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Abscisic acid coordinates dose-dependent developmental and hydraulic responses of roots to water deficit

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1 **Short title: ABA Mediates Root Responses to Water Deficit**

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3

4 **Article title: Abscisic Acid Coordinates Dose-dependent Developmental and**
5 **Hydraulic Responses of Roots to Water Deficit**

6

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9 **France.**

10 **One-sentence summary:** Similar bell-shaped dose-dependent effects of water deficit
11 (WD) and ABA on root development and hydraulics as well as analysis of ABA synthesis
12 and response mutants suggest that ABA acts as a coordinator of root responses to WD.

13

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19 writing of the article.

20 The authors responsible for distribution of materials integral to the findings presented
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28

29 **ABSTRACT**

30 Root water uptake is influenced by root system architecture, which is determined by
31 root growth and branching and the hydraulics of root cells and tissues. The
32 phytohormone abscisic acid (ABA) plays a major role in the adaptation of plants to
33 water deficit (WD). Here we addressed at the whole-root level in *Arabidopsis thaliana*
34 the regulatory role of ABA in mechanisms that determine root hydraulic architecture.
35 Root system architecture and root hydraulic conductivity (L_{pr}) were analyzed in
36 hydroponically-grown plants subjected to varying degrees of WD induced by various
37 polyethylene glycol (PEG) concentrations. The majority of root traits investigated,
38 including first- and second-order lateral root production and elongation and whole-root
39 hydraulics, had a bell-shaped dependency on WD, displaying stimulation under mild WD
40 conditions (25 g PEG L⁻¹) and repression under more severe conditions. These traits also
41 showed a bell-shaped dependency on exogenous ABA, and their regulation by WD was
42 attenuated in genotypes altered in ABA biosynthesis and response. Thus, we propose
43 that ABA acts as a coordinator and an integrator of most root responses to mild and
44 moderate WD, whereas responses to strong WD (150 g PEG L⁻¹) are largely ABA-
45 independent. We also found that roots exhibit different growth responses to both WD
46 and ABA depending on their rank and age. Taken together, our results give further
47 insights into the coordinated water acquisition strategies of roots deployed in relation to
48 WD intensity.

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56 INTRODUCTION

57 Water deficit (WD) is now recognized as the abiotic stress with the greatest effect
58 on crop productivity (Comas et al., 2013). Thus, understanding how plants use water for
59 optimal biomass production has become a fundamental issue worldwide (Koevoets et
60 al., 2016). Plants are sessile organisms that cannot escape from environmental
61 constraints and, as a result, have evolved numerous adaptive responses at molecular,
62 cellular and physiological levels. When exposed to WD, plants first respond by a strict
63 regulation of stomatal aperture together with rapid changes in root hydraulic
64 conductivity (L_{pr}) (Maurel et al., 2010; Sutka et al., 2011; Rosales et al., 2012). Over a
65 longer term, plants change both shoot growth (to restrict water loss) and root growth
66 and differentiation to modulate their capacity to take up soil water (Deak and Malamy,
67 2005; Koevoets et al., 2016).

68 Root system architecture (RSA) refers to the three-dimensional organization of
69 the root system. The model plant *Arabidopsis thaliana* has been widely used to unravel
70 the molecular and genetic bases of root traits and adaptive responses. In this species, the
71 root system is formed through a reiterative program in which lateral roots (LRs) are
72 produced along the primary root (first-order LRs) or along LRs themselves (second-,
73 third-, etc., order LRs). Despite this apparent simplicity, root development is highly
74 flexible and can adjust to the environment to optimise soil foraging and nutrient and
75 water uptake. More generally, the depth of rooting is an important parameter for
76 foraging for water when deep water is available (Lynch, 1995; Uga et al., 2013), but the
77 overall distribution of roots has received a lesser attention as it displays a significant
78 degree of plasticity in response to heterogeneous distribution of soil resources
79 (Koevoets et al., 2016). When water availability is limited, plants firstly reduce shoot
80 development leading to a shift in their allometry (metrics of root to shoot relationships)
81 and then reduce root development depending on the constraint extent and duration
82 (reviewed by Comas et al., 2013; Pierick and Testerink, 2014; Koevoets et al., 2016).
83 However, several studies in maize (*Zea mays*) (Sharp and Davies, 1979, Dowd et al.,
84 2018) and *Arabidopsis* (Van der Weele et al., 2000, Li et al., 2017) have reported that a
85 mild WD can transiently stimulate root growth. There is, however, no consensus on the
86 mechanisms underlying these responses.

87 Whereas long-distance water transport mostly occurs through xylem vessels,
88 water uptake by roots first requires transport through living tissues that is mainly
89 mediated by aquaporins, which are water channel proteins that facilitate water
90 transport across cell membranes. Many reports show that Lp_r is highly dependent on the
91 content of the soil in water, nutrients, or oxygen and on biotic interactions. This control
92 largely relies on the coordination of transcription, post-translational modifications and
93 sub-cellular trafficking of aquaporins (Maurel et al., 2015). For instance, direct exposure
94 of roots to water stress results in inhibition of aquaporin activity and hydraulic
95 conductivity at the cell or root system levels (Boursiac et al., 2005; Hachez et al., 2012;
96 Sutka et al., 2015). However, some plant species or *Arabidopsis* accessions showed an
97 enhancement of Lp_r under moderate stress (Hachez et al., 2012; Sutka et al., 2015).

98 Abscisic acid (ABA) has been recognised as the main plant stress hormone
99 playing a major role during drought responses (Parent et al., 2009; Cutler et al., 2010;
100 Wilkinson et al., 2012; Hong et al., 2013). Under WD conditions, ABA rapidly
101 accumulates inducing stomatal closure to reduce water loss through transpiration
102 (Zhang and Davies, 1987; Wilkinson et al., 2012) and, subsequently, a general inhibition
103 of plant growth (Parent et al., 2009; Cutler et al., 2010; Rowe et al., 2016). Furthermore,
104 high levels of exogenous ABA inhibit shoot and root growth under well-watered
105 conditions (Ghassemian et al., 2000; van der Weele et al., 2000; De Smet et al., 2003;
106 Deak and Malamy, 2005; Xiong et al., 2006, Harris, 2015; Rowe et al., 2016). Besides
107 these well-characterised responses, analysis in *Medicago truncatula* suggested a positive
108 role for ABA in the establishment and maintenance of root meristem function (Liang et
109 al., 2007) and in stimulation of root elongation (Yang et al., 2014). Similarly, root
110 development of maize plants with reduced endogenous ABA content was more
111 repressed in response to drought than that in control plants, indicating that ABA plays a
112 role in maintaining root elongation under low water potentials (Saab et al., 1990). Other
113 studies have indicated a complex biphasic effect of exogenous ABA application on root
114 growth under well-watered conditions where low concentrations of ABA stimulated
115 root growth of *Medicago* (Gonzalez et al., 2015), rice (*Oryza sativa*) and *Arabidopsis* (Xu
116 et al., 2013). As this response was similar to the biphasic effect of increasing levels of
117 WD, Xu et al. (2013) and Li et al. (2017) proposed that primary root adaptive response
118 to WD was controlled by ABA. More recently, ABA has also been identified as a key
119 component of several major adaptive responses to local WD (reviewed by Scharwies and

120 Dinneny, 2019). Firstly, Dietrich et al. (2017) have shown that local activation of ABA
121 signalling in cortical cells of the elongation zone of Arabidopsis roots controls their
122 hydrotropic response *i.e.* their growth towards water. Secondly, Orman-Ligeza et al.
123 (2018) reported that transient water deficit at the tip of cereal roots resulted in
124 xerobranching, a local inhibition of lateral root formation that is dependent on the
125 PYR/PYL ABA signalling pathway. Finally, ABA and auxin signalling pathways have been
126 shown to control hydropatterning *i.e.* preferential positioning of lateral roots towards
127 higher water availability (Bao et al., 2014, Orsa-Puente et al., 2018 and Robbins and
128 Dinneny 2018)

129 ABA also exerts contrasting effects on root hydraulic conductance (Hose et al.,
130 2000; Thompson et al., 2007; Parent et al., 2009). When applied in the micromolar range
131 (0.1–1 μM), ABA rapidly enhances the hydraulic conductivity of both cortical cells (Hose
132 et al., 2000) and whole roots of maize (Parent et al 2009, Fan et al., 2015), whereas
133 higher ABA concentrations (5–100 μM) inhibit the L_{pr} of maize (Aroca et al., 2003) and
134 soybean (*Glycine max*; Markhart et al., 1979) The hydraulic effects of ABA are largely
135 mediated through tissue-specific regulation of aquaporins. For instance, hydraulic
136 conductance in maize roots closely follows the amount of aquaporin transcripts (Parent
137 et al., 2009; Caldeira et al., 2014) and proteins (Fan et al., 2015) upon changes in ABA
138 content following water deficit. A key role of phosphorylation in ABA-dependent
139 regulation of aquaporins was also observed in Arabidopsis guard cells (Grondin et al.,
140 2015).

141 In the present work, we investigated how WD alters the ability of the plant root
142 system to acquire water, by connecting effects on root hydraulics and root growth and
143 development. The latter effects were addressed from an elementary developmental
144 process, LR formation, up to whole-root architecture in Arabidopsis adult plants grown
145 in hydroponics under increasing PEG concentrations to induce a wide range of WD
146 levels. This experimental set-up allowed us to observe a stimulation of both root
147 development and hydraulics under mild WD whereas severe WD had a repressive effect.
148 Our results showed that ABA controls and coordinates both the developmental and
149 physiological responses. These findings bring further insights into the mechanisms and
150 significance of adaptive control of root hydraulic architecture depending on WD
151 intensity.

153 **RESULTS**

154 **Root Development Exhibits a Bell-shaped Dose-dependent Response to Water**
155 **Deficit**

156 To analyze the adaptive responses of root development to WD, Arabidopsis
157 plants were grown hydroponically under control conditions for 18 days after sowing (18
158 DAS) and also for an additional 5 days (23 DAS) in the presence of various
159 concentrations of high molecular weight polyethylene glycol (PEG-8000). With respect
160 to experiments in soil or agar plate, this procedure provides access to the RSA of fully
161 developed plants and allows examining the effects of water stress, independently of any
162 ion stress, mechanical impedance, or stress heterogeneity. In addition, the procedure
163 allows a parallel monitoring of root water transport capacity. Whereas a control
164 hydroponic solution shows a water potential of -0.034 MPa, three WD treatments were
165 imposed by addition of 25, 75 or 150 g L^{-1} PEG yielding water potentials of -0.047 MPa
166 (25PEG), -0.103 MPa (75PEG) and -0.332 MPa (150PEG), respectively. Pronounced
167 modifications in root growth and architecture were observed after 5 days of treatment
168 (Fig. 1A). Total root length and root biomass (Fig. 1B-C) showed a bell-shaped dose
169 dependency, with an increase up to 10% in response to 25PEG whereas a reduction was
170 recorded after exposure to 75PEG or 150PEG. To explore the developmental
171 mechanisms underlying this response, we analysed in detail the morphology of root
172 parts developed within the 5 days of PEG treatment, considering independently the
173 primary root and the firstly-formed (oldest) LRs. These parts will be further referred to
174 as Primary Root After Treatment (PRAT) and Lateral Root After Treatment (LRAT),
175 respectively. The PRAT and LRAT were identified based on three criteria: (i) the length
176 of the primary root and oldest LRs before the PEG treatments, (ii) root morphological
177 modifications (i.e. root curvature, root thickening, root hair length, etc.) induced by PEG
178 and visualised under a microscope at the end of the experiment, and (iii) changes in the
179 slope between the position of the LRs and their distance to the root tip (Supplemental
180 Fig. S1) (see Experimental Procedures for more details).

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182 The PRAT length showed a progressive reduction by 18 to 84% with increasing
183 PEG concentrations (Fig. 1D). On the contrary, the LRAT length was stimulated by 12%

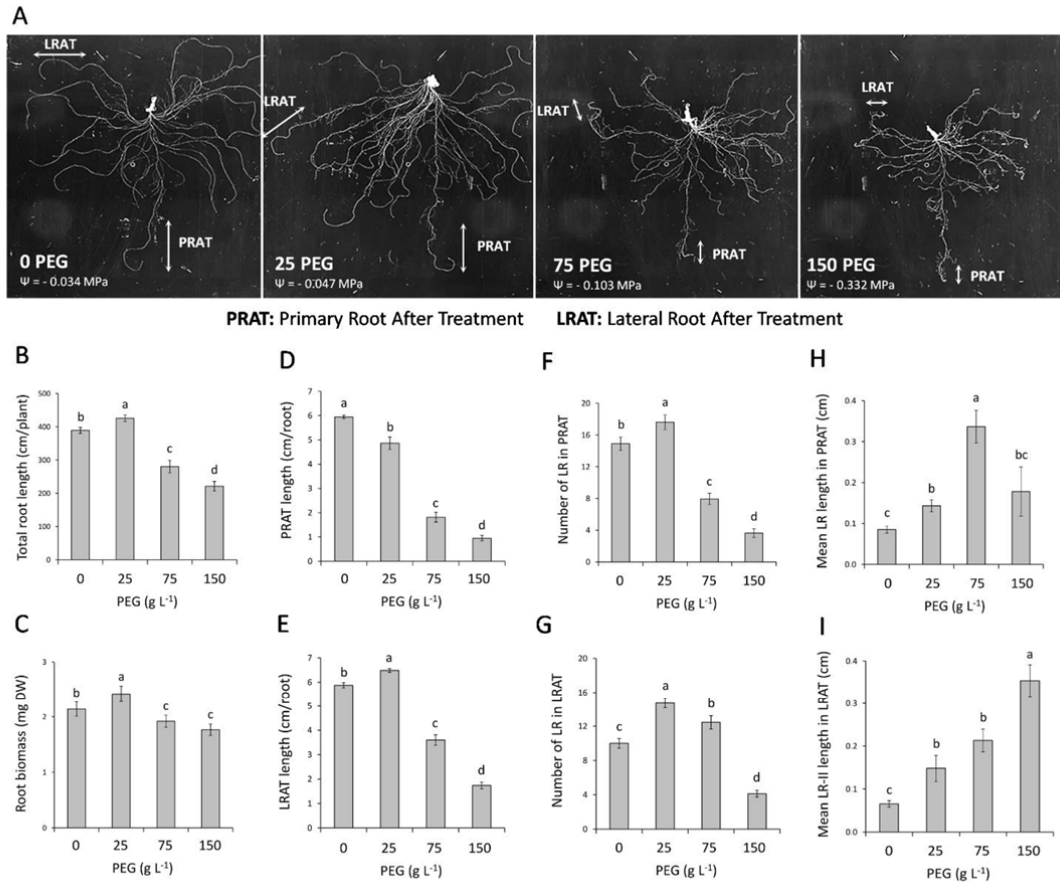


Figure 1. Changes of RSA in response to WD.

Plants were grown until 18DAS in hydroponics under standard conditions and subjected for 5 additional days (23DAS) to the indicated PEG-8000 treatments. (A) Representative images showing the whole root system architecture and the parts of the primary root grown after treatment (PRAT) and of the oldest LR grown after treatment (LRAT) used for specific analyses. Whole root systems were used to determine total root length (B) and biomass production (C) per plant. The length of the PRAT (D) and the LRAT (E), the number of LRs formed in the PRAT (F), the number of second order LRs formed in the LRAT (G), the mean length of LRs formed in the PRAT (H), and the mean length of second order LRs formed in the PRAT (I) per root are shown. Mean values \pm SE were obtained from plants grown in three independent experiments ($n = 20-25$). ‘Homogeneous group’ statistics were calculated through ANOVA, where mean values with different letters are significantly different according to LSD test at $P \leq 0.001$.

184 at 25PEG and repressed by 38 and 67% at 75PEG and 150PEG, respectively (Fig. 1E).
 185 The number and mean length of LRs in both PRAT and LRAT parts also exhibited a bell-
 186 shaped dose-dependent response to WD (Fig. 1F-I). Yet, we observed both a stronger
 187 stimulation and a lower sensitivity for inhibition of LR development in LRAT compared
 188 to that for PRAT. For instance, 75PEG caused 47% repression (Fig. 1F) and 20%
 189 stimulation (Fig. 1G) of LR number in PRAT and LRAT, respectively. Similarly, 150PEG

190 had no-significant effect on mean LR length in PRAT (Fig. 1H) whereas it induced
191 stimulation by 450% in LRAT (Fig. 1I). We also investigated the LR development in
192 sections of the primary root produced prior to the PEG treatment (Supplemental Fig.
193 S2A). The 75PEG and 150PEG treatments stimulated the mean LR length (up to 86%) in
194 the 0–2 cm section whereas a repression of 60% was induced in the 2–4 cm section
195 (Supplemental Fig. S2B-C). On the contrary, the WD did not change the LR number in
196 these sections (Supplemental Fig. S2F-G). Taken together, our results indicate that RSA
197 exhibits bell-shaped dose-dependent responses to WD, with overall stimulation
198 responses at mild (25PEG) or moderate (75PEG) WD, and a general repression under
199 severe WD (150PEG). In addition, a differential sensitivity to WD was observed between
200 primary and oldest LRs, the former showing lower stimulatory responses associated
201 with a higher sensitivity to WD.

202 **ABA Accumulates under WD and Exerts both Stimulatory and Repressive Effects** 203 **on RSA**

204 To investigate whether ABA participates in the root growth responses described
205 above, we first quantified ABA accumulation in the same conditions. At the whole-root
206 level, no significant alteration in ABA concentrations was induced by mild or moderate
207 WD whereas a three-fold increase was detected at 150PEG (Fig. 2A). We next analysed
208 the expression of three ABA responsive genes: *ras-related small GTPase homologue B18*
209 (*RAB18*), *responsive to desiccation 29A (RD29A)* and *responsive to desiccation 29B*
210 (*RD29B*) (Leonhardt et al., 2004; Fujii et al., 2007). The mRNA abundance of these genes
211 showed a gradual increase with increasing WD levels, up to 15-fold at 150PEG (Fig. 2B).
212 Therefore, our results suggest that PEG treatments induce an accumulation and gradual
213 response to ABA in relation to WD intensity.

214 Several studies have shown that exogenous ABA, usually delivered at high
215 concentrations, can dramatically modify root growth and architecture (reviewed in
216 Harris, 2015; Rowe et al., 2016). In order to thoroughly explore the role of ABA in
217 shaping root developmental responses to WD, we investigated the long-term (5 days)
218 effects of low exogenous ABA concentrations (up to 250 nM) in hydroponically grown
219 plants. qRT-PCR showed that the expression levels of *RAB18*, *RD29A* and *RD29B* were
220 gradually enhanced with increasing ABA concentrations to reach a 3 to 4-fold increase in
221 mRNA abundance in response to the 250 nM ABA treatment (Supplemental Fig. S3).

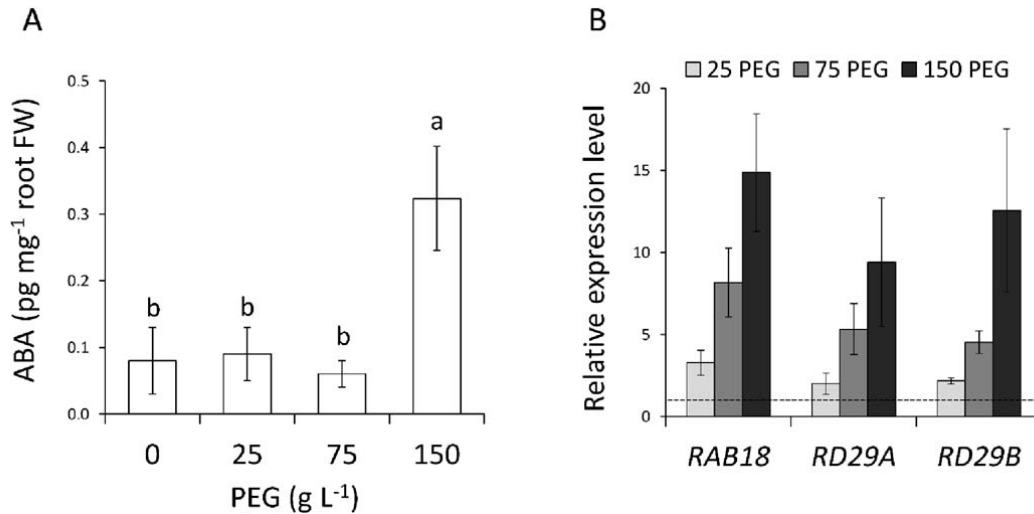


Figure 2. Effects of WD on ABA abundance and expression of ABA-regulated genes.

ABA concentrations (A) and transcript abundance of ABA-regulated genes *RAB18*, *RD29A* and *RD29B* in roots (B) of plants subjected from 18 DAS to a 5-day-long treatment with the indicated PEG concentrations. The dotted line in (B) represents the normalized expression level in control conditions. Mean values \pm SE were obtained from 15 plants grown in three independent experiments ($n = 3$). ‘Homogeneous group’ statistics were calculated through ANOVA where mean values with different letters are significantly different according to LSD test at $P \leq 0.05$ (A). FW, fresh weight.

222 Exogenous ABA application revealed bell-shaped dose-dependent responses of growth,
 223 with an increase in the total root length and root biomass at the lowest ABA
 224 concentration (15 nM) and an inhibition by up to 25% at the highest ABA concentrations
 225 (Fig. 3A-B). These responses were further dissected at the levels of PRAT and LRAT.
 226 Whereas PRAT length essentially showed an inhibition at 250 nM (Fig. 3C), a significant
 227 stimulation of LRAT length was recorded along the three lowest ABA treatments (15–
 228 100 nM) (Fig. 3D). With respect to LR number, PRAT showed a mild (+22%) stimulation
 229 only at 15 nM ABA whereas LRAT exhibited a more pronounced (up to 71%) stimulation
 230 at all ABA concentrations investigated (Fig. 3F). Mean LR length was stimulated by ABA
 231 in both PRAT and LRAT (Fig. 3G-H), but with a distinct dose-dependency since a
 232 stimulation (+116%) or a lack of effect was observed at the highest ABA treatment for

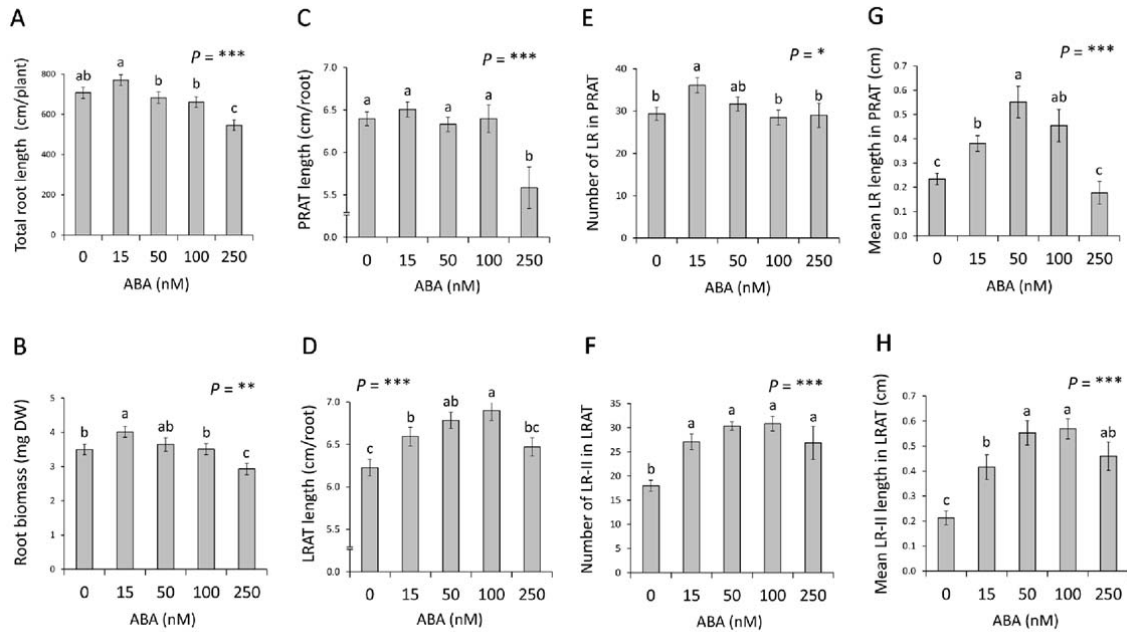


Figure 3. Effects of exogenous ABA on RSA.

Plants grown in hydroponics were subjected at 18 DAS for 5 additional days (23DAS) to the indicated concentrations of exogenous ABA. The figure shows the total root length (A) and the root biomass (B) per plant, the length of PRAT (C) and LRAT (D), the number of LRs formed in the PRAT (E), the number of second order LRs formed in the LRAT (F), the mean length of LRs formed in the PRAT (G), and the mean length of second order LRs formed in the LRAT (H) per root. Mean values \pm SE were obtained from plants grown in three independent experiments ($n = 15-20$). ‘Homogeneous group’ statistics were calculated through ANOVA, where mean values with different letters are significantly different according to LSD test at $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$.

233 LRAT (Fig. 3H) and PRAT (Fig. 3G), respectively. Finally, when considering the primary
 234 root formed prior to ABA treatment, a strong stimulation of mean LR length (up to 80%)
 235 was induced in the 0–2 cm section by the 50 nM ABA treatment whereas no response
 236 was recorded in the 2–4 cm section (Supplemental Fig. S2D-E). LR number was also
 237 insensitive to ABA in both sections (Supplemental Fig. S2H-I). Taken together, our
 238 results demonstrate that RSA shows bell-shaped responses to exogenous ABA, with
 239 stimulation at low ABA concentrations (15–100 nM) and repression at higher
 240 concentrations (250 nM). In addition, dose-response curves point to a lower response
 241 amplitude but a higher sensitivity for LR formation and elongation in the primary root
 242 (PRAT) compared to that in the preformed LRs (LRAT).

243 ABA Mediates the Effects of WD on RSA

244 The responses of RSA to exogenous ABA, which largely recapitulate those
245 observed in response to WD (Fig. 1), prompted us to investigate further the involvement
246 of ABA in the root adaptive responses to WD. Our analyses were based on comparison of
247 wild-type plants (Col-0) with mutants deficient in ABA synthesis (*aba2-1*; Schwartz et
248 al., 1997) or with altered ABA signalling. In the latter case, we used *snrk2.2 snrk2.3* (Fujii
249 et al., 2007) and *hab1-1 abi1-2* (Saez et al., 2006) double mutants, which show a lower
250 and higher sensitivity to ABA, respectively. Under control conditions, the root and shoot
251 growth of *aba2* was strongly affected with respect to that in Col-0 (Supplemental Fig.
252 S4A-C). A significant but lower shoot and root growth reduction (by approximately
253 25%) was also observed in *snrk2.2 snrk2.3*, whereas growth was unchanged in the *hab1-1*
254 *abi1-2* hypersensitive mutant (Supplemental Fig. S4A-C and Supplemental Table 2).
255 Under mild WD (25PEG), all mutant lines lost the growth stimulation observed for Col-0
256 (Supplemental Fig. S4A-C). On the contrary, under severe WD (150PEG), *hab1-1 abi1-2*
257 showed a higher growth reduction by up to 50% (under 150PEG) in comparison to that
258 in Col-0, whereas growth of both *aba2* and *snrk2.2 snrk2.3* was not or only slightly
259 altered (Supplemental Fig. S4A-C). Root development was further dissected at the levels
260 of PRAT and LRAT. Considering PRAT length (Fig. 4A), all genotypes but *snrk2.2 snrk2.3*
261 showed qualitatively similar sensitivity to mild WD (Supplemental Table 2). In contrast,
262 when considering LRAT length and LR number in PRAT and LRAT (Fig. 4B-D), both *aba2*
263 and *snrk2.2 snrk2.3* lacked the stimulation of LR growth and formation that was induced
264 by mild WD (25PEG) in Col-0, whereas a significant LR inhibition was found in *hab1-1*
265 *abi1-2* (Fig. 4B-D). In response to moderate WD (PEG75), *aba2* showed a lower
266 inhibition of the LR growth and formation than that in Col-0, whereas *hab1-1 abi1-2* had
267 an enhanced repression of the root development (Fig. 4B-D).

268 To further investigate these differences in sensitivity, we performed a more
269 detailed analysis of LR growth and development (Fig. 4E-F). As described previously,
270 increasing WD induced a stimulation of the LR elongation in Col-0 PRAT. This response
271 was lost in *aba2* and *snrk2.2 snrk2.3* and strongly attenuated in *hab1-1 abi1-2*. However,
272 the latter genotype showed a greater repression for highest WD levels (Fig. 4E). The
273 most striking difference was observed in LRAT, where moderate and severe WD
274 strongly stimulated the LR elongation in Col-0 whereas they dramatically repressed the
275 LR elongation in *hab1-1 abi1-2* (Fig. 4F). We also investigated the LR development in
276 sections of the primary root that were produced prior to the PEG treatment (see

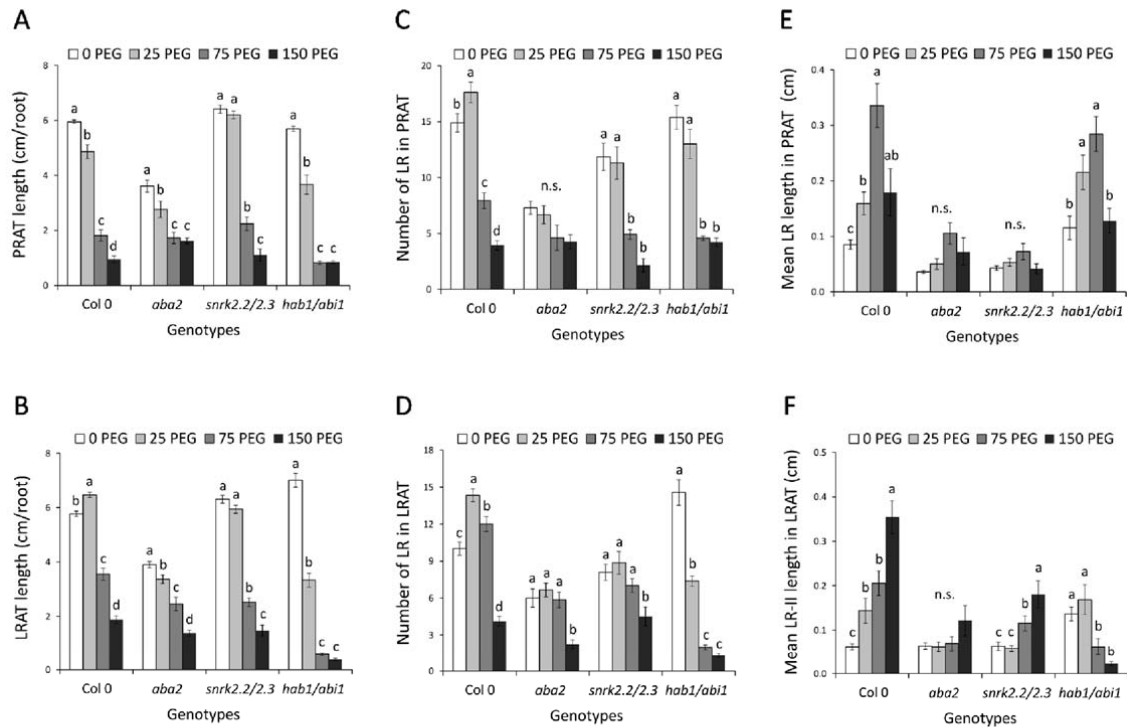


Figure 4. Effects of WD on RSA of ABA biosynthesis (*aba2*) and response (*snrk2.2 snrk2.3*; *hab1 abi1*) mutants.

Plants grown in hydroponics were subjected at 18 DAS for 5 additional days (23DAS) to the indicated PEG concentrations. Three mutants including *aba2* (an ABA biosynthesis deficient mutant), *snrk2.2 snrk2.3* (an ABA signaling mutant) and *hab1 abi1* (an ABA signaling hypersensitive mutant), were used in addition to the Col-0 WT control. The RSA was analysed by several parameters: the length of the PRAT (A) and the LRAT (B), the number of LRs formed in PRAT (C), the number of second order LRs formed in the LRAT (D), the mean length of LRs formed in the PRAT (E), and the mean length of second order LRs formed in the LRAT (F) per root. Open boxes, light grey, dark grey and black boxes represent values for plants grown in PEG25, PEG75 and PEG150 conditions, respectively. Mean values \pm SE were obtained from plants grown in three independent experiments ($n = 10-20$). 'Homogeneous group' statistics were calculated through ANOVA for each genotype, where mean values with different letters are significantly different according to LSD test at $P \leq 0.05$, representing n.s. as not significant ($P > 0.05$).

277 Supplemental Fig. S2A for illustration). Whereas increasing WD stimulated the mean LR
 278 length in the 0–2 cm section of Col-0 as described above (Supplemental Fig. S2B-C and
 279 Supplemental Table S2), this bell-shaped response was lost in ABA mutants
 280 (Supplemental Fig. S4E). By contrast, the WD-induced inhibition of the LR length
 281 observed in the 2–4 cm section of Col-0 (Supplemental Fig. S2F) was unaffected in the
 282 mutant genotypes (Supplemental Fig. S4F and Supplemental Table 2). Therefore, the

283 distinct WD phenotypes of ABA biosynthesis and sensitivity mutants confirm that ABA
284 plays a crucial role in most of the adaptive responses of RSA to WD.

285 **Root Hydraulics Exhibits an ABA-dependent Bell-shaped Dose Response Curve to** 286 **WD**

287 To investigate a putative coordination of root developmental and physiological
288 responses to WD, we next investigated the root hydraulics of the same genotypes under
289 PEG and ABA treatments as above. In agreement with these ideas, the whole-root
290 hydraulic conductance (L_o) of Col-0 plants showed a bell-shaped response to increasing
291 PEG concentrations (Supplemental Fig. S5A). The L_{p_r} , as deduced by normalization of L_o
292 to root size (dry weight), exhibited a similar dose-response curve with stimulation and
293 inhibition by 30% and 51% at 25PEG and 150 PEG, respectively (Fig. 5A). When the
294 measured water flow was normalized to the total root length instead of the dry weight,
295 the maximal stimulation and inhibition of L_{p_r} were of 40% and 36%, respectively
296 (Supplemental Fig. S5B). Interestingly, L_{p_r} also showed a bell-shaped dependency to
297 exogenous ABA concentrations with a mild stimulation (12%) at 15 nM and an
298 inhibition down to 20% at 100 and 250 nM ABA (Fig. 5B). Similar effects were observed
299 when considering L_o (Supplemental Fig. S5C) or normalizing L_{p_r} to the root length
300 (Supplemental Fig. S5D).

301 Under control conditions, and by comparison to that in Col-0, *aba2* and *snrk2.2*
302 *snrk2.3* showed a reduction in L_{p_r} by 56 and 21%, respectively, whereas *hab1-1 abi1-2*
303 showed stimulation by 30% (Fig. 5C and Supplemental Table 3). However, L_{p_r} of the
304 three mutant genotypes was insensitive to mild and moderate WD (25PEG and 75PEG)
305 whereas it showed a strong repression in response to 150PEG (Fig. 5C). These combined
306 physiological and genetic approaches indicate that ABA is involved in the stimulation of
307 L_{p_r} by mild WD (25PEG) and can reduce L_{p_r} at the highest concentration investigated. In
308 contrast, ABA plays a minor role, if any, in L_{p_r} inhibition under severe WD (150PEG).

309 Water uptake in the Arabidopsis root is mediated in large part by aquaporin
310 water channels. To estimate their contribution to the modulation of L_{p_r} by WD and ABA,
311 we applied azide, a well-characterized aquaporin blocker (Sutka et al., 2011), and
312 measured the residual L_{p_r} . Under control conditions, aquaporins accounted for more
313 than 75% of L_{p_r} (Fig. 5A) as described earlier (Sutka et al., 2011). Furthermore, we

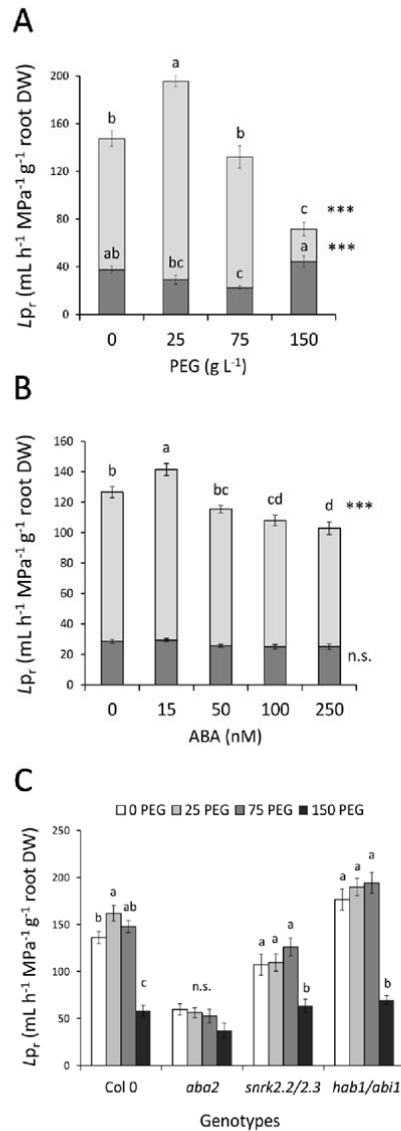


Figure 5. Effects of WD and exogenous ABA on root hydraulic properties of wild-type and ABA biosynthesis (*aba2*) and response (*snrk2.2 snrk2.3; hab1 abi1*) mutants.

Plants grown in hydroponics were subjected at 18 DAS and for 5 additional days (23 DAS) to the indicated concentrations of PEG (A and C) and ABA (B), and different genotypes (C) were used as described previously. For A and B, the light and dark grey boxes represent the measured L_{p_r} and residual L_{p_r} after the aquaporin activity inhibition, respectively. For C, open boxes, light grey, dark grey and black boxes represent L_{p_r} values for plants grown in PEG25, PEG75 and PEG150 conditions, respectively. Mean values \pm SE were obtained from plants grown in three independent experiments ($n = 20-25$). 'Homogeneous group' statistics were calculated through ANOVA, where mean values with different letters are significantly different according to LSD test at $P \leq 0.05$ (C). For A and B, levels of significance are represented as *** $P \leq 0.001$, and n.s. as not significant ($P > 0.05$).

314 found a mild effect of WD on residual L_{p_r} specifically at 75PEG (Fig. 5A), whereas none
 315 of the ABA treatments altered this parameter (Fig. 5B). Thus, the bell-shaped effects of
 316 PEG and ABA application on L_{p_r} can be mostly accounted for by the modulation of root

317 aquaporin function. To address this point further, we analysed the mRNA abundance of
318 all members of the *Plasma membrane Intrinsic Protein (PIP)* subfamily and of four highly
319 expressed members of the *Tonoplast Intrinsic Protein (TIP)* subfamily. No significant
320 variation was observed in response to either mild WD (PEG25) (Supplemental Fig. S6A)
321 or exogenous ABA (Supplemental Fig. S6B). In contrast, a moderate or severe WD
322 (PEG75 or 150) reduced the mRNA abundance of several aquaporin genes, with the
323 strongest repression observed for *PIP1;5*, *PIP2;4*, *PIP2;7* and two *TIPs* (*TIP1;1*, *TIP1;2*).
324 Noticeably, *PIP1;4* and *PIP2;5* expression was induced 2 and 3 fold, respectively.
325 Interestingly, expression of *PIP1,4* but not *PIP2,5* was significantly induced by the
326 highest ABA concentration (Supplemental Fig. S6B).

327 Our results indicate that, similar to its effects on RSA, WD induces a double effect
328 on root hydraulics, with a stimulation of aquaporin-dependent water transport at mild
329 WD (PEG25) and a strong repression of the aquaporin pathway at severe WD (PEG150).
330 Under mild or moderate WD, these responses seem to be largely mediated by ABA. In
331 contrast, the inhibition of L_p_r at 150 PEG is ABA-independent and seems to involve an
332 overall repression of aquaporin expression.

333

334 **DISCUSSION**

335 Understanding the mechanisms that underlie root development and hydraulics
336 under drought is of major importance to maintain plant growth under suboptimal
337 conditions. However, the analysis of RSA under drying soil conditions is extremely time
338 consuming and not compatible with fine morphological or functional analyses. Thus,
339 many studies have been conducted on *Arabidopsis* seedlings grown in agar plates and
340 mostly subjected to severe osmotic stress, as induced by high mannitol, sorbitol, NaCl or
341 KCl concentrations. To have a more comprehensive view of adaptive and functional
342 responses of mature plants to WD, we analysed both root architecture and hydraulics of
343 23-day-old adult plants grown in hydroponics in the presence of a wide range of PEG
344 concentrations. PEG is a non-permeant molecule that can consistently reduce water
345 potential in plates (van der Weele et al., 2000; Rowe et al., 2016). As PEG can also induce
346 anoxia (Verslues et al., 1998), the nutrient solution was constantly oxygenated and
347 circulated around roots. These optimized culture conditions triggered a wide range of
348 highly reproducible responses to WD.

349 **WD Exerts Contrasting Dose-Dependent Effects on Root Development**

350 Inhibition of plant growth and development is a common response to WD (Zhu et
351 al, 1998; Deak and Malamy, 2005; Xiong et al 2006; Claeys et al, 2014; Thalmann et al,
352 2016 and reviewed in Comas et al., 2013). Accordingly, we observed here that a severe
353 WD (150PEG) caused a significant reduction in both shoot and root growth (Fig. 1;
354 Supplemental Fig. S7). Unlike in most fields experiments, no change in shoot:root ratio
355 was observed (Supplemental Fig. S7) probably due to the fact that our culture conditions
356 temperature and hygrometry are tightly controlled and nutrient availability is
357 unchanged under WD whereas it is dramatically reduced when soil water content drops.
358 In addition, we observed that a mild WD (25PEG) significantly promoted both shoot and
359 root development giving rise to bell-shaped dose-responses to WD. Fine analysis
360 revealed that this type of response was observed for nearly all analysed RSA parameters
361 (Fig. 1; Supplemental Fig. S2 and S7). Such positive effects of mild WD on root
362 development have been punctually reported for the primary root of *Arabidopsis* (van
363 der Weele et al., 2000; Claeys et al., 2014), or roots of rice (Henry et al., 2011) or maize
364 (Zhu et al., 2010; Dowd et al., 2018) in soil. Interestingly, our fine analysis showed that
365 the adaptive responses to WD were shifted depending on root order, with the primary

366 root being more sensitive than the oldest LR (compare for instance root length or LR
367 numbers in PRAT and LRAT in Fig. 1D and E, F and G, and H and I). Differential
368 responses to WD were also recorded for LR already initiated prior to WD treatment
369 (Supplemental Fig. S2B-C). Thus, elongation of non-emerged or juvenile LR was
370 stimulated by mild and moderate WD in the 0–2 cm part of the primary root whereas it
371 was repressed for older LR in the 2 cm section above. We also note that growth
372 stimulation of Col-0 roots by a 25PEG treatment was not unspecific, as this response was
373 lost in ABA mutants (Fig. 4) and could be recapitulated in Col-0 seedlings grown in agar
374 plates using small concentrations (25 mM) of sorbitol (Supplemental Fig. S8).

375 Taken together, these results highlight a complex response of the root system to
376 WD with different sensitivity depending of root age and branching level (see model in
377 Figure 6). In brief, i) the primary root appears to be more sensitive than LR to WD, ii)
378 first-order LR are more sensitive than second-order LR and, iii) LR elongation is less
379 repressed than LR emergence. Accordingly, combination of these WD responses gives
380 rise to a more compact root system, which is highly branched and carries elongated LR
381 at its periphery (see PEG75 and PEG150 in Fig. 1A).

382 A similar differential adaptive response of primary and LR growth has been
383 reported by Julkowska et al. (2014) in Arabidopsis plants under salt stress. In contrast,
384 studies in maize (Gao and Lynch, 2016), rice (Uga et al., 2013; Sandhu et al., 2016) and
385 wheat (Awad et al., 2018) showed that WD dramatically reduced shoot-born roots and
386 LR development whereas it favoured main root elongation. Such difference may reflect
387 distinct adaptive strategies between monocots and dicots. Indeed, the fibrous root
388 system of monocots, with post-embryonic roots developing from the stem provides a
389 unique opportunity for plant foraging of late season precipitations which is absent in
390 dicots.

391 When considering common drought scenarios, WD usually starts in the upper soil
392 layers and progresses gradually in deeper layers. Our observation that LR formed in
393 both primary and oldest LR are more tolerant to WD than their mother root fits with
394 the idea that progression of the primary root in deep soil layers protects it from severe
395 WD and that newly formed LR experience stronger WD than their genitors as they
396 develop in a soil area already foraged for water by the latter. Thus, when extrapolating
397 our observations under homogenous and constant WD to a developing WD in soil, we

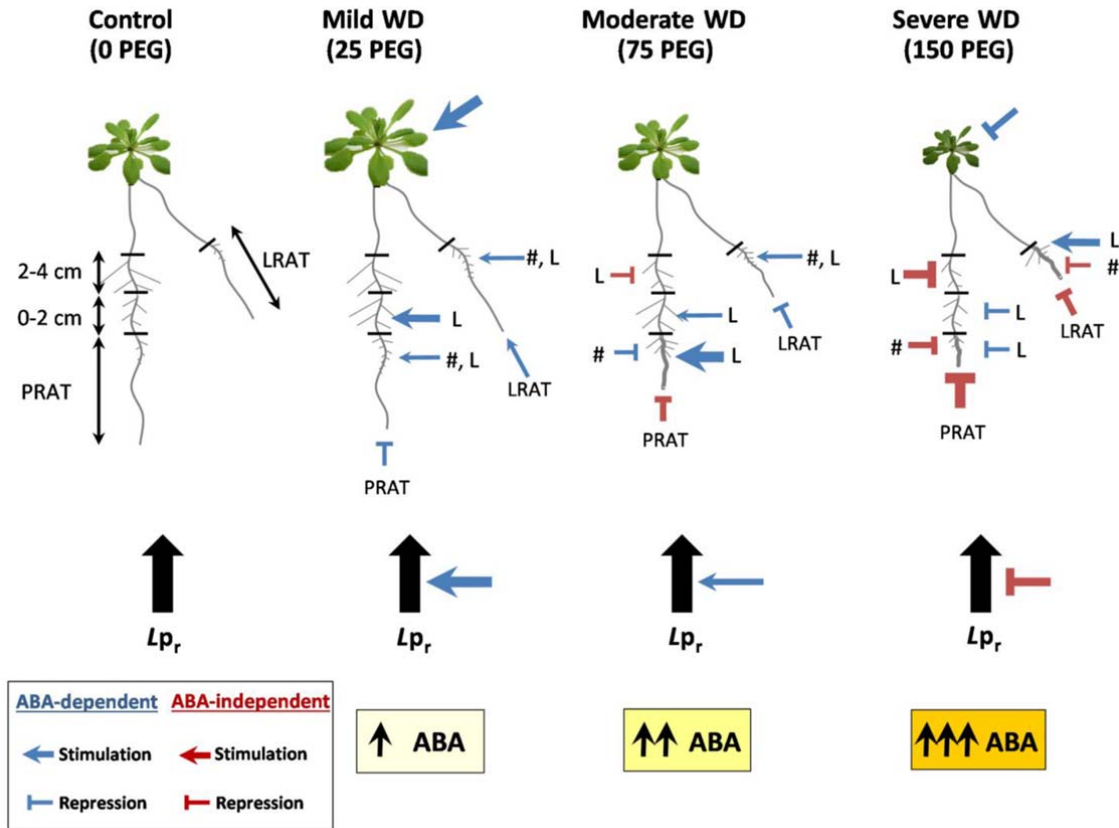


Figure 6. Integrative model of root developmental and hydraulic responses to representative WD intensities.

The different WD treatments are represented by four plants. The root system is schematically represented by a primary root, an old LR and first and second order LRs on the primary and the old LR. The root parameters analyzed are schematically represented and their response to WD is represented by an arrow or a bar for stimulation or repression, respectively. The intensity of the responses is shown by the thickness of the line. PRAT is the part of the primary root grown after treatment, LRAT is the part of the old LR grown after treatment, L and # stand for mean LR length and LR number, respectively. L_{p_r} stands for root hydraulic conductivity. The blue and red color codes represent ABA-dependent or -independent responses, respectively. The thickness of blue and red lines and arrows represent the intensity of response (thicker line represent stronger response). The three yellow boxes represent the root ABA content. Color intensity and number of arrow schematize ABA concentrations.

398 can predict a massive stimulation of RSA mainly due to the progressive development of
 399 LRs from different orders to explore a larger volume of soil. The observed adaptive
 400 responses are also relevant to more severe WD. As surface soil dries, increase of root
 401 length and density in the deepest layers significantly contribute to improving the water
 402 and nutrient acquisition with a limited carbon investment. This adaptive response to
 403 WD agrees with traits found in crops. For instance, cultivars of rice and soybean with
 404 increased root length density (root length per soil volume) in the 35–45 cm layer

405 appeared to have increased water uptake and improved resistance to drought (Carter et
406 al., 1999; Henry et al., 2011).

407 **WD also Exerts Contrasting Dose-Dependent Effects on Root Hydraulics**

408 Root hydraulics (L_{p_r}) is known to rapidly respond to environmental cues, such as
409 water availability, supporting its role in plant adaptation to fluctuating environments
410 (Steudle, 2000; Vandeleur et al., 2009). Accordingly, our measurements indicated that a
411 mild WD enhances Arabidopsis L_{p_r} whereas a severe WD had a repressive effect (Fig. 5).
412 The generally observed decline of L_{p_r} under drought conditions can be interpreted as a
413 means for hydraulically promoting stomatal closure or for limiting a counterflow of
414 water from the root to the drying soil (reviewed in Aroca et al., 2012). Drought-induced
415 increases of L_{p_r} have been reported under more specific conditions, generally under
416 mild drought stress (Siemens and Zwiazek, 2004). This response is thought to promote
417 water uptake as long as the water potential remains higher in the soil than in the root.

418 Previous work from our group has shown that aquaporins contribute to
419 approximately 75–80% of Arabidopsis L_{p_r} (Tournaire-Roux et al., 2003; Boursiac et al.,
420 2005; Sutka et al., 2011; Li et al., 2015). Here, we provide pharmacological evidence that
421 aquaporins can account for most of the stimulation and repression of L_{p_r} under mild and
422 severe WD, respectively (Fig. 5). Yet, no significant change in *PIP* or *TIP* gene expression
423 was observed in response to mild or moderate WD (Supplemental Fig. S6). In contrast,
424 severe WD reduced the mRNA abundance of most aquaporin genes, possibly explaining
425 the parallel inhibition of L_{p_r} . These contrasting results are consistent with other studies
426 indicating that numerous post-transcriptional mechanisms can account for aquaporin
427 regulation in roots under stress (reviewed in Aroca et al., 2012; Li et al., 2015).

428 The observation that both root developmental and hydraulic parameters
429 exhibited similar bell-shaped dose-dependent responses to WD points to an obvious
430 coordination of these responses. Under severe WD (150PEG) for instance, both root
431 development and hydraulics were dramatically repressed to restrict water exchange
432 between roots and their harsh environment. Conversely, a mild WD (25PEG) induced a
433 dual stimulation of root development and root hydraulics to optimize water uptake and
434 support shoot biomass production during the onset of drought. Yet, we found a
435 stimulation of total root length specifically under mild WD (Fig. 1B) whereas L_{p_r} (as

436 normalized by total root length instead of dry weight) was significantly stimulated
437 under both mild and moderate (75PEG) WD (Supplemental Fig. S5B). Thus, moderate
438 WD represses root development (Fig. 1B) while maintaining root hydraulics (Fig. 5A) to
439 possibly maintain shoot hydration (Suppl. Fig. 4A). These responses illustrate different
440 adaptation strategies to increasing levels of WD with differential carbon investment.
441 Under mild WD, carbon is invested in the production of new and longer roots, whereas
442 under moderate WD, carbon is invested in the production of second-order LRs and root
443 thickening to prevent water loss. On the contrary, aquaporin-dependent water uptake
444 that mainly recruits pre-existing proteins is stimulated to improve water uptake. Similar
445 responses to moderate and severe WD were also proposed by Parent et al., (2009)
446 Vandeleur et al. (2009) and Laur and Hacke (2013) who suggested that when soil water
447 is scarce, increased aquaporin abundance and activity play a major role in compensating
448 for reductions in root elongation and surface area production.

449 Another explanation of the increase in L_{pr} under moderate WD may account for
450 the modification of root architecture that under these conditions present a significant
451 increase in number and elongation of newly formed roots (mainly second-order LRs)
452 that may be more active in water uptake. This hypothesis is supported by several
453 reports suggesting that fine or LRs are the most active portion of root system in water
454 uptake (reviewed in Comas et al 2013). Recently, Ahmed et al. (2016; 2018) used heavy
455 water (D_2O) labelling to show that, in maize, up to 80% of water absorption can be
456 accounted for by laterals. Improved hydraulic measurements will have to be developed
457 in the Arabidopsis root to directly test this hypothesis.

458

459 **ABA Acts as an Integrator and Coordinator of Root Responses to WD**

460 It well established that drought and osmotic stress induce an accumulation of
461 ABA in both roots and shoots (Schachtman and Goodger, 2008; Wilkinson et al., 2012,
462 Clays and Inzé, 2013). Besides stomatal closure (Trejo and Davies, 1995; Schroeder et
463 al., 2001), ABA can positively or negatively act on shoot and root growth depending on
464 its concentration or the plant model investigated (Saab et al., 1990; Hooker and Thorpe,
465 1998; Barrero et al., 2005; Zhang et al., 2010; Leach et al., 2011; reviewed by Harris,
466 2015). Root hydraulics is also controlled by ABA but again stimulatory or inhibitory
467 effects can be observed, depending on the experimental conditions and genotypes

468 (reviewed by Aroca et al., 2012). Our aim here was to evaluate how ABA may coordinate
469 the developmental and hydraulic responses of roots over a wide range of WD.

470 When quantifying ABA accumulation at the whole-root level, a significant
471 increase was observed under severe WD only, whereas the expression level of ABA
472 responsive genes was enhanced even under mild WD (Fig. 2B). Although this increase
473 remained limited compared to related studies in *in vitro* grown seedlings (Rowe et al.,
474 2016), it potentially reflects a typical response to WD with possibly undetectable and
475 local accumulations of low ABA amounts. These ideas prompted us to analyse the impact
476 of exogenous ABA applications in the nanomolar range instead of the micromolar range
477 as usually used. Interestingly, expression of the ABA reporter genes showed a slight
478 stimulation that was comparable to that measured under mild WD (Supplemental Fig.
479 S3).

480 When considering RSA, low exogenous ABA concentrations revealed bell-shaped
481 dose-response curves that were reminiscent of those observed under low and moderate
482 WD. Moreover, all developmental parameters positively affected by mild WD were also
483 positively regulated by low ABA concentrations (Fig. 3; Supplemental Fig. S2 and S4).
484 Finally, the differential developmental responses of roots to WD, with young tissues
485 being more responsive than older ones (Fig. 1 and Supplemental Fig. S4), were also
486 observed in the context of exogenous ABA application (Fig. 3 and Supplemental Fig. S4).

487 The idea that ABA mediates a large range of RSA responses to WD was further
488 supported by the analysis of ABA mutants with *aba2* and *snrk2.2 snrk2.3* having an
489 attenuated response of most root developmental parameters to WD whereas the
490 hypersensitive *hab1 abi1* mutant showed enhanced responsiveness.

491 We note, however, that some root responses to WD seem to be ABA-independent
492 (Fig. 6). When considering the primary root (PRAT), its elongation was dramatically
493 repressed by increasing levels of WD (Fig. 1D and Fig. 3A), but to a similar extent in the
494 different ABA mutants (Fig. 4A). Accordingly, exogenous ABA application had little
495 impact on PRAT (Fig. 3C) suggesting that this WD adaptive response is mainly ABA
496 independent. A similar conclusion can be drawn for LRs formed in the 2–4 cm part of the
497 primary root present before transfer (see Supplemental Fig. S2A). On the contrary,
498 elongation of roots in the 0–2 cm part was stimulated by WD (Supplemental Fig. S2C)
499 and this response was typically dependent on exogenous ABA (Supplemental Fig. S2E)

500 and dramatically modified in the ABA mutants (Supplemental Fig. S4E). Taken together,
501 these results suggest that ABA-dependent responses to WD are mainly observed in
502 young roots whereas the adaptive responses of older tissues become ABA independent.

503 Similar to the adaptive response of RSA to WD, we showed that the response of
504 root hydraulics to WD is in large part mediated by ABA. In brief, L_{p_r} of Col-0 showed a
505 bell-shaped dose-dependent response to both WD and exogenous ABA (Fig. 5A and B)
506 and L_{p_r} response to WD was dramatically attenuated in ABA mutants (Fig. 5C). These
507 results establish that ABA coordinates both root developmental and hydraulic response
508 to mild and moderate WD. The complex interactions between RSA, hydraulics and ABA
509 are summarized in the integrative model presented in Figure 6. When considering
510 severe WDs, both the RSA and hydraulic responses were ABA-independent (as
511 illustrated on Fig. 6 by a large number of red symbols). We speculate that, due to the
512 severity of the stress, strong inhibitory responses are governed by local signals,
513 independent of the overall hormonal status of the plant.

514 CONCLUSION

515 In the present study, we showed that mild and somewhat moderate WD stimulate
516 both root growth and root hydraulics. In addition, the fine analysis of root architecture
517 revealed that mild WD mainly stimulates elongation and to a lower extent production of
518 LRs, this response being markedly enhanced in pre-existing LRs. Concomitantly, mild
519 WD significantly stimulates root hydraulics in an aquaporin-dependent manner. Both
520 responses are mediated by ABA. Since under WD, ABA is mainly synthesized in shoots
521 and transported to roots via the phloem (review by Osakabe et al., 2013), we assume
522 that the differential adaptive responses observed in our experiments can be accounted
523 for by either a differential accumulation of ABA due to local regulation of ABA
524 transporters or by differential ABA responses depending on WD level and root tissue
525 types. This non-linear response is well documented for phytohormones and has been
526 extensively studied for auxin. Identifying the molecular basis of these local ABA
527 responses will be critical to better understand how the plant coordinates its RSA and
528 root hydraulic responses, and how these responses can be reshaped to possibly improve
529 the tolerance of crops to water stress. Our work also shows that a severe WD
530 dramatically modifies both RSA and hydraulics and, again, the molecular bases of these
531 ABA-independent regulations remain to be identified. Overall, the experimental

532 framework established in the present work will be crucial to explore the adaptive
533 significance of the multiple responses of roots to WD.

534

535 **MATERIALS AND METHODS**

536 **Plant Materials and Growth Conditions**

537 The *Arabidopsis thaliana* accession Columbia-0 (Col-0 N60000) and the *aba2-1*
538 (Léon-Kloosterziel et al., 1996), *snrk2.2 snrk2.3* (Fuji et al., 2007), and *hab1-1 abi1-2*
539 (Saez et al., 2006) mutants in Col-0 background were used in this work. Seeds were
540 surface sterilized and sown on 1/2 MS agar vertical plates (2.2 g L⁻¹ MS, 1% w/v sucrose,
541 0.05% w/v MES, and 0.8% w/v agar, pH 5.7). Plates were incubated for 2 days at 4°C in
542 dark for stratification. Plants were germinated and further grown on these plates for 9–
543 11 days in a growth chamber at 70% relative humidity (RH) and 20°C, with 16 h/8 h
544 light/dark cycles (250 μE m⁻² s⁻¹). Subsequently, seedlings were transferred to a
545 hydroponic medium containing: 1.25 mM KNO₃, 0.75 mM MgSO₄, 1.5 mM Ca(NO₃)₂, 0.5
546 mM KH₂PO₄, 0.1 mM MgCl₂, 50 μM Fe-EDTA, 50 μM H₃BO₃, 12 μM MnSO₄, 0.7 μM CuSO₄,
547 1 μM ZnSO₄, 0.24 μM MoO₄Na₂, 0.01 μM CoCl₂, 100 μM Na₂SiO₃, 1 mM MES, pH 5.7
548 adjusted using KOH. At 18 days after sowing (DAS), seedlings were transferred for 5
549 days to a fresh medium containing different concentrations (25, 75, or 150 g L⁻¹) of
550 polyethylene glycol 8000 (PEG-8000) to reduce the water potential (Ψ) of the nutrient
551 solution. To avoid anoxia, culture solutions were constantly bubbled with air and
552 recirculated in each basin. The solution was replaced at the middle of the treatment
553 period (at 2.5 days) to avoid bacterial contamination. Ψ was measured with a vapor
554 pressure osmometer WESCOR 5520 (USA) as described by Turner (1981). For ABA
555 treatments, 18-day-old plants were transferred for 5 days to a fresh hydroponic solution
556 containing different concentrations of ABA (15, 50, 100 and 250 nM), obtained using
557 ABA stock solutions in ethanol. Control plants grown in the absence of ABA were mock-
558 treated with a same amount of ethanol (final concentration of 0.00033%). The
559 hydroponic solution was replaced once during the treatment period (at 2.5 days). All
560 experiments were performed at least three independent times. All chemicals were
561 provided by Sigma-Aldrich (Saint Quentin, France).

562 **Analysis of Root System Architecture (RSA)**

563 Root systems were excised at 23 DAS from plants grown in hydroponics (growth
564 was performed for 18 days under control conditions followed by 5 days in the presence
565 of PEG or ABA, as described above), and immediately used for root hydraulic
566 measurements (see below). Subsequently, roots were washed and stored in 20% v/v
567 ethanol prior to analysis of RSA. For imaging, each root system was manually separated
568 on a 240 × 240 mm Petri dish containing water, and scanned with an Epson Perfection
569 V850 Pro scanner (Epson Europe BV, Netherlands). RSA was analysed using the
570 OPTIMAS image analysis software (Adept Turnkey Pty Ltd, Osborne Park WA, Australia).
571 Several parameters were measured. Total root length was quantified on the whole-root
572 system. The PRAT (primary root after treatment) and LRAT (lateral root after
573 treatment) correspond to the parts of primary and lateral roots that were produced
574 during the 5 days treatment. The PRAT and LRAT were precisely observed at the end of
575 the culture using a binocular microscope. Root length measured before transplanting
576 was combined to these microscopic observations in order to identify at the cellular level
577 the morphological changes induced by the PEG or ABA treatment (Supplemental Fig. S1).
578 Microscopic morphological changes (curvature of the root, changes in cell size and a
579 transient increase in distance between lateral roots) were not only observed in response
580 to PEG or ABA but also, though at a much lower extent, in plants grown on a control
581 medium. These morphological changes were found to be very reproducible, probably
582 due to a transient stress induced by manipulating the plants. Finally, plants with no clear
583 differentiation between the initially formed root parts and the PRAT and/or LRAT were
584 discarded for the RSA analysis. Overall, the length of PRAT and LRAT as well as the
585 number and mean length of lateral roots formed in these parts during the treatment
586 were measured. The number and mean length of lateral roots were also determined in
587 the 0–2cm and 2–4 cm parts of the primary root that was produced before treatment
588 application (see Fig. 1A and S2A).

589 **ABA Quantification**

590 For ABA quantification, root and shoot tissues were frozen in liquid nitrogen and
591 ABA was extracted and quantified by the Metatoul platform
592 (https://www6.toulouse.inra.fr/metatoul_eng/, Toulouse, France). Three biological
593 replicates from three independent experiments were used.

594 **Measurement of Root Hydrostatic Hydraulic Conductivity (L_{pr})**

595 A freshly detopped root system was inserted into a pressure chamber filled with
596 the same hydroponic medium as used for each treatment. The hypocotyl was tightly
597 sealed inside a combination of plastic and metallic seals using a low-viscosity dental
598 paste (President Light, Coltene, Switzerland). The rate of pressure (P)-induced sap flow
599 (J_v) exuded from the hypocotyl section was recorded using high-accuracy flow meters
600 (Bronkhorst, France) in an automated manner using a LabVIEW-derived application. In
601 practice, roots excised at 23 DAS from plants grown in standard conditions were
602 subjected to a pre-treatment at 350 kPa for 10 min to attain flow equilibration, and J_v
603 was measured successively at 320, 160, and 240 kPa for about 5 min. For PEG
604 treatments, pressure increments corresponding to the Ψ of the hydroponic solution
605 were applied in order to counteract osmotically-induced water efflux from the root. Root
606 dry weight (DW_r) was analysed after measurement of J_v and RSA. The L_{p_r} ($\text{mL g}^{-1} \text{h}^{-1}$
607 MPa^{-1}) of an individual root system was calculated using the following equation:

$$608 \quad L_{p_r} = J_v / (DW_r \cdot P)$$

609 In NaN_3 experiments, L_{p_r} was derived from continuous J_v measurement at 320 kPa as
610 described in Sutka et al. (2011).

611 **Total RNA Isolation and Analysis**

612 Root samples were frozen in liquid nitrogen and disrupted for 1 min at 30
613 oscillations s^{-1} in a Retch mixer mill MM301 (Switzerland) homogenizer. Total RNA was
614 extracted using TRI REAGENT (Molecular Research Center Inc, OH, USA) and DNA was
615 eliminated by RQ1 RNase-Free DNase (Promega, Madison, WI, USA) according to the
616 manufacturer's instructions. RNA was purified by ethanol precipitation. Two μg of total
617 RNA were used as a template for first strand cDNA synthesis, which was performed
618 using M-MLV Reverse Transcriptase, RNase H Minus, Point Mutant (Promega Madison,
619 WI, USA) and Oligo(dT)15 Primer (Promega Madison, WI, USA) in a final volume of 20
620 μL , according to the manufacturer's instructions.

621 First strand cDNA was diluted 10 times and 2 μL of diluted cDNA solution were
622 used as template for gene expression level quantification by reverse transcription
623 quantitative PCR (RT-qPCR). The latter was performed in 384-well plates with a
624 LightCycler 480 Real-Time PCR System (Hoffmann-La Roche AG, Basel, Switzerland).
625 SYBR Premix Ex Taq (TaKaRa, Ozyme France) was used to monitor cDNA amplification

626 at an annealing temperature of 57°C according to the manufacturer's instructions. SYBR
627 Green Primer efficiencies for each pair were evaluated from the analysis of dilution
628 series of 1, 1:5, 1:10, 1:100 and 1:1000 of a mix of all the diluted first strand cDNA
629 samples and derived from Cp values calculated according to the second derivative
630 maximum method (LightCycler® LC480 II, Hoffmann-La Roche AG, Basel, Switzerland).
631 Three to six biological replicates from three independent plant cultures and two
632 technical repeats were used per treatment in every run. Expression data were
633 normalized to expression of three housekeeping genes, TIP41-like protein (At4g34270),
634 CLATHRIN (At4g24550), and SAND family protein (At2g28390), which were selected as
635 reference genes according to (Czechowski et al., 2005). Data from mutant and control
636 plants were compared using a two-tailed Student's *t*-test. The primer sequences used
637 are listed in Supplemental Table S1.

638 **Statistical Analyses**

639 Statistical analysis was performed using the STATGRAPHICS Centurion XVI
640 software (StatPoint Technologies, Warrenton, VA, USA). One-way ANOVA was
641 performed to determine significant differences between groups of samples, as indicated
642 by different letters. Levels of significance are indicated in the figures by asterisks:
643 * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. Not significant (n.s.) differences correspond to $P > 0.05$.
644 Multiple range comparisons of means were determined by the least significant
645 difference (LSD) test included in the above-mentioned software. Values represent the
646 mean of 10–40 Arabidopsis plants in each treatment, and come from at least 3
647 independent plant cultures.

648 **ACCESSION NUMBERS**

649 Sequence data from this article can be found in the GenBank/EMBL data libraries under
650 accession numbers ABA 2 : AT1G52340; SnRK2.2, At3g50500; SnRK2.3, At5g66880;
651 ABI1 : AT4G2542 and HAB1 : AT1G72770

652

653 **SUPPLEMENTAL DATA**

654 **Supplemental Figure S1.** Distribution of LRs along the primary root in response to
655 various WD levels.

656 **Supplemental Figure S2.** LR elongation and number in response to WD or exogenous
657 ABA.

658 **Supplemental Figure S3.** Expression of ABA-regulated genes in response to exogenous
659 ABA.

660 **Supplemental Figure S4.** Effects of WD on biomass production and RSA of ABA
661 biosynthesis and response mutants.

662 **Supplemental Figure S5.** Effects of WD and exogenous ABA on root hydraulic
663 properties of wild-type and ABA biosynthesis and response mutants.

664 **Supplemental Figure S6.** Expression of aquaporin genes in response to WD and
665 exogenous ABA.

666 **Supplemental Figure S7.** Shoot and root biomass production in response to WD or
667 exogenous ABA.

668 **Supplemental Figure S8.** Changes of RSA of seedlings in response to sorbitol (WD).

669 **Supplement Table S1.** Sequences of gene primer pairs used for RT-qPCR.

670 **Supplement Table S2.** Statistical analysis of the effects of WD on RSA of wild-type and
671 ABA 2 biosynthesis (*aba2*) and response (*snrk2.2 snrk2.3; hab1 abi1*) mutants.

672 **Supplement Table S3.** Statistical analysis of the effects of WD on root hydraulic
673 properties of wild-8 type and ABA biosynthesis (*aba2*) and response (*snrk2.2 snrk2.3;*
674 *hab1 abi1*) mutants.

675

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688

689 **FIGURE LEGENDS**

690 **Figure 1. Changes of root system architecture in response to water deficit.**

691 Plants were grown until 18 days after sowing (DAS) in hydroponics under standard
692 conditions and subjected for 5 additional days (23 DAS) to the indicated PEG-8000
693 treatments. A, Representative images showing the whole root system architecture, the
694 parts of the primary root grown after treatment (PRAT), and the oldest lateral root
695 grown after treatment (LRAT) used for specific analyses. B and C, Whole root systems
696 were used to determine total root length (B) and biomass production (C) per plant. D to
697 I, The length of the PRAT (D) and the LRAT (E), the number of LRs formed in the PRAT
698 (F), the number of second-order LRs formed in the LRAT (G), the mean length of LRs
699 formed in the PRAT (H), and the mean length of second-order LRs formed in the PRAT
700 (I) per root are shown. Mean values \pm SE were obtained from plants grown in three
701 independent experiments ($n = 20-25$). 'Homogeneous group' statistics were calculated
702 through ANOVA, where mean values with different letters are significantly different
703 according to LSD test at $P \leq 0.001$.

704

705 **Figure 2. Effects of water deficit on ABA abundance and expression of ABA-** 706 **regulated genes.**

707 A and B, ABA concentrations (A) and transcript abundance of ABA-regulated genes
708 RAB18, RD29A and RD29B in roots (B) of plants subjected from 18 days after sowing to
709 a 5-day-long treatment with the indicated PEG concentrations. The dotted line in (B)
710 represents the normalized expression level in control conditions. Mean values \pm SE were
711 obtained from 15 plants grown in three independent experiments ($n = 3$).
712 'Homogeneous group' statistics were calculated through ANOVA where mean values
713 with different letters are significantly different according to LSD test at $P \leq 0.05$ (A). FW,
714 fresh weight.

715

716 **Figure 3. Effects of exogenous ABA on root system architecture.**

717 A to H, Plants grown in hydroponics were subjected at 18 days after sowing (DAS) for 5
718 additional days (23 DAS) to the indicated concentrations of exogenous ABA. The figure
719 shows the total root length (A) and the root biomass (B) per plant, the length of PRAT
720 (C) and LRAT (D), the number of LRs formed in the PRAT (E), the number of second-
721 order LRs formed in the LRAT (F), the mean length of LRs formed in the PRAT (G), and
722 the mean length of second-order LRs formed in the LRAT (H) per root. Mean values \pm SE
723 were obtained from plants grown in three independent experiments (n = 15–20).
724 ‘Homogeneous group’ statistics were calculated through ANOVA, where mean values
725 with different letters are significantly different according to LSD test at *P \leq 0.05, **P \leq
726 0.01, ***P \leq 0.001.

727

728 **Figure 4. Effects of water deficit on root system architecture of ABA biosynthesis**
729 **(*aba2*) and response (*snrk2.2 snrk2.3; hab1 abi1*) mutants.**

730 A to F, Plants grown in hydroponics were subjected at 18 days after sowing (DAS) for 5
731 additional days (23 DAS) to the indicated PEG concentrations. Three mutants including
732 *aba2* (an ABA biosynthesis deficient mutant), *snrk2.2 snrk2.3* (an ABA signaling mutant)
733 and *hab1 abi1* (an ABA signaling hypersensitive mutant), were used in addition to the
734 Col-0 WT control. The root system architecture was analysed by several parameters: the
735 length of the primary root grown after treatment (PRAT; A) and the oldest lateral root
736 grown after treatment (LRAT; B), the number of lateral roots (LRs) formed in PRAT (C),
737 the number of second-order LRs formed in the LRAT (D), the mean length of LRs formed
738 in the PRAT (E), and the mean length of second-order LRs formed in the LRAT (F) per
739 root. Open boxes, light grey, dark grey and black boxes represent values for plants
740 grown in PEG25, PEG75 and PEG150 conditions, respectively. Mean values \pm SE were
741 obtained from plants grown in three independent experiments (n = 10–20).
742 ‘Homogeneous group’ statistics were calculated through ANOVA for each genotype,
743 where mean values with different letters are significantly different according to LSD test
744 at P \leq 0.05, representing n.s. as not significant (P > 0.05).

745

746 **Figure 5. Effects of water deficit and exogenous ABA on root hydraulic properties**
747 **of wild-type and ABA biosynthesis (*aba2*) and response (*snrk2.2 snrk2.3; hab1***
748 ***abi1*) mutants.**

749 A to C, Plants grown in hydroponics were subjected at 18 days after sowing (DAS) and
750 for 5 additional days (23 DAS) to the indicated concentrations of PEG (A and C) and ABA
751 (B), and different genotypes (C) were used as described previously. For A and B, the light
752 and dark grey boxes represent the measured Lpr and residual Lpr after the aquaporin
753 activity inhibition, respectively. For C, open boxes, light grey, dark grey and black boxes
754 represent Lpr values for plants grown in PEG25, PEG75 and PEG150 conditions,
755 respectively. Mean values \pm SE were obtained from plants grown in three independent
756 experiments (n = 20–25). ‘Homogeneous group’ statistics were calculated through
757 ANOVA, where mean values with different letters are significantly different according to
758 LSD test at $P \leq 0.05$ (C). For A and B, levels of significance are represented as *** $P \leq$
759 0.001, and n.s. as not significant ($P > 0.05$).

760

761 **Figure 6. Integrative model of root developmental and hydraulic responses to**
762 **representative water deficit intensities.**

763 The different water deficit (WD) treatments are represented by four plants. The root
764 system is schematically represented by a primary root, an old lateral root (LR) and first
765 and second-order LRs on the primary and the old LR. The root parameters analyzed are
766 schematically represented and their response to WD is represented by an arrow or a bar
767 for stimulation or repression, respectively. The intensity of the responses is shown by
768 the thickness of the line. PRAT is the part of the primary root grown after treatment,
769 LRAT is the part of the old LR grown after treatment, L and # stand for mean LR length
770 and LR number, respectively. Lpr stands for root hydraulic conductivity. The blue and
771 red color codes represent ABA-dependent or -independent responses, respectively. The
772 thickness of blue and red lines and arrows represent the intensity of response (thicker
773 line represents stronger response). The three yellow boxes at the bottom of the model
774 represent the root ABA content. Color intensity and number of arrows are a schematic
775 representation of ABA concentrations.

776

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