in the vasculature[45], endodermal localisation of its accumulation in roots[32] and our mapping of ABA response to the adjacent cortical tissue leads us to propose a 'perimeter fence model' (Fig. 6). We hypothesise that roots regulate hydrotropism by linking asymmetric ABA distribution with differential water uptake into the root elongation zone. Differential water movement could carry ABA from inner tissues to cortical cells (on the low water potential side) or away from cortical cells towards the stele (on the high water potential side) as described in other plant species[46]. The resulting radial ABA concentration difference could then promote differential cortical expansion, triggering the root to re-orient its growth direction towards a source of higher water potential. Testing of this hypothesis awaits the development of ABA sensors/detection methods more sensitive than those currently available.

METHODS

Ablation of root-tip cells using laser-microscopy systems

For micro-beam laser irradiation, 4-day-old seedlings were aligned in a micro-chamber comprising two glass coverslips (25×60 mm^2 and 24×24 mm^2, Matsunami) and a seal (TaKaRa Slide Seal for in situ PCR, Takara Bio). The micro-chamber was filled with low-melting agar (half-strength MS medium, 0.4% (w/v) sucrose [Wako Pure Chemical Industries], 0.2% (w/v) low-melting agarose [SeaPlaque; FMC BioProducts]). These samples were put on the stage of a microscope (Nikon ECLIPSE TiE, Nikon) and irradiated with a N_2 pulsed micro-beam laser through Coumarin 440 with an averaged power of 330 kW for a 3 to 5 nanosecond pulse (MicroPoint PIJ-3-1; Andor Technology). For femtosecond laser irradiation, seedlings were placed on the half-strength MS medium on a glass slide. Amplified femtosecond laser pulses from a re-generatively amplified Ti:sapphire femtosecond laser system (IFRIT; 780 ± 5 nm, 230 fs, < 1 mJ/pulse, 1 kHz, Cyber Laser Inc.) were focused onto root cap cells through a 10x objective lens (UPlanSApo NA 0.4, Olympus) on a confocal laser scanning microscope (FV1000-BX51, Olympus). Laser pulses (200) were detected with a mechanical shutter (gate time: 200 ms) and delivered to the sample. The laser pulse was collimated by dual convex lenses before the microscope, and the laser focal point was tuned to the plane of the image. The diameter of the laser focal point, which is consistent with the beam waist, was about 1 μm. A neutral density filter was put between the laser and microscope and used to tune the laser pulse energy to around 400 nJ/pulse, which is about 4 times larger than the threshold energy for cavitation bubble generation in water (100 nJ/pulse). Laser-ablated seedlings were incubated on half-strength MS medium for 1 h in a vertical position before performing further assays.

Root tropism and growth assays

The hydrotropism assay shown in Fig. 1 and Supplementary Fig. 5b-e were performed as described previously using a split-agar system with 812 mM sorbitol[11]. Gravitropism assays shown in Fig. 1 and Supplementary Fig. 2 were performed using 1% agar medium with or without 0.5x MS medium as described previously[16]. Hydrotropism assays shown in Fig. 2g and Supplementary Fig. 2g-r, 4e-f, 5 f-g and 6 were performed using a moisture gradient in

air as described previously[11]. Four-day-old seedlings were used for all tropism assays described above.

Hydrotropism assays shown in Fig. 2b and d, 4 and 5 and Supplementary Fig. 2s, 3, 4d and 8 were performed as previously described[47] using 5-day-old seedlings in a split-agar system with 400 mM sorbitol.

For gravitropism assays shown in Fig. 5, 5-day-old seedlings were transferred to new plates containing 0.5x MS medium with 1% agar. After acclimatisation for 2 hours in the controlled environment room, plates were rotated by 90°. Images of seedlings were acquired using an automated imaging platform[48] and root tip angle and length determined using the Fiji image processing package (http://fiji.sc/Fiji).

For assessing root growth response to ABA, 5-day-old seedlings were transferred to new plates containing 0.5x MS medium with the indicated amount of ABA (Sigma). To determine meristem cell number and length, longitudinal images of root tips clearly showing the cortex cell file were taken with a confocal laser scanning microscope, using propidium iodide to stain cell walls. Starting from the QC, the length of individual cortex cells was determined using the Cell-o-Tape macro[49] for Fiji. The mean length of meristem cells was calculated using ten cells from the rapid amplifying region of the meristem (cells 10-19 counting shootward from the QC), and the end of the meristem deemed to have been reached when consecutive cells had reached and/or exceeded the mean length by two. Cell production rates were calculated as previously described[50].

Modelling ABA and water potential gradients

Changes in growth rate under the addition of 100 nM ABA (Fig. 3a) and measurements of root tip angle change (Fig. 2b) were used to estimate required differences in ABA concentration between the cortical cells on the wet and dry side of the root.

From geometrical considerations (see Supplementary Note 1, Section 1 for further details), the difference in growth rates between the cortical cells on the wet and dry side of the root needed to generate the observed rate of change of tip angle over the first hour of the

hydrotropism assay was calculated. Under the assumption that the effect of ABA on growth is linear in the concentration range from 0 to 100 nM, the necessary difference in ABA concentration between cortical cells on the wet and dry sides was calculated to be 4 nM.

The water potential gradient experienced by the root was estimated by the solution of the diffusion equation for sorbitol in agar, combined with the van 't Hoff equation which relates water potential and solute concentration. From the geometry of the hydrotropism assay, away from the edges of the agar plate, the resulting water potential distribution can be treated as a function purely of the distance from the cut, namely

$$\Phi = -A \ \operatorname{erfc}\left(\frac{x}{2\sqrt{Dt}}\right)$$

Here x is distance from the cut, t is time after sorbitol is added to the plate, $D = 10^{-5} cm^2/hr$ and $A = 1MPa$. Using the distance of the initial placement of the root tip from the cut (2.5 mm) as an estimate of the distance of the root centre line from the cut and 100 μm as an estimate of the root diameter, the relative difference in water potential across the root was estimated through the evaluation of the water potential distribution at $x = 2.5 \pm 0.05$ mm.

Modelling root bending

A mechanical model has been developed to describe hydrotropism-associated root bending. The approach[38] exploits the large aspect ratio of the root, which allows a relatively simple description of bending in terms of the stretch and curvature of the root midline. A viscoplastic constitutive relation is adopted (viscous flow where the yield stress is exceeded), with the yield stress of cortical cells on the dry side of the root modified in response to a hydrotropic stimulus; the resulting partial differential equations for the dependence of midline stretch and curvature in terms of time and arc length are solved numerically by a finite-difference approach. Further details are given in the Supplementary Note 1, Section 2**.**

Note: Supplementary Information (Supplementary Methods, Supplementary Figures and two Supplementary Notes) is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

D.D., L.P., A.K., J.F., V.B., R.B., R.A., T.N., S.H., T.-W.B., Y.M., D.M.W., S.T., C.J.S. performed experimental work and data analysis and mathematical modelling. D.M.W., M.R.O., L.R.B., R.D., O.J., J.R.K., S.J.M., J.R., R.B., J.D., P.L.R., T.I.B., T.P., L.D.V., N.F., Y.M., A.N., Y.H., H.T. and M.J.B. oversaw project planning and discussed experimental results and modelling simulations. D.D., L.P., A.K., N.F., Y.M., T.I.B., H.T. and M.J.B. wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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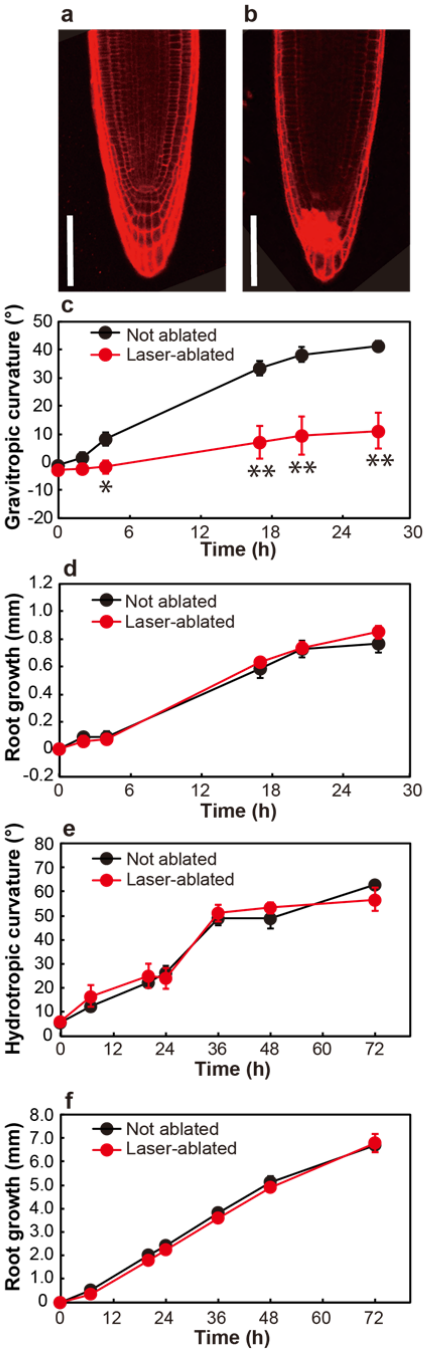


Figure 1 Laser ablation of columella cells affects the gravitropic but not the hydrotropic response of roots

Confocal fluorescence micrograph of propidium iodide-stained primary root tips before (**a**) and after (**b**) femtosecond-laser ablation of the columella, scale bar = 100 µm. Time-course study of root gravitropic curvature (**c**) and root growth (**d**). In c, 0° equals horizontal. Time-course study of root hydrotropic curvature (**e**) and root growth (**f**). In e, 0° equals vertical. The hydrotropism assay was performed using the split agar system with 812 mM sorbitol. Values are mean ± SEM of a representative experiment, $n = 3 – 6$, from three independent experiments. Asterisks indicate statistically significant differences (*$p < 0.05$, **$p < 0.01$, Student's t-test).

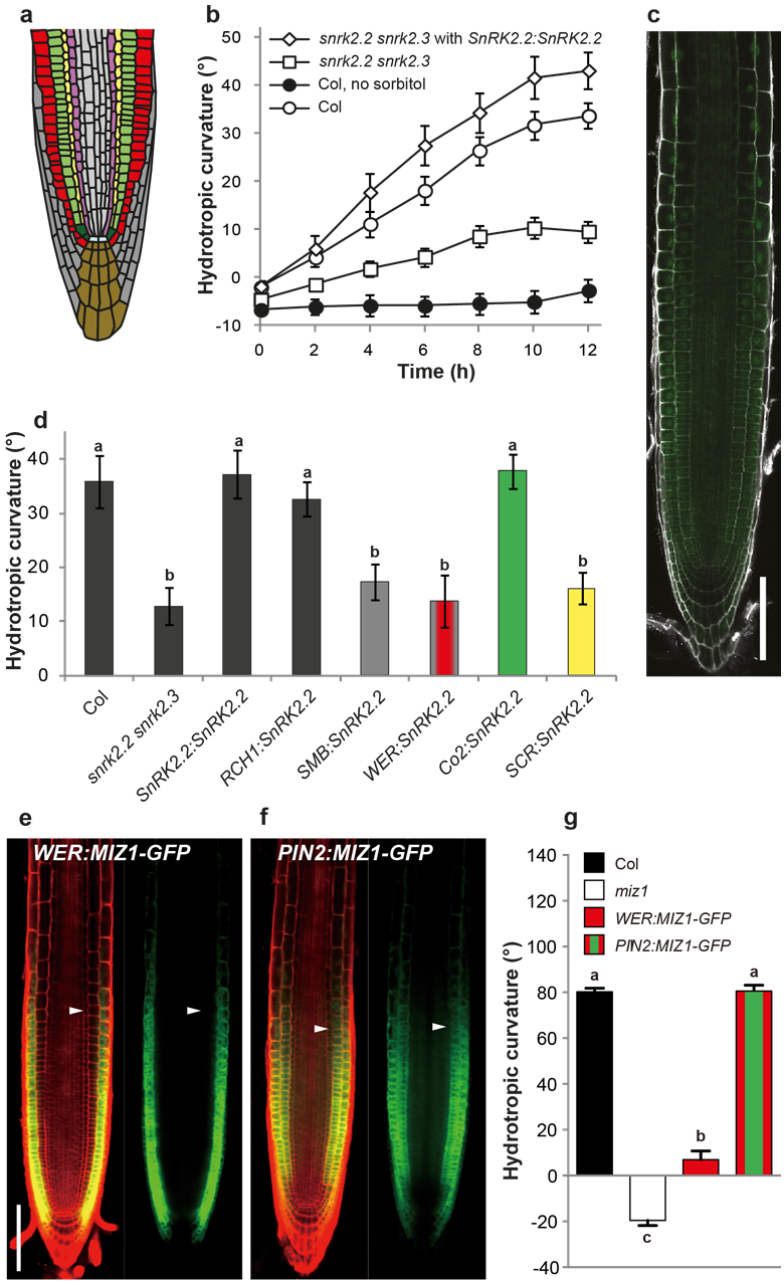


Figure 2 ABA signalling in the cortex is crucial for root hydrotropism

a Schematic drawing indicating tissues in the root tip, grey: lateral root cap, red: epidermis, green: cortex, yellow: endodermis. **b** Kinetics of hydrotropic curvature after transferring seedlings to split agar plates with 400 mM sorbitol. Values are mean ± SEM, *n* = 29 - 40. **c** Expression of SnRK2.2:SnRK2.2-GFP in the root tip, scale bar = 100 µm. **d** Hydrotropic curvature 12 h after transfer to split agar plates with 400 mM sorbitol. Values are mean ± SEM, *n* = 24-31. Different letters indicate statistically significant differences ($p < 0.05$, Fisher's LSD). **e, f** Expression pattern of MIZ1-GFP fusion protein under control of (e) the *WER* and (f) *PIN2* promoters with *HSP* terminator. Left-hand image shows an over lay of fluorescence from GFP (green) and PI (red), right-hand image shows GFP only. Arrowhead indicates the approximate rootward boundary of the elongation zone, scale bar = 100 µm. **g** Hydrotropic curvature 12 h after transfer of seedlings to the moisture gradient in air assay system. Values are mean ± SEM of three independent experiments, *n* = 35-44. Different letters indicate statistically significant differences ($p < 0.05$, Tukey HSD test).

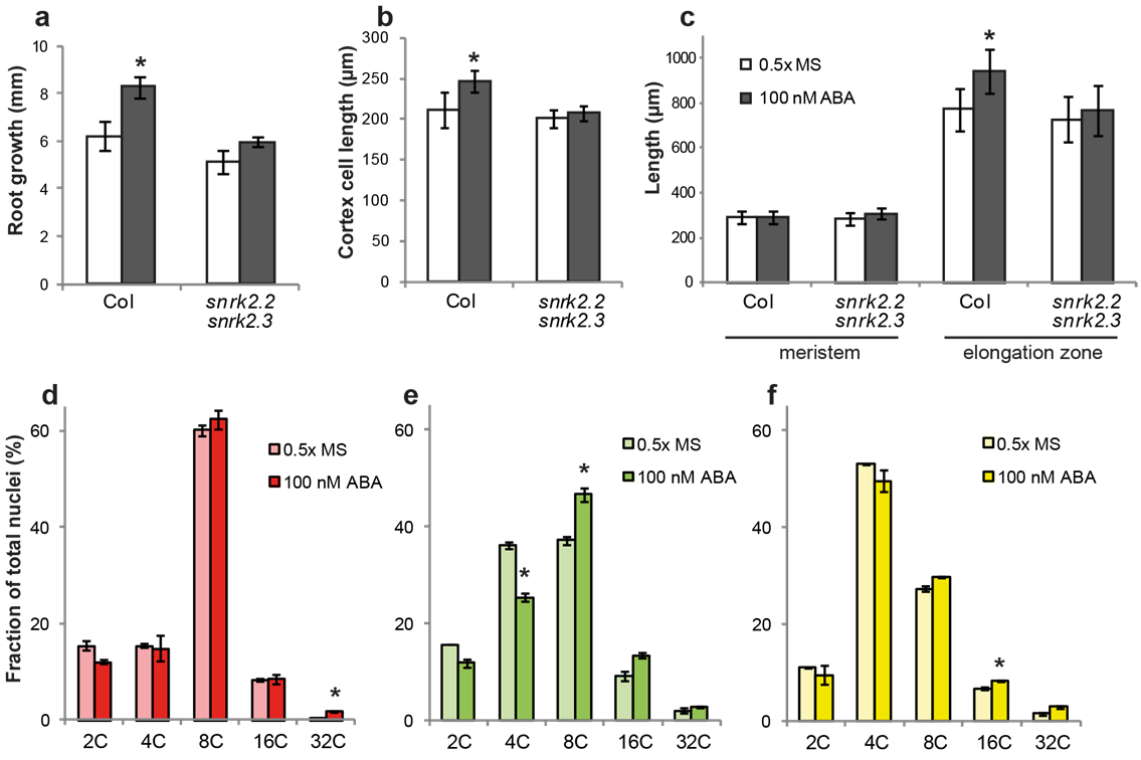


Figure 3 Root growth and cortical endoreplication are induced by low levels of ABA
a – c Root growth and histology. **a** Root growth without (0.5x MS) or with 100 nM ABA 24 h after transfer. Values are mean of three experiments ± SD, *n* = 12-40. **b**, **c** Seedlings treated as in (a) were stained with propidium iodide and images taken with a confocal microscope. **b** Cell length of mature cortex cells. Values are mean ± SD, *n* = 18-47 cells for 10 roots per line and treatment. **c** Meristem length was determined using Cell-o-Tape and an arithmetic method to determine the meristem end. Elongation zone length was determined by measuring the distance from the end of the lateral root cap until the first root hair bulge. Values are mean ± SD, *n* = 11-28. For a - c: * statistically significant different ($p < 0.01$, Student's *t*-test). **e – f** Endoreplication. DNA content of nuclei isolated from (d) the epidermis (non-hair cells), (e) cortex and (f) endodermis of roots treated for 24 h without (0.5x MS, light bars) or with 100nM ABA (dark bars). Values are mean ± SD. For d - f: * statistically significant different ($p < 0.05$, Student's *t*-test).

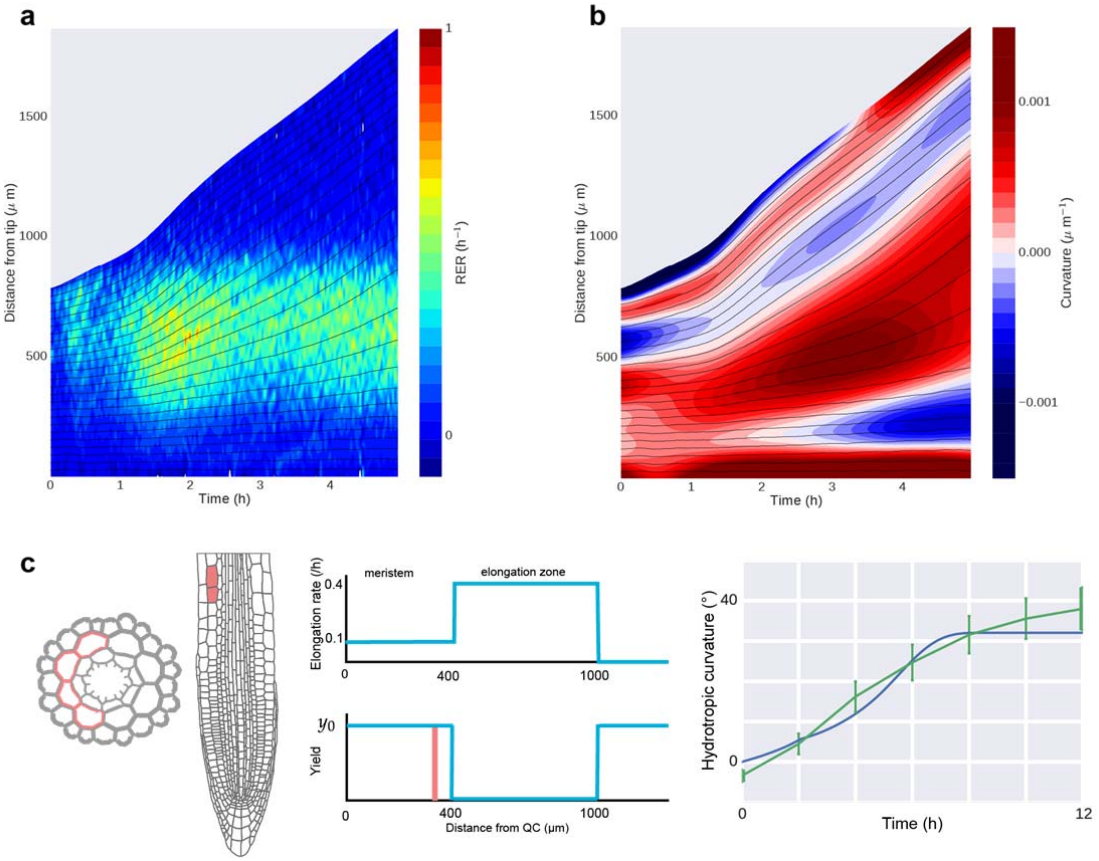


Figure 4 Changes in root growth in response to hydrotropism

a Relative elongation rate and **b** curvature of a hydrotropically bending root during the first 5 h after transfer to a split agar plate with 400 mM sorbitol. Solid lines show the trajectories of points equally spaced at time zero. Representative data from four independent repeats shown. **c** Modelling the hydrotropism response: The transition between the meristem and elongation zone is marked by a drop in yield stress leading to a rise in elongation rate (centre); the large yield stress y_0 in the meristem inhibits cell expansion; cortical cells on the dry side of the root (pink in transverse and axial cross-sections, left) enter elongation early for the first 2 h, with asymmetric softening across the root generating a bend. Simulated (blue) and experimental (green) hydrotropic curvature profiles are compared (right).

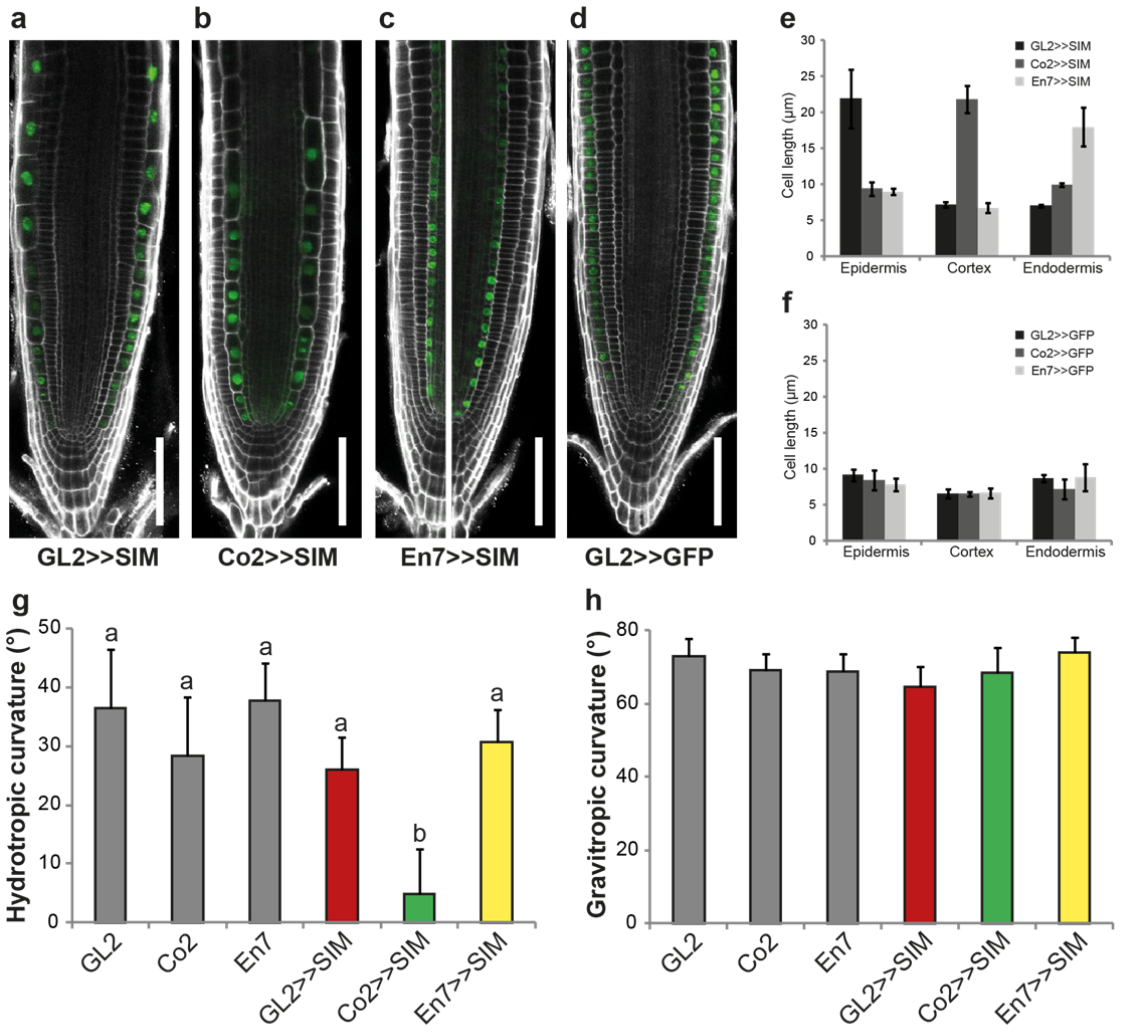
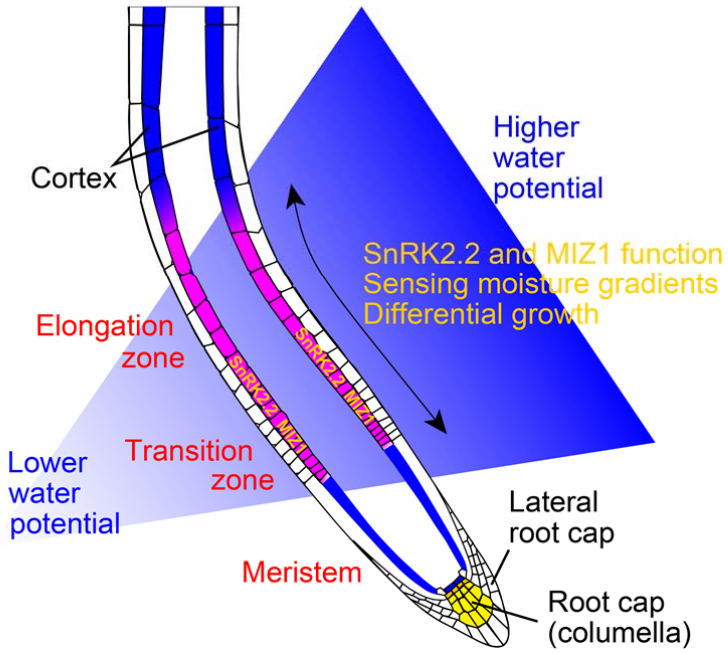


Figure 5 Inhibition of differential cell elongation in the cortex prevents hydrotropism but not gravitropism

a – d Confocal images of root tips co-expressing *SIM* and *NLS-GFP* (green) in (a) epidermis, (b) cortex, (c) endodermis, or (d) expressing *NLS-GFP* in epidermis. Cell walls were stained with propidium iodide (white). In c, two images of the same root are shown, for better visualization of the endodermis cell file. Scale bars for a - d = 100 µm. **e, f** Quantification of cell lengths for epidermis, cortex and endodermis files in the meristem. Values are mean ± SD, *n* = 7-52 cells from three plants for each line and tissue. **g** Hydrotropic curvature 10 h after transfer to split agar plates with 400 mM sorbitol. Values are mean ± 2x SEM, *n* = 14-15 for parental lines (GL2, Co2, En7) and *n* = 56 for *SIM* expression lines (GL2>>SIM, Co2>>SIM, En7>>SIM). Different letters indicate statistically significant differences ($p < 0.05$, Fisher's LSD). **h** Gravitropic curvature 8 h after plates were rotated by 90°. Values are mean ± 2x SEM, *n* = 30-31.



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674 **Figure 6 Conceptual model for root hydrotropism**
675 SnRK2.2 and MIZ1 expression in cortex cells of the transition and elongation zone are required to
676 mediate the ABA-dependent differential growth response to a water potential gradient. Perception of
677 the water potential gradient does not require tissues in the root cap or meristem, but takes place in
678 the transition and elongation zones where the differential growth response occurs.
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