1 **Hydrotropism – How Roots Search for Water**

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3 Daniela Dietrich

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5 Centre for Plant Integrative Biology and Plant & Crop Sciences, School of

6 Biosciences, University of Nottingham, Nottingham, LE12 5RD, UK

7 daniela.dietrich@nottingham.ac.uk

8 Tel: 01159516108

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17 **Hydrotropism – How Roots Search for Water**

18

19 **Abstract**

20 Fresh water is an increasingly scarce resource for agriculture. Plant roots mediate

21 water uptake from the soil and have developed a number of adaptive traits like

22 hydrotropism to aid water foraging. Hydrotropism modifies root growth to respond to

23 a water potential gradient in soil and grow towards areas with a higher moisture

24 content. Abscisic acid (ABA) and a small number of genes, including ABA signal

25 transducers, MIZ2/GNOM and the hydrotropism-specific MIZ1 are known to be

26 necessary for the response in *Arabidopsis thaliana*, whereas the role of auxin in

27 hydrotropism appears to vary depending on the plant species. This review will

28 describe recent progress characterising the hormonal regulation of hydrotropism.

29 Recent advances in identifying the sites of hydrotropic perception and response,

30 together with its interaction with gravitropism, will also be discussed. Finally, I will

31 describe putative mechanisms for perception of the water potential gradient and a

32 potential role for hydrotropism in acclimatising plants to drought conditions.

33

34 **Introduction**

35 Plants need to respond to a constantly changing environment and use tropisms to

36 reposition organs for resource capture. Tropisms are directional growth movements

37 that allow plants to respond to gravity, light, touch, water, salt and oxygen (Gilroy

38 and Masson, 2008; Galvan-Ampudia *et al.*, 2013; Eysholdt-Derzso and Sauter, 2017;

39 Su *et al.*, 2017). In plant roots, gravity is described as the main driver determining

40 the direction of root growth. Gravity is perceived in the columella cells of the root

41 cap, where displacement of statoliths leads to a lateral gradient in the shootward

42 auxin flux. More auxin flows through the lateral root cap and epidermis on the lower

43 side of the root, leading to differential growth in the epidermis of the elongation zone,

44 ultimately resulting in the root tip growing downwards (Blancaflor *et al.*, 1998;

45 Ottenschläger *et al.*, 2003; Swarup *et al.*, 2005; Friml, 2010; Rahman *et al.*, 2010). In

46 comparison, relatively little is known about hydrotropism, the directional growth of

47 plant roots towards a water source. Plant roots are able to perceive a water potential

48 gradient in their surroundings and change the direction of the root tip through

49 differential growth in the elongation zone.

Water is getting increasingly scarce on our planet and agriculture, which uses around 70% of all freshwater globally, claims the biggest share of this limited resource (WWAP, 2015; Davies and Bennett, 2015). Making crop plants more resilient to drought stress has been highlighted as an important goal in a recent report on sustainable water use (WWAP, 2015). Current strategies to improve water use efficiency through changes in root system architecture focus on increasing the steepness of roots to exploit water resources in lower soil horizons (Henry *et al.*, 2011; Lynch, 2013; Uga *et al.*, 2013; Rogers and Benfey, 2015; Gao and Lynch, 2016). Hydrotropism allows roots to grow actively towards water sources which may be located in any direction. Understanding and modifying this response in plants should be considered as an additional strategy to pursue the goal of sustainable water use in agriculture.

Early descriptions of hydrotropism exist (Bonnet, 1754; Knight, 1811) and in the 19th century Sachs, Molisch, Darwin, Wiesner and others conducted experiments to determine which part of the root tip is necessary for the perception of the water signal (Sachs, 1872; Darwin and Darwin, 1880; Wiesner, 1881; Molisch, 1883). Growing plant roots through a sieve with moist sawdust suspended at an angle or along the outside of a clay funnel, these experiments employed moisture gradients in air to observe the bending response of roots (Fig. 1A, B). Roots were covered in a mixture of olive oil and lamp black or cauterized with silver nitrate to determine the site of perception (Darwin and Darwin, 1880). Although some of those studies came to the conclusion that the root tip is the site of perception for both gravity and water, others observed that the elongation zone is also able to perceive the water signal - for a review of early hydrotropism literature, see Hooker (1915).

Loomis and Ewan (1936) conducted the first assessment of how plant roots grow in soil with different water availability. Utilising the fact that soil of a certain moisture content does not lose water to adjacent, drier soil through capillary action (Veihmeyer and Hendrickson, 1927), they created water gradients in soil to test the growth response of plant roots. Placing germinating seeds at the border between soil with either 4 or 11.8% moisture (the wilting coefficient of that soil was 7.1%), roots growing into the dry soil stopped their growth, whereas roots growing in the wet soil continued to grow and produced lateral roots (Loomis and Ewan, 1936). This is not a directional growth response in the strictest sense, but slight modifications allowed hydrotropism to be observed. When the border between wet and dry soil was set at a

84 45° angle and seeds were placed in wet soil some distance away from the border

85 between wet and dry soil, several plant species, including beans (*P. limensis* and *P.*

86 *vulgaris*), buckwheat (*Fagopyrum esculentum*) and foxtail millet (*Setaria italica*), had

87 roots that did not grow along the gravity vector into dry soil but were bending to

88 follow the line between dry and wet soil (Loomis and Ewan, 1936).

89 In recent years, there has been renewed interest in hydrotropism (reviewed in

90 Monshausen and Gilroy, 2009; Cassab *et al.*, 2013; Moriwaki *et al.*, 2013; Shkolnik

91 and Fromm, 2016) and hydrotropic responses have been shown for pea, cucumber,

92 wheat, maize, rice, birdsfoot trefoil (*Lotus japonicus)*, sitka spruce (*Picea sitchensis)*

93 and *Arabidopsis thaliana* (Table 1) (Jaffe *et al.*, 1985; Takahashi and Scott, 1991;

94 Coutts and Nicoll, 1993; Oyanagi *et al.*, 1995; Mizuno *et al.*, 2002; Takahashi *et al.*,

95 2002; Nakajima *et al.*, 2017). Although other methods to test hydrotropism exist

96 (Tsuda *et al.*, 2003; Eapen *et al.*, 2015), most hydrotropism assays are currently

97 performed using two systems (Fig. 1C-H). For the moisture in air assay (Fig. 1C, E,

98 F), seedlings are mounted on a support, usually foam or agar blocks, in such a way

99 that just the very root tip is suspended in air. The support acts as source of water

100 and the moisture gradient to the surrounding air is increased by placing the mounted

101 seedlings in an enclosed environment with a concentrated salt solution (Takahashi *et*

102 *al.*, 2002; Morohashi *et al.*, 2017). Alternatively, a water potential gradient can be

103 imposed in a split-agar based system by adding an osmolyte, e.g. sorbitol, to growth

104 medium and place this in direct contact with growth medium without additives (Fig.

105 1D, G, H). Seedlings are transferred to these plates and placed with their root tips a

106 set distance away from the border between the two growth media (Antoni *et al.*,

107 2016). In both cases, roots will experience a water potential gradient, with a wet (in

108 contact with the foam/agar support or closer to the growth media without osmolyte)

109 and dry (facing air or closer to the growth media with osmolyte) side to the root.

110 Roots showing a hydrotropic response will change the growth direction of the root

111 tip, either bending around the supporting block or towards the medium with higher

112 water potential. The resulting angle of deflection from vertical, gravitropic growth is

113 then measured. These assays have been used to identify genes involved in

114 hydrotropism and characterize cellular and molecular events of the response.

115 This review will give an overview of the signalling pathways and genes involved in

116 hydrotropism, species-specific differences in the response and putative mechanisms

117 for perception of the water gradient, describe interaction between hydrotropism and

4

118   gravitropism, possible contributions of hydrotropism to drought resilience and

119   concludes with a series of future directions for hydrotropism research.

120

121   **Plant hormones and hydrotropism**

122   Auxin plays a central role in several tropisms and might be involved in hydrotropism

123   too. However, the requirement for auxin in the hydrotropic response varies

124   depending on the plant species examined (Table 1). In *A. thaliana*, the agravitropic

125   auxin transport mutants *aux1* and *pin2* are not impaired in their hydrotropic response

126   (Takahashi *et al.*, 2002). Likewise, the auxin transport inhibitors 2,3,5-triiodobenzoic

127   acid (TIBA), 1-naphthylphthalemic acid (NPA) and 3-chloro-4-hydroxyphenylacetic

128   acid (CHPAA) are unable to block hydrotropism, in fact treatment with TIBA or NPA

129   leads to an earlier increase in root tip angle in hydrotropism assays, even though

130   final angles remain the same (Kaneyasu *et al.*, 2007; Shkolnik *et al.*, 2016). In

131   addition, expression of the auxin reporters DII-Venus and DR5 remains unchanged

132   throughout the hydrotropism response (Ponce *et al.*, 2008*b*; Takahashi *et al.*, 2009;

133   Shkolnik *et al.*, 2016). Even though hydrotropism in *A. thaliana* does not require

134   auxin transport, a functioning response to auxin appears to be necessary for

135   hydrotropism. Treatment with auxin response inhibitors led to contrasting results.

136   Whereas *p*-chlorophenoxyisobutylacetic acid (PCIB) led to a decrease in hydrotropic

137   response, addition of auxinole or α-(phenylethyl-2-oxo)-indole acetic acid (PEO-IAA)

138   accelerated the response (Kaneyasu *et al.*, 2007; Shkolnik *et al.*, 2016). These

139   contrasting results could be due to different modes of action and specificities of

140   inhibitors. Auxinole and PEO-IAA have been shown to bind to the auxin receptor

141   TRANSPORT INHIBITOR RESPONSE 1 (TIR1), whereas the mode of action of

142   PCIB is still unclear (Oono *et al.*, 2003; Hayashi *et al.*, 2008, 2012). PCIB is unable

143   to reverse the effects of exogenous IAA application on root growth, which both

144   auxinole and PEO-IAA are able to do (Oono *et al.*, 2003; Hayashi *et al.*, 2008, 2012).

145   The more specific inhibitors auxinole and PEO-IAA indicate that auxin has a negative

146   influence on hydrotropism in *A. thaliana*, but this awaits independent confirmation

147   from experiments with auxin response mutants.

148   Auxin's function in hydrotropism has been explored in four other plant species,

149   cucumber (*Cucumis sativus), rice (Oryza sativa*), birdsfoot trefoil (*Lotus japonicus*)

150   and pea (*Pisum sativum*). Gravitropism usually masks the hydrotropism response in

151   both cucumber and pea, hence experiments with pea use the *ageotropum* mutant

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which is completely agravitropic whereas experiments with cucumber seedlings are conducted either under microgravity, clinorotation or after removal of the root tip (Jaffe *et al.*, 1985; Morohashi *et al.*, 2017). In cucumber, the Aux/IAA gene *CsIAA1* (sometimes also referred to as *CsIAA12*) is differentially expressed within 30 minutes of exposure to a gravity or water stimulus, with increased expression occurring on the concave side of the bending root (Mizuno *et al.*, 2002). Increased expression on the concave side of hydrotropically bending roots has also been observed for other *CsIAA* genes (Morohashi *et al.*, 2017). Treatment with the auxin transport inhibitors TIBA and 9-hydroxyfluorene-9-carboxylic acid (HFCA) strongly reduces the hydrotropic response in cucumber, while PCIB and brefeldin A (BFA) have a less strong inhibitory effect (Morohashi *et al.*, 2017). *CsPIN5*, which is localised in the epidermis and lateral root cap and like *AtPIN2* may function in shootward transport of auxin from the root tip, is decreased on the convex side of gravitropically bending roots and on the dry side of roots exposed to a water potential gradient (Morohashi *et al.*, 2017). Surprisingly, this differential CsPIN5 localisation also takes places in hydrotropically stimulated roots that show no response because they are exposed to normal gravity (Morohashi *et al.*, 2017). Auxin efflux transport inhibitors (HFCA, NPA and TIBA) disrupt hydrotropism in the pea *ageotropum* mutant, whereas inhibitors of auxin influx (CHPAA, 1-NOA) do not seem to have a discernible effect on the response (Nakajima *et al.*, 2017). In rice, inhibitors of auxin transport (CHPAA, TIBA), response (PCIB) and biosynthesis (kynurenine) inhibit hydrotropism, and the effect of the latter can be rescued by exogenous application of IAA (Nakajima *et al.*, 2017). Interestingly, the hydrotropic response of birdsfoot trefoil is only inhibited by kynurenine application, which again can be rescued by IAA application, whereas CHPAA, TIBA and PCIB do not affect hydrotropism (Nakajima *et al.*, 2017). It seems puzzling that auxin biosynthesis, but not signalling is necessary for hydrotropism in birdsfoot trefoil. Signal transduction through ABP1, which recently has been shown not to be involved in auxin signalling (Enders *et al.*, 2015; Gao *et al.*, 2015), has been invoked to explain this discrepancy (Nakajima *et al.*, 2017). An alternative explanation may be that PCIB is not specific enough to inhibit the response in *L. japonicus*, and that a more potent inhibitor, e.g. auxinole, could prove the necessity for auxin signalling. In summary, the involvement of auxin in hydrotropism varies widely in a plant species-specific manner. Plants usually have species-specific water requirements for successful completion of their life cycle, which might explain why

gravitropism overrides hydrotropism in some species, whereas in others, e.g. rice, hydrotropism is independent of gravitropism, but still requires auxin. Understanding the role of auxin in hydrotropism will be important to understanding how gravi- and hydrotropic signals are integrated to determine the growth direction of the root tip.

Abscisic acid (ABA) is involved in many processes in plant development and physiological responses, but is perhaps best known for its function in the response to drought and osmotic stress (Yamaguchi-Shinozaki and Shinozaki, 2006; Cutler *et al.*, 2010). The core components of the ABA signalling pathway consist of cytosolic receptors of the START-domain superfamily (PYR/PYL/RCAR), clade A, type 2C protein phosphatases (PP2C) and a subclass III Snf1-related kinases (SnRK2) (Cutler *et al.*, 2010). ABA leads to the formation of a ternary receptor-hormone-phosphatase complex that relieves the inhibition of SnRK2 kinases by PP2C phosphatases, allowing the phosphorylation of downstream targets (Fujii *et al.*, 2009; Ma *et al.*, 2009; Park *et al.*, 2009). In *A. thaliana*, the ABA biosynthesis mutant *aba1-1* has a reduced hydrotropic response, but this defect is rescued by the exogenous application of ABA (Takahashi *et al.*, 2002). ABA signal transduction mutants also have an altered hydrotropic response, with the gain-of-function PP2C mutant *abi2-1* and a hextuple receptor mutant showing a reduced response whereas it is increased in a loss-of-function quadruple pp2c mutant (Takahashi *et al.*, 2002; Antoni *et al.*, 2013).

The most detailed exploration of the role of ABA signalling in hydrotropism has been conducted for the SnRK2 kinases. Three family members, *SnRK2.2*, *SnRK2.3* and *SnRK2.6*, are known to be involved in ABA signalling, and the *snkr2.2 snrk2.3* double mutant has a strongly reduced hydrotropism (Mustilli *et al.*, 2002; Fujii *et al.*, 2007; Dietrich *et al.*, 2017). Tissue-specific expression of *SnRK2.2* in the double mutant background showed that expression in the cortex alone is able to rescue the response (Dietrich *et al.*, 2017). Exogenous ABA at low concentrations promotes root elongation through increasing the length of root cells at maturity and in the *snkr2.2 snrk2.3* mutant, *SnRK2.2* expression in the cortex was able to rescue this effect (Dietrich *et al.*, 2017). A mathematical model examining the contribution of the cortex to root bending predicted that differential elongation in the cortex could be the driving force behind hydrotropic bending (Dietrich *et al.*, 2017). This was further confirmed by blocking differential elongation in a tissue-specific manner, which only

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220 blocked hydrotropism if the cortex was affected (Dietrich *et al.*, 2017). Together, this

221 led to the proposal that ABA-mediated differential elongation in the cortex is the

222 driving force behind the changes in growth direction observed in hydrotropism

223 (Dietrich *et al.*, 2017) (Fig. 2). With auxin transport and response in the lateral root

224 cap and epidermis driving gravitropism (Swarup *et al.*, 2005), the distinct role of the

225 cortex in hydrotropism indicates that there are a tissue-specific and

226 mechanisticdifferences between responses to gravity and water. The position of ABA

227 in the signalling cascade for hydrotropism is currently unclear. The rescue of the

228 hydrotropic defect of the *aba1-1* mutant by application of exogenous ABA, which is

229 non-directional, could be taken as indication that hydrotropic signalling does not

230 involve an ABA gradient across the root. On the other hand, hydrotropic signalling

231 could involve changes in ABA sensitivity on the dry and wet side of the root. It is also

232 still unknown if the water potential gradient across the root affects the radial transport

233 of water and signalling molecules. It seems possible that a water potential gradient

234 could lead to changes in the direction of water flow on the dry and wet side of the

235 root, with water flowing towards the stele on the wet side and away from the stele on

236 the dry side of the root. This differential water flow could affect the transport direction

237 of signalling molecules, including ABA. These different hypotheses about the

238 mechanism of ABA in the hydrotropic response still await experimental verification.

239 In addition, the requirement for ABA in hydrotropism of plant species other than *A.*

240 *thaliana* still needs to be examined.

241

242 **Key hydrotropism genes**

243 Forward genetic screens have only led to the isolation of a few hydrotropism-related

244 genes in *A. thaliana. no hydrotropic response 1* (*nhr1*) and *altered hydrotropic*

245 *response 1* (*ahr1*) are semi-dominant mutants affected in hydrotropism (Eapen *et al.*,

246 2003; Saucedo *et al.*, 2012). Homozygous *nhr1* plants never reach the reproductive

247 stage, and the genes affected in both mutants have not been cloned yet (Eapen *et*

248 *al.*, 2003; Saucedo *et al.*, 2012; Salazar-Blas *et al.*, 2017).

249 *mizu-kussei 1* (*miz1*), described by Kobayashi et al. (2007), is caused by a recessive

250 mutation in At2g41660. Apart from a complete absence of hydrotropism and slightly

251 reduced root phototropism and waving, *miz1* plants grow normally and in particular

252 show a normal gravitropism response and root tip anatomy (Kobayashi *et al.*, 2007).

253 Overexpression of *MIZ1* leads to increased root curvature in hydrotropism assays

254 (Miyazawa *et al.*, 2012). Unfortunately, MIZ1 is a protein of unknown function,

255 containing only a conserved domain of uncharacterized function (DUF617 domain).

256 Homologs containing a DUF617 domain have been found in rice and physcomitrella

257 but not in algae, suggesting that acquisition of MIZ1 function may have taken place

258 during the evolution of land plants (Kobayashi *et al.*, 2007). It is still unclear at which

259 step of the hydrotropism response *MIZ1* functions, but subcellular localisation of

260 MIZ1 showed that it is a soluble protein associated with the cytosolic side of the

261 endoplasmatic reticulum membrane (Yamazaki *et al.*, 2012). ABA and blue light are

262 both able to upregulate *MIZ1* expression (Moriwaki *et al.*, 2012). *MIZ1* itself appears

263 to influence auxin accumulation, as free IAA concentrations in *miz1* and *MIZ1*

264 overexpressing roots increase and decrease, respectively (Moriwaki *et al.*, 2011).

265 Whether this is directly linked to the role of MIZ1 in hydrotropism is unclear, and

266 overexpression or loss of MIZ1 function does not affect PIN gene expression and

267 localisation (Moriwaki *et al.*, 2011). A MIZ1-GFP fusion under the control of its own

268 promoter has shown that the protein is strongly expressed in cortex cells around the

269 transition zone between meristem and elongation zone, the lateral root cap and

270 columella and also, to a lesser extent, in the epidermis and stele, but MIZ1-GFP

271 intensity and localisation does not change during the hydrotropic response

272 (Yamazaki *et al.*, 2012; Moriwaki *et al.*, 2013). Recently, it was shown that

273 expression of *MIZ1* in the cortex alone is able to rescue the hydrotropism response

274 of *miz1* mutants, highlighting the important role of this tissue in hydrotropism

275 (Dietrich *et al.*, 2017) (Fig. 2).

276 A second mutant isolated through forward screens, *miz2*, is a weak *GNOM* allele

277 (G951E) (Miyazawa *et al.*, 2009*b*). GNOM is a GDP/GTP exchange factor for small

278 G proteins of the ARF class (ARF-GEF) regulating intracellular vesicle trafficking,

279 whose best characterised function is polar targeting of PIN proteins to the plasma

280 membrane (Geldner *et al.*, 2003). Importantly, *miz2* does not affect auxin response

281 or PIN localisation (Miyazawa *et al.*, 2009*a*,*b*). The G951E mutation of *miz2* is

282 downstream of the Sec7 domain and affects an amino acid conserved in GNOM

283 homologs in other plant species (Miyazawa *et al.*, 2009*b*). Treatment with BFA, a

284 known inhibitor of ARF-GEFs, phenocopies *miz2*. In addition, the hydrotropic

285 response of the BFA resistant GN^{M696L} allele cannot be blocked by BFA whereas the

286 weak gnom$^{B/E}$ allele is ahydrotropic (Miyazawa *et al.*, 2009*b*). Supporting evidence of

287 the importance of vesicle trafficking for hydrotropism comes from a phospholipase D

288 mutant that is slightly impaired in hydrotropism (Taniguchi *et al.*, 2010).

289 There appears to be no direct interaction between MIZ2 and MIZ1, as MIZ1-GFP is

290 still correctly localised in the *miz2* mutant (Moriwaki *et al.*, 2011). Interestingly

291 though, *miz2* plants that overexpress *MIZ1* show an ahydrotropic phenotype,

292 demonstrating that *MIZ2* is epistatic to *MIZ1* (Miyazawa *et al.*, 2012).

293

294 **Where and how do roots sense water?**

295 Which part of the root is able to sense a gradient in water availability is a question

296 that has fascinated people since the early days of hydrotropism research. The

297 Darwins describe experiments where covering the root tip with a mixture of olive oil

298 and lamp black abolishes the hydrotropic response, concluding that the very root tip

299 is necessary for the perception of gravity and water. This led them to coin the since

300 then much repeated phrase of the root tip as the "brain of the root" (Darwin and

301 Darwin, 1880). Darwin's contemporaries already criticised those experiments,

302 especially with regards to the effect of the applied mixture on root growth rates and

303 the difficulties in applying the mixture in an even manner and to a precise region of

304 the root (Wiesner, 1881; Molisch, 1883). Similar problems affect more recent

305 experiments. A role for the root cap in hydrotropism perception was reported for pea

306 and maize, but root growth rates were not always recorded (Takahashi and Scott,

307 1991; Takahashi and Suge, 1991; Takahashi and Scott, 1993; Takano *et al.*, 1995;

308 Hirasawa *et al.*, 1997). In addition, while surgical ablation experiments record the

309 length of root tip removed, usually no relation to anatomical markers along the root

310 axis is given and it is therefore difficult to know whether just the columella or larger

311 parts, including the meristem or perhaps even the elongation zone, were removed.

312 Miyazawa et al. (2008) used heavy-ion microbeam irradiation and laser ablation to

313 ablate either the columella or what is described as the elongation zone of *A. thaliana*

314 roots and report conflicting results. While irradiation of the elongation zone led to a

315 reduction in hydrotropic bending, the same treatment of the columella did not

316 (Miyazawa *et al.*, 2008). On the other hand, laser ablation of the columella did

317 reduce the hydrotropic response (Miyazawa *et al.*, 2008). However, root growth rates

318 following both treatments were extremely slow, so that these results have to be

319 considered with caution. Lately, laser ablation and microdissection were again used

320 to determine the root tissue responsible for hydrotropismus perception in *A. thaliana*.

321   Root growth rates were reported for these experiments and were in an expected

322   range. Whereas laser ablation of the columella inhibited the gravitropic response as

323   reported by Blancaflor et al. (1998), the hydrotropic response was not perturbed

324   (Dietrich *et al.*, 2017). Removal of the root cap and meristem by either laser ablation

325   or microdissection also did not inhibit hydrotropism, demonstrating that the

326   elongation zone of the root is able to perceive and respond to the hydrotropic signal

327   (Dietrich *et al.*, 2017) (Fig. 2). While some contribution from the columella and root

328   cap in hydrotropism perception cannot be totally excluded, these results place

329   perception for hydro- and gravitropism in separate tissues. In addition, removal of the

330   columella in rice and cucumber does not impair hydrotropism, demonstrating that in

331   other plant species hydrotropism perception also does not depend on this tissue

332   (Morohashi *et al.*, 2017; Nakajima *et al.*, 2017; Fujii *et al.*, 2018).

333

334   How could roots be able to sense a water potential gradient in the elongation zone?

335   The difference in water potential across the root is rather small, and was calculated

336   to reach a maximum of less than 10 kPa across a 100 µm wide *A. thaliana* root

337   during a standard split-agar hydrotropism assay, which is less than 3% of the

338   maximum absolute water potential experienced at the root midline (Dietrich *et al.*,

339   2017). Mechanosensitive ion channels could potentially be triggered by changes in

340   cell volume if a root is exposed to a water potential gradient (Hamilton *et al.*, 2015).

341   Pea is the only plant species where turgor measurements have been performed

342   during hydrotropism, but differences in turgor between the wet and dry side of the

343   root were not observed (Hirasawa *et al.*, 1997; Miyamoto *et al.*, 2002).

344   The *miz2* (GN^{G951E}) phenotype strongly implies that membrane proteins play an

345   important part in the hydrotropism response (Miyazawa *et al.*, 2009*b*). Although

346   GNOM is best known for its role in endosomal recycling of PIN proteins (Geldner *et

347   al.*, 2003), localisation of PIN1 is unaffected in *miz2* (Miyazawa *et al.*, 2009*a*).

348   Hydrotropism may rely on endosomal recycling of other proteins, trafficking from the

349   ER to the Golgi or endocytosis, processes that also rely on GNOM (Paez Valencia *et

350   al.*, 2016).

351   It is highly likely that hydrotropism is intricately linked to water uptake and transport

352   in the root. Radial water uptake from the soil towards the xylem vessels in the

353   vasculature follows two paths, the apoplastic route along cell walls and the cell-to-

354   cell path that is comprised of transcellular (across membranes) and symplastic

355 (through plasmodesmata) transport (Li *et al.*, 2014). Root hydraulic conductivity (Lp_r)

356 is a measure for water transported through the root. Aquaporins are membrane

357 channels that transport water and small neutral molecules and one subfamily, the

358 Plasma membrane Intrinsic Proteins (PIPs), contributes significantly to Lp_r (Sutka *et*

359 *al.*, 2011; Li *et al.*, 2014). How could aquaporins and changes in Lp_r contribute to

360 hydrotropic signalling? Root hydraulic conductivity is reduced by abiotic stress in

361 many plant species (Aroca *et al.*, 2012). PIP activity is regulated at many levels -

362 transcriptionally, translationally, through gating of the channel itself by

363 phosphorylation, protons or divalent cations and by cellular trafficking (Li *et al.*, 2014)

364 – and reduction of root hydraulic conductivity under abiotic stress could be achieved

365 using any of these regulatory mechanisms. For salt stress, it was demonstrated that

366 treatment with 100 mM sodium chloride reduces Lp_r by around 60% within one hour

367 and decreases aquaporin transcript abundance (Boursiac *et al.*, 2005).

368 Downregulation of aquaporin gene expression however takes longer than the

369 decrease in Lpr, but other regulation mechanisms respond more rapidly to salt

370 stress. 45 minutes after the start of salt treatment, a substantial amount of a PIP2;1-

371 GFP fusion protein had become internalized and removal from the plasma

372 membrane involved clathrin and membrane raft-associated pathways (Boursiac *et*

373 *al.*, 2008; Li *et al.*, 2011). Another pathway for removal of aquaporins from the

374 plasma membrane involves tryptophan-rich sensory protein/translocator (TSPO),

375 which is induced by abiotic stress and was shown to interact with PIP2;7, leading to

376 internalization and autophagic degradation of the aquaporin (Hachez *et al.*, 2014). In

377 addition, PIP1;2 and PIP2;1 were recently shown to directly interact with receptor-

378 like kinases (RLKs) in the plasma membrane and were regulated in their water

379 transporting activity by this interaction (Bellati *et al.*, 2016). Similar to the examples

380 for salt and TSPO regulating PIPs at the plasma membrane, the low water potential

381 during hydrotropism could affect the membrane presence of aquaporins through

382 endosomal recycling, which would explain the requirement for MIZ2/GNOM (Fig. 3).

383 The interaction of aquaporins with RLKs could also be affected by low water

384 potential, potentially leading to changes in cell elongation through signalling via the

385 RLKs in addition to regulation of PIP activity by the RLKs (Fig. 3). These hypothetical

386 regulation mechanisms of aquaporin activity or membrane presence could lead to a

387 change in hydraulic conductivity, with two possible outcomes. Cell or tissue growth

388 could be affected by changes in Lp_r, as was demonstrated for lateral root primordia

emergence (Péret *et al.*, 2012). Alternatively, hydraulic conductivity was shown to affect radial ABA transport along the apoplastic pathway through solvent drag (Freundl *et al.*, 1998). This could lead to changes in ABA concentration on the dry and wet side of the root, driving differential cell elongation.

These hypothetical perception mechanisms linked to aquaporins would not necessarily require sensing of the water potential gradient at opposing sides of the root, but could utilise a water potential set point, below which aquaporin membrane presence or activity changes, setting in motion the signalling cascade leading to cell elongation. However, at the moment the identity of the hydrotropic signal perceived by the root is still unclear.

Interaction between hydro- and gravitropism

Several tropisms can adjust the growth direction of the root tip and interaction and competition between the responses to different environmental cues will determine the final growth direction. To understand hydrotropism, its interaction with gravitropism is central. It has been argued that gravitropism determines the "default" growth direction of the root, which is then adjusted by tropic responses to other environmental cues (Blancaflor and Masson, 2003; Rosquete and Kleine-Vehn, 2013; Krieger *et al.*, 2016). In the interaction between hydro- and gravitropism, a clear distinction has to be drawn between plant species that depend on auxin and its transport for their hydrotropic response and those where hydrotropism is independent of development of a lateral auxin gradient. In those plant species which require auxin transport, the gravitropic response can be assumed to counteract hydrotropism, unless the water potential gradient aligns with the gravity vector. This would explain why hydrotropism in pea and cucumber can only be observed if gravitropism has been removed. Still, there are plants, e.g. rice, that rely on auxin transport for both tropisms but react to a water gradient in the presence of gravity. How can such differences be explained? It is still unclear whether plant species requiring auxin for hydrotropism develop a lateral auxin gradient during the response. If they do, species-specific differences in the interaction between gravi- and hydrotropism could be due to differences in the establishment of these auxin gradients.

Another factor influencing the interaction between the two tropisms could be timing and sensitivity of each response. Presentation time, defined as the minimum

423 exposure time needed to elicit a response, has been determined for the gravitropism
424 response of various plant roots (Kiss and Sack, 1989; Kiss *et al.*, 1996; Hou *et al.*,
425 2003). Usually, root curvature in response to a 90° stimulus is plotted against
426 stimulation time and the presentation time determined by regression analysis (Kiss *et*
427 *al.*, 1996). For hydrotropism, the presentation time has so far only be determined for
428 *ageotropum* peas following the method described for gravitropism (Stinemetz *et al.*,
429 1996). Equally, data on the strength of the water potential gradient necessary for
430 triggering hydrotropism is scarce (Takano *et al.*, 1995). Natural variation has been
431 reported to exist for gravitropic presentation times (Tanimoto *et al.*, 2008; Moulia and
432 Fournier, 2009), and a more detailed examination of presentation times and
433 response strength for both hydro- and gravitropism should help to understand
434 species-specific differences in the interaction between those tropisms.
435 In *A. thaliana* hydrotropism is independent of the development of a lateral auxin
436 gradient (Shkolnik *et al.*, 2016). Plants treated with auxin transport and response
437 inhibitors (Shkolnik *et al.*, 2016) and the *pgm1* mutant which lacks statoliths
438 (Takahashi *et al.*, 2003) show a faster hydrotropic response. Together with the
439 observation that statolith degradation occurs in roots exposed to a water potential
440 gradient in *A. thaliana* and *Raphanus sativus* (Takahashi *et al.*, 2003; Ponce *et al.*,
441 2008*a*), this has been taken as evidence to support the hypothesis that gravitropic
442 responsiveness needs to be reduced so that hydrotropism can take place. In
443 contrast, exposure of roots to 150 mM sodium chloride leads to agravitropic growth
444 and degradation of statoliths but several *salt overly sensitive* mutants, which display
445 the same agravitropic growth on medium with salt, retain their statoliths (Sun *et al.*,
446 2008). This indicates that statolith degradation on exposure to environmental stress
447 may be a mere correlation and not causative for the response. In addition, the
448 agravitropic *pin2* and *aux1* mutants do not have an accelerated hydrotropic response
449 (Takahashi *et al.*, 2002). Detailed analysis of the kinetics of gravitropism shows that
450 the rate of gravitropic root bending in *A. thaliana* depends on the stimulation angle,
451 with smaller stimulation angles resulting in reduced bending rates (Mullen *et al.*,
452 2000). In addition, a threshold angle of 15° from the vertical has to be reached
453 before 50% of a population of seedlings respond gravitropically (Mullen *et al.*, 2000).
454 Therefore a water potential gradient can lead to a substantial change in root angle
455 before a gravity response is triggered. Furthermore, this gravitropic response will be
456 slow to begin with, as the stimulation angle is small.

Recently, a study investigated the interaction between hydro- and gravitropism and the role of reactive oxygen species (ROS) (Krieger *et al.*, 2016). A very interesting observation of this study was that hydro- and gravitropism lead to bending of the root tip in different regions, with gravitropic bending initiating relatively close to the root tip in the distal elongation zone whereas hydrotropic bending takes place in a more shootward region of the elongation zone (central elongation zone) (Krieger *et al.*, 2016), which provides further confirmation that gravitropism and hydrotropism employ different tissues in their bending mechanisms.

Auxin-induced ROS production is necessary for gravitropism (Joo *et al.*, 2001) and using the fluorescent dye dihydrorhodamine-123, Krieger et al. (2016) demonstrated that two hours after gravistimulation a transient ROS increase was visible on the concave side of the distal elongation zone of the bending root. Using the moisture in air assay, a ROS increase was observed on the concave side of the central elongation zone of hydrotropically bending roots (Krieger *et al.*, 2016). However, when calcium chloride was replaced with distilled water in the assay, i.e. under conditions that do not induce hydrotropic bending in roots, a similar ROS increase in the same location was observed (Krieger *et al.*, 2016). ROS distribution however was unchanged when hydrotropism was induced in roots using the split-agar assay and the authors attribute the spurious ROS accumulation in the moisture in air assay to the mechanical tension the roots were under (Krieger *et al.*, 2016). Treatment with ROS scavengers and NADPH oxidase inhibitors showed that ROS production in fact inhibited hydrotropism (Krieger *et al.*, 2016). Ascorbate peroxidase (*apx1-2*) and respiratory burst oxidase homolog (*rbohC*) mutants showed decreased and increased hydrotropic curvature, respectively, further confirming the inhibition of hydrotropism by ROS (Krieger *et al.*, 2016). How ROS inhibits hydrotropism is currently unknown. Interestingly though, the same study also showed that after four hours of hydrostimulation, a 90° gravitropic stimulus was unable to elicit an increase in ROS or a lateral auxin gradient (Krieger *et al.*, 2016). Clearly more work is still necessary to understand exactly how hydro- and gravitropism interact, but this is an exciting first glimpse that hydrotropism is able to influence the gravitropic response.

Can hydrotropism improve drought acclimation?

Drought stress is a major limiting factor in crop production and complex plant responses exist to escape, avoid or tolerate limited water availability (Wery, 2005;

491 Gaur *et al.*, 2008). Drought can lead to an increase in the root to shoot ratio of
492 plants, usually due to shoot growth being more strongly affected by drought (Blum,
493 2005) and maintaining yield under drought conditions can be linked to a well-
494 developed root system, particularly in those regions of the soil still containing water
495 (Comas *et al.*, 2013). Irrigation is used to prevent drought stress in crops and
496 agriculture uses 70% of globally available freshwater, mostly for this purpose (Du *et*
497 *al.*, 2015; WWAP, 2015). Climate change however will make water availability more
498 unpredictable, with increased likelihoods for extreme weather events and changes in
499 rainfall patterns (IPCC, 2014). A variety of strategies are pursued to make
500 agricultural water use more sustainable and "produce more crop per drop" (Morison
501 *et al.*, 2008; Du *et al.*, 2015). How could hydrotropism, which allows roots to forage
502 for water in soil, contribute to this? Conservation tillage, which minimises the amount
503 of soil disturbance, increases soil water availability through improved physical soil
504 properties, increased organic matter and reduced evaporation due to crop residue
505 left on the surface (Triplett and Dick, 2008) and is now widely adopted in many rain-
506 fed agriculture systems (Brunel *et al.*, 2013; Peiretti and Dumanski, 2014). Currently
507 little is known about water distribution in soils under conservation tillage, but water
508 may be more heterogeneously distributed than under conventional tillage, which
509 would make crops with an increased hydrotropism response more efficient. For
510 agricultural systems using irrigation, deficit and partial root zone drying (PRD)
511 irrigation systems have been demonstrated to increase the water use efficiency in a
512 number of crops (Kang and Zhang, 2004). In these systems, less water than is
513 needed to cover evapotranspiration demand is supplied, sometimes only to part of
514 the root system (PRD). As a result, plants produce less shoot biomass and decrease
515 stomatal conductance, whilst still producing similar or slightly reduced yields
516 compared to fully irrigated crops. Under PRD, it is thought that the drying part of the
517 root system produces a signal that regulates stomata conductance, whereas the
518 irrigated part supplies the shoot with sufficient water to produce the crop (Kang and
519 Zhang, 2004; Sobeih *et al.*, 2004). The applied effects of PRD on plant growth have
520 been extensively studied and are reviewed elsewhere (Kang and Zhang, 2004). For
521 root growth, it was shown that PRD leads to an increase in tomato root dry weight,
522 particularly in those parts of the rootsystem that were rewatered after a previous
523 drying period (Mingo *et al.*, 2004), and an increase in the root surface area of maize
524 (Zhenchang *et al.*, 2016).

525   That hydrotropism can contribute to directional root growth in soil has been

526   demonstrated for *A. thaliana* grown in soil microcosms with a lateral water gradient

527   (Iwata *et al.*, 2013). Plants showed increased root growth in the area with higher

528   water content (Iwata *et al.*, 2013). This behaviour was dependent on a functioning

529   hydrotropism response, as plants overexpressing *MIZ1* had an increased tendency

530   to grow roots in soil with high water content whereas *miz1* plants grew roots in a

531   random fashion, unrelated to water distribution in the soil (Iwata *et al.*, 2013). In

532   maize, a recent study tried to link hydrotropic responsiveness to yield under PRD

533   irrigation and drought (Eapen *et al.*, 2017). The hydrotropic response of part of a

534   collection of maize hybrid lines from the Drought Tolerance Maize for Africa project

535   was analysed at four days after germination and representative lines with strong and

536   weak hydrotropic responses tested in field trials (Eapen *et al.*, 2017). Although one

537   line with a strong hydrotropic response showed increased yield under PRD irrigation

538   and drought stress, the results were more ambiguous for other lines (Eapen *et al.*,

539   2017). Interestingly though there seemed to be a stronger correlation between root

540   weight and grain yield in the lines with a strong hydrotropic response compared to

541   those lines which only weakly responded to the stimulus (Eapen *et al.*, 2017). Root

542   biomass and root system architecture traits might have been confounding factors in

543   this study, and highlight the need for rigorous experimental design when assessing

544   the contribution of hydrotropism to crop performance. These new developments are

545   an indication that crops with an improved hydrotropic response could be beneficial in

546   agricultural systems using conservation tillage or deficit/PRD irrigation systems,

547   contributing to improved water use efficiency.

548

Conclusions and future directions

549

550   Hydrotropism research has taken a leap forward in the last few years with a number

551   of discoveries describing the site of perception, bending mechanism and interaction

552   with gravitropism. Hydrotropism has now been shown to exist in an increasing

553   number of plant species and interestingly, species-specific mechanistic differences in

554   the response exist. New techniques will allow us to understand this tropism and how

555   it contributes to water uptake and drought responses in plants.

556   Although progress has been made in understanding hydrotropism, many more

557   questions still remain open. Most importantly, it is still unclear what the water signal

558   is and how it is perceived. With the recent discovery that the columella may not be

necessary for hydrotropism and that the signal can be perceived by the elongation zone of *A. thaliana* (Dietrich *et al.*, 2017), the pool of potential candidates for hydrotropism perception has widened and changed again. Development of sensors for calcium, ABA, pH, ROS and other signalling molecules has improved dramatically over recent years (Nagai *et al.*, 2004; Jones *et al.*, 2014; Waadt *et al.*, 2014; Krieger *et al.*, 2016), but these sensors may still not be sensitive enough to detect changes during hydrotropism. It might be necessary to indirectly determine the signal, and a better understanding of hydrotropism response kinetics may help in this respect. The presentation time for the hydrotropic signal has been determined so far only for pea, and water potential gradients used in assays are usually chosen on the basis of returning the maximum response without affecting root growth (Stinemetz *et al.*, 1996; Takahashi *et al.*, 2002). A systematic evaluation of presentation times and the strength of the water potential gradient needed to trigger the response may inform the search for the elusive water signal. New developments in microfluidic devices now allow precise delivery of stimuli at high spatial and temporal resolution and will be instrumental in determining these parameters (Meier *et al.*, 2010; Stanley *et al.*, 2018). Once the signal for hydrotropism has been found, it should be easier to connect genes known to be involved in hydrotropism, e.g. *MIZ1*, *MIZ2/GNOM* and ABA genes, to the signal transduction pathway of the hydrotropic response. Alternatively, a reverse approach could be used, starting from these known components to search for interaction partners that are specific to hydrotropism. In *A. thaliana*, the cortex tissue has been shown to play an important role in hydrotropism, with evidence that differential elongation in this tissue drives the bending response (Dietrich *et al.*, 2017). Other plant species in which hydrotropism has been observed have a cortex consisting of multiple cell layers and it will be interesting to see whether hydrotropic bending in those species uses the same mechanistic principle to drive the bending response.

The interaction of hydrotropism with other tropisms, gravitropism in particular, is another area of great interest. For those plant species that require auxin transport for hydrotropism, it will be important to determine whether a lateral auxin gradient develops during the response and how such a gradient is affected by gravitropism. Mathematical modelling has given new insights into gravitropism (Swarup *et al.*, 2005; Band *et al.*, 2012) and has been used to investigate the bending response in hydrotropism (Dietrich *et al.*, 2017). Development of new models that combine

593 hydro- and gravitropic responses will be an important part of understanding how

594 these tropisms interact and direct root tip growth angles.

595 Until now, all hydrotropism experiments have been performed on primary roots of

596 plants. Wiesner and Molisch already observed that lateral roots grow more easily in

597 the direction of water than primary roots (Wiesner, 1881; Molisch, 1883).

598 Hydrotropism research needs to extend its scope and investigate the response of

599 lateral roots. Lateral roots, which have a different gravitropic set point angle and are

600 therefore less responsive to gravity than primary roots (Roychoudhry *et al.*, 2013,

601 2017), are in theory more responsive to water potential gradients. Lateral roots make

602 up the majority of any plant root system and although hydrotropism assays for lateral

603 roots will be technically more difficult, these should give us a better appreciation if

604 hydrotropism is able to increase water uptake.

605 Ultimately, hydrotropic responses will have to be assessed in soil. Methods now exist

606 that allow the visualisation of roots and water in soil and to compute water fluxes into

607 the root (Daly *et al.*, 2015, 2017). Development of a hydrotropic assay in soil will be a

608 necessity to understand the true contribution of this tropism to water uptake and

609 drought acclimation in plants.

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Table 1 Plant species-specific differences in hydrotropism

Plant species	Gravitropism masks hydrotropism	Root cap needed for hydrotropism	Auxin transport inhibitor blocks hydrotropism	Auxin response inhibitor blocks hydrotropism	Auxin biosynthesis inhibitor blocks hydrotropism	Hydro-tropism genes	References
Pea	yes	nd	yes/no	only at 100 μM PCIB	nd	nd	Jaffe et al., 1985 Nakajima et al., 2017
Cucumber	yes	no	yes	yes (PCIB)	nd	*IAA1* *PIN5*	Mizuno et al., 2002 Morohashi et al., 2017
A. thaliana	no	no	no	yes (PCIB) no (auxinole, PEO-IAA)	nd	*MIZ1* *MIZ2* *PYR/PYL* *PP2CA* *SnRK2.2* *ABA1* *PLDζ2*	Takahashi et al., 2002 Kaneyasu et al., 2007 Kobayashi et al., 2007 Miyazawa et al., 2009b Taniguchi et al., 2010 Antoni et al., 2013 Shkolnik et al., 2016 Dietrich et al., 2017
Rice	no	no	yes	yes (PCIB)	yes	nd	Nakajima et al., 2017
Lotus japonicus	no	nd	no	no (PCIB)	yes	nd	Nakajima et al., 2017

987 nd, not determined

988

989

Figure legends

Figure 1 Historic and modern assays for hydrotropism

A Cross-section of assay described by Sachs and used by the Darwins, Mesh covers the bottom of a round metal frame. Filled with moist sawdust and suspended at an angle, roots can grow through the mesh and need to bend in order to maintain contact with moisture provided by the saw dust. Redrawn from Sachs (1872). **B** Molisch's hydrotropism assay. Roots grow through holes in the rim of a clay funnel connected to a water reservoir. Once roots reach the edge of the funnel, they have to bend in order to stay in contact with the moisture provided by the funnel surface. Redrawn from Molisch (1883). **C** Moisture in air assay. Inside a box, seedlings are mounted on a water-soaked foam or agar bloc with the root tip pointing down and suspended in air. The water potential gradient between the moisture containing support and the surrounding air is further increased by a dish containing a concentrated salt solution. Roots need to bend around the edge of the support in order to stay in contact with their water supply. **D** Split-agar assay. Seedlings are placed in a square petri dish on growth medium which is in direct contact with another growth medium containing an osmolyte. Diffusion of the osmolyte establishes a water potential gradient that is able to deflect root tip growth from following the gravity vector. **E, F** Rice root bending hydrotropically in moisture in air assay (Nakajima et al., Auxin transport and response requirements for root hydrotropism differ between plant species, J Exp Bot, 2017, **68** (13), 3441-3456, by permission of Oxford University Press and the Society of Experimental Biology). **G, H** *A. thaliana* roots bending hydrotropically in split-agar assay. The white dashed line indicates the border between the two different growth media. The arrow labelled g indicates the gravity vector in all assays.

Figure 2 Hydrotropism mechanism in *Arabidopsis thaliana*

A. thaliana roots exposed to a water potential gradient perceive reduced water availability through an as yet unknown mechanism in the elongation zone. Reactive oxygen species (ROS) are able to inhibit hydrotropism, but currently the stage at which the response is affected is unknown. Abscisic acid and MIZ2/GNOM are required for hydrotropism and could be involved in perception and differential growth. The role of auxin is currently unclear, but a lateral auxin gradient does not develop during hydrotropism in *A. thaliana*. Bending of the root tip is achieved by differential

1024 elongation of cortex cells; abscisic acid and expression of *MIZ1* and *SnRK2.2* in the
1025 cortex cell file are required for this. Hydrotropic bending of the root tip will trigger a
1026 gravitropic response through statolith relocalisation, which provides feedback
1027 inhibition. Statoliths and differentially expanding cortex cells have been drawn for
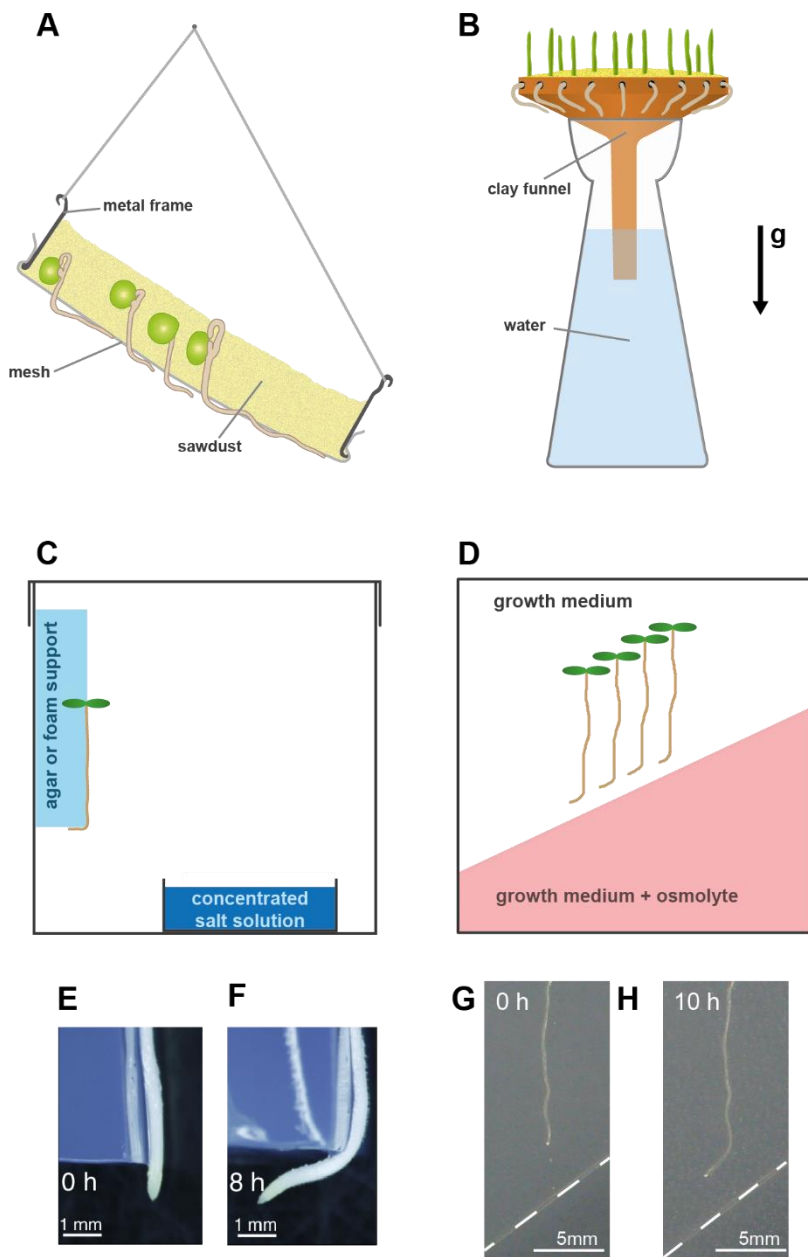1028 emphasis and are not to scale.

1029

1030 **Figure 3 Potential mechanisms for perception and response to low water**
1031 **potential.**

1032 Low water potential could affect the membrane presence or activity of Plasma
1033 membrane Intrinsic Proteins (PIPs). This could affect cell elongation through several
1034 independent pathways: PIPs were shown to directly interact with receptor-like
1035 kinases (RLK) in the plasma membrane. This interaction was shown to regulate PIP
1036 activity, but could potentially also affect signalling from the RLK to change cell
1037 elongation. Changes in AQP activity or presence due to low water potential will also
1038 lead to a change in hydraulic conductivity, with two possible outcomes. Hydraulic
1039 conductivity could affect cell elongation directly (as demonstrated for lateral root
1040 primordia), but can also affect radial ABA transport in the root. Changes in local ABA
1041 concentration could be the driver of differential cell elongation, leading ultimately to
1042 root bending. Perception would not necessarily require sensing of a water potential
1043 gradient at opposing sides of the root, but could work through a water potential set
1044 point, below which PIP membrane presence or activity changes, initiating the signal
1045 cascade leading to cell elongation. MIZ2/GNOM is required to facilitate cycling of
1046 PIPs (and RLKs) to and from the plasma membrane in this model. Aquaporin
1047 regulation in a single layer or all tissue layers of the root may be necessary for this
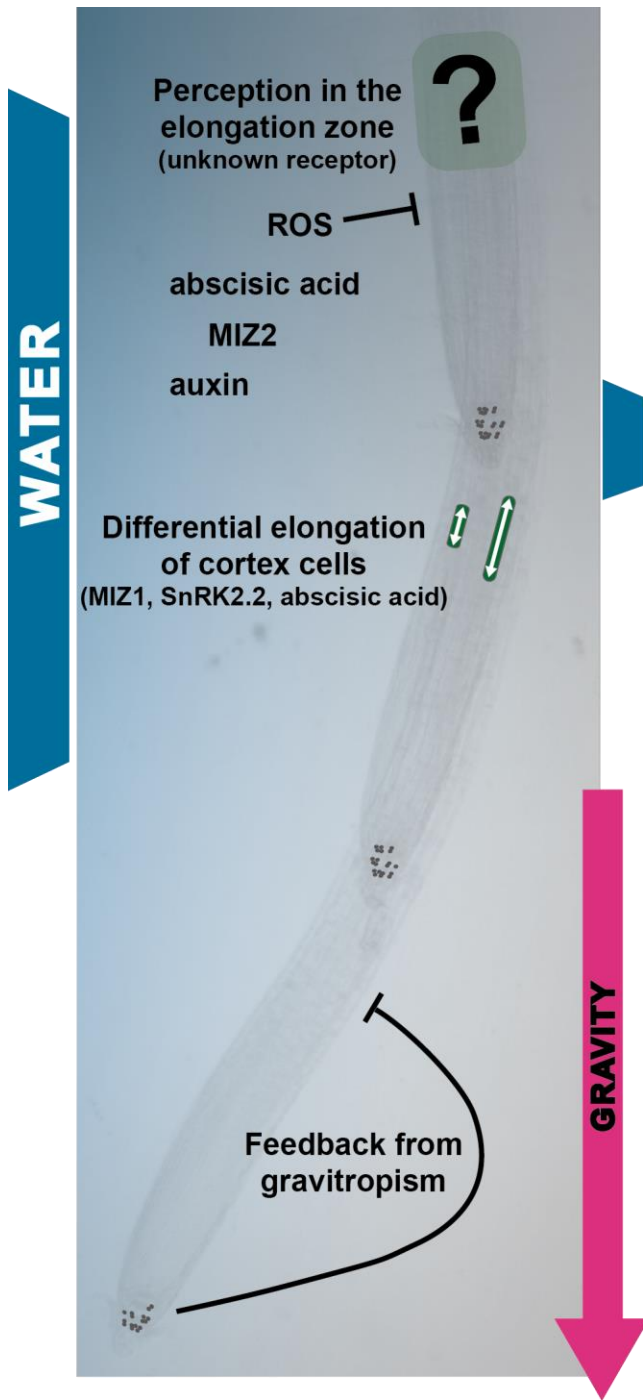1048 mechanism.

1049

Figure 1

1054   **Figure 2**

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1056

Figure 3