Title

Natural Genetic Variation Shapes Root System Responses to Phytohormones in Arabidopsis

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ABSTRACT

Plants adjust their architecture by modulating organ growth. This ability is largely dependent on phytohormones. While many genes involved in phytohormone pathways have been identified, it remains unclear to which extent and how these pathways are modulated in non-reference strains and whether this is relevant for local adaptation. Here we assess variation of root traits in response to perturbations of the auxin, cytokinin, and abscisic acid pathways in 192 Arabidopsis accessions. We identify common response patterns, uncover the extent of their modulation by specific genotypes, and find that the Col-0 reference accession is not a good representative of the species in this regard. We conduct GWAS and identify 114 significant associations, most of them relating to ABA treatment. The numerous ABA candidate genes are not enriched for known ABA associated genes indicating that we largely uncovered unknown players. We then study two associated regions in detail and identify the *CRF3* gene as a modulator of multiple hormone pathways. Finally, we show that natural variation in root traits is significantly associated with climate parameters relevant for local adaption in *Arabidopsis thaliana* and that, in particular, ABA regulated lateral root traits are likely to be relevant for adaptation to soil moisture.

34 **Author Summary**

35 The root system is a key component for plant survival and productivity. Apart from anchoring

36 the plant, its architecture determines which parts of the soil are foraged for water and nutrients,

37 and it serves as an interface for interaction with microbes and other soil organisms. Plant

38 hormones play crucial roles in the development of root system architecture and its plasticity.

39 However, while there is substantial natural variation of root architectures, it is not clear to which

40 extent genetic variation in hormone related pathways contribute. Using the model species

41 *Arabidopsis thaliana* we quantitatively explore the breadth of natural variation in plant hormone

42 responses to three major plant hormones: auxin, cytokinin, and abscisic acid. The Col-0

43 reference strain can be quite different from a large proportion of the natural accessions of the

44 species, illustrating a severe caveat of relying on a single reference strain in model species and

45 drawing generalizations from it. Using GWAS, we further identify a large number of loci

46 underlying the variation of responses to plant hormones, in particular to abscisic acid, find links

47 between local adaptation and root responses to hormones, and finally using mutants for GWAS

48 candidate genes, identify novel players involved in regulating hormone responses.

49

50 **INTRODUCTION**

51

52 To grow and survive plants need access to sunlight, nutrients and water resources that are

53 not evenly distributed in the environment. As sessile organisms plants adjust their architecture

54 according to the distribution of resources by modulating organ growth and development.

55 Consequently, plant architecture is of major adaptive relevance (Ackerly et al., 2000). Directed

56 plant growth responses, as well as the resulting plant architecture, are largely dependent on

57 phytohormones, systemic signals that are interpreted in a cellular context (Malamy, 2005). Work

58 related to the phytohormone auxin in the model species *Arabidopsis thaliana* has shown that this

59 pathway is subject to extensive natural variation at the transcriptional level (Delker et al., 2010).

60 However, it is unclear how this relates to plant architecture phenotypes, how the numerous other

61 phytohormone pathways are affected by natural variation, and what the genetic bases for these

62 alterations are. Moreover, it is not known whether the large number of phenotypic and associated

63 molecular responses to phytohormones described in the reference accession Col-0 are

64 representative for *Arabidopsis thaliana* as a species.

65

66   The root is an excellent system for studying the dependence of plant architecture on
67   phytohormone pathways. Not only is it technically feasible to perform hormonal perturbations on
68   a large number of roots, but root traits are also of high adaptive relevance as the root system
69   represents the backbone for plant growth and productivity. It anchors the plant to the soil,
70   uptakes water and nutrients, and interacts with soil microorganisms (Den Herder et al., 2010,
71   Lynch, 1995). There are three key developmental processes that determine the most important
72   properties of the spatial distribution of roots, known as root system architecture (RSA): (i) the
73   rate of proliferation and differentiation, which are the two main processes shaping how quickly
74   roots grow; (ii) root growth direction, which determines in which direction the root system is
75   expanding; (iii) the formation of lateral roots, which determine the lateral extensiveness of the
76   root system. All of these processes are under the control of multiple phytohormone pathways (for
77   an overview see (Satbhai et al., 2015)). These hormonal pathways play a key role in adjusting
78   RSA in response to environmental conditions, including availability of the three main
79   macronutrients: nitrogen (Gifford et al., 2013, Krouk et al., 2010, Rosas et al., 2013, Ruffel et al.,
80   2011, Vidal et al., 2010), phosphate (Lopez-Bucio et al., 2005, Nacry et al., 2005, Perez-Torres
81   et al., 2008, Singh et al., 2014), and potassium (Vicente-Agullo et al., 2004) as well as stresses
82   such as salt (Ding et al., 2015, Kumar & Verslues, 2015, Liu et al., 2015, Zhao et al., 2011),
83   drought (Du et al., 2012, Hong et al., 2013, Kang et al., 2002), and exposure to excess metals
84   (Hu et al., 2013, Sun et al., 2010, Yuan et al., 2013).

85   Here we assess natural variation of RSA traits in response to perturbations of the auxin
86   (IAA), cytokinin (CK), and abscisic acid (ABA) phytohormone pathways by generating and
87   analyzing a comprehensive atlas of these responses in a large set of accessions covering most of
88   the genetic diversity in *Arabidopsis thaliana*. We identify common response patterns and linked
89   RSA traits that are dominated by specific phytohormone pathways, and uncover the extent of
90   their modulation by specific genotypes. Using expression analysis we show that the expression
91   of key genes for multiple hormone pathways are frequently altered in accessions with contrasting
92   RSA responses to hormones. We further study two regions of the genome that are significantly
93   associated with responses in hormone treatments and identify the *CRF3* gene as a being a
94   modulator of multiple hormone pathways. Finally, we show a potential relevance of natural
95   variation of root traits for local adaptation, as we identify significant overlaps between

96   associations for the root traits in this study and environmental variables at the sites of origin of

97   the accessions

98

99   **RESULTS**

100

101   **The Response to Perturbation of Phytohormone Pathways is Subject to Extensive Natural**

102   **Variation**

103   In order to study and catalogue natural variation of phenotypic responses to phytohormones, we

104   grew a diverse panel of 192 Arabidopsis accessions covering most of the Arabidopsis genetic

105   diversity (Supplemental Figure 1, Supplemental Table 1) on vertical plates for 7 days on 1/5

106   strength MS medium. We then carefully transferred individual seedlings to different plates

107   supplemented with auxin (IAA), cytokinin (CK), abscisic acid (ABA), or no hormone (C)

108   (Figure 1, Supplemental Figure 2A). Plates were scanned directly after the transfer (day 7) and

109   then again 3 days later (day 10). These images were used to quantify ten root traits

110   (Supplemental Figure 2B and 2C). Overall, we quantified 5 seedlings of 192 accessions in 4

111   different conditions (3 hormone treatments plus control), totaling 3840 seedlings and 38400 root

112   measurements. There was a very broad spectrum of responses of root growth and development

113   upon perturbation of the endogenous hormone pathways (Figure 1, Supplemental Figure 3).

114   Importantly, these responses were clearly dependent on the genotype, as the broad-sense

115   heritability of the traits was high with an average of 57% for all traits and conditions, ranging

116   from 22% for lateral root density in the branching zone under control conditions to 77% for the

117   primary root length on cytokinin treatment (Supplemental Table 2). In our treatment conditions,

118   we observed strong effects of IAA and ABA on most of the traits, while the impact of CK was

119   rather subtle (Supplemental Figure 3). IAA had strong negative effects on root growth rate (P2)

120   and strong positive effects on lateral root related traits (R, LR.No, LRD_P, TLRL and LRR).

121   ABA had strong negative effects on lateral root related traits (R, LRL, LR.No, LRD_P, LRD_R,

122   TLRL, and LRR), the opposite effect to IAA

123   (Figure 2B). The strongest effects of CK were a negative effect on root growth (P2 and LRL);

124   although we didn't explicitly quantify it, we also observed a highly increased stimulation of root

125   hair growth upon CK treatment in most of the accessions, especially on the elongated primary

126   root (Figure 2A). Despite being quite genetically diverse, most accessions followed these general

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127 response patterns with only some accessions showing strong deviations with regard to specific

128 hormonal perturbations or traits. Surprisingly, Col-0, the reference accession on which most of

129 the previous studies of hormonal effects had been performed, is among the accessions whose

130 root growth responses are frequently quite different from the bulk of Arabidopsis accessions. On

131 average it is in the upper quartile of the accession distribution and belonged to the 1% most

132 extreme phenotypes for some traits in some conditions (Supplemental Figure 3, Supplemental

133 Table 3). Col-0 is therefore not a good representative of the diverse panel of accessions that we

134 have investigated.

135 In conclusion, we have generated the first comprehensive atlas of root responses to perturbation

136 of hormonal pathways. Our data show that while there are clear trends of phenotypic responses

137 to perturbation of specific hormonal pathways, there is significant natural variation in these

138 responses. Overall, this suggests that hormonal signaling pathways within the *Arabidopsis*

139 *thaliana* species are largely acting on the same traits, but that the extent of the hormonal control

140 of specific traits is genotype dependent. We therefore conclude that hormonal pathways are

141 subject to natural genetic variation. Importantly, the effects of hormones on root growth in the

142 Col-0 reference accession are not representative of the major proportion of Arabidopsis

143 accessions.

144

145 **Distinct Hormonal Pathways Dominate Distinct Traits**

146 The dependence or independence of traits in diverse genotypes is highly relevant for inferring the

147 genetic architecture of a trait; a strong dependence suggests a common genetic, and possibly

148 molecular, basis underlying the regulation of the respective traits. Our comprehensive atlas of

149 RSA traits of a large-number of Arabidopsis genotypes upon perturbation of phytohormone

150 pathways allowed us to ask which of the hormonally regulated RSA traits were linked and to

151 which extent. We used our trait data from the 192 accessions and calculated pairwise Pearson's

152 correlation coefficients of all traits for each condition (Figure 2A-D). To determine whether the

153 extent of trait correlation was similar in all conditions, or whether perturbations of phytohormone

154 pathways affected a particular trait correlation, we also calculated the variance of each trait

155 correlation between all conditions. We reasoned that if this variance was high, the observed trait

156 correlation would be strongly dependent on a subset of phytohormone pathways (Figure 2E). The

157 highest variation of correlations between independent traits was observed for the length of

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158 branching zone and the lateral root density of the primary root ($\sigma=0.13$). While these traits are
159 highly correlated in control (0.67), CK (0.63) and ABA (0.67) conditions, this correlation
160 reverses upon IAA treatment (-0.31). This strong effect can be traced back to the impact of IAA
161 on root growth rate and its impact on the branching zone, which are key for the second and third
162 ranked correlation variances respectively. Under control conditions, there is a very high positive
163 correlation between primary root growth rate before treatment and growth rate after treatment
164 ($r=0.9$), indicating that root length after 7 days is generally a very good predictor for root length
165 after 10 days. IAA treatment, however, breaks the correlation of these two traits almost
166 completely ($r=0.19$). ABA and CK treatments diminish this correlation only slightly, suggesting
167 that the observed effect of IAA on root growth rate is highly specific. Overall, this strongly
168 suggests that the regulation of root growth rate is strongly dominated by the auxin pathway, as
169 demonstrated by multiple studies (Evans et al., 1994; Coenen and Lomax, 1997; Tian and Reed,
170 1999; Friml, 2003; Swarup and Bennett, 2003; Robert and Friml, 2009; De Smet et al., 2012;
171 Pacifici et al., 2015). While primary root length and the length of the branching zone are
172 moderately positively correlated under control conditions ($r=0.4$), this correlation increases to
173 very high levels under IAA treatment ($r=0.91$), suggesting that auxin generally promotes lateral
174 root formation or outgrowth. ABA treatment renders these two traits almost completely
175 uncorrelated ($r=0.07$), suggesting that ABA generally represses the formation or outgrowth of
176 lateral roots, a finding that is consistent with the literature (De Smet et al., 2003).
177 Overall, these examples not only demonstrate the significance of specific hormone pathways for
178 specific traits, but also support the view that RSA traits such as branching and primary root
179 growth rate can be regulated independently of each other, dependent on the condition, and over a
180 large number of genetic backgrounds, a phenomenon previously shown for the impact of
181 different nitrogen environments on root traits (Gifford et al., 2013).
182

183 **Genotypes can Alter Phenotypic Responses to Hormones**
184 While our correlation analysis revealed common trends over a large number of genotypes, our
185 atlas of root responses to perturbation of hormonal pathways also allowed us to study which
186 genotypes modulate the responses to perturbations of hormone pathways. To do so, we
187 performed a hierarchical-clustering of our ten traits separated by condition. Different clusters
188 contain groups of accessions with genotypes that generate a similar RSA under the respective

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189   conditions (Figure 3, and Supplemental Table 4). We observed that perturbation of specific

190   hormone pathways shift RSA to a distinct state (for instance, IAA causes roots to become shorter

191   and more branched) but that the genotype determines the extent to which this state is reached.

192   For instance, IAA treatment cluster 5 is shifted to the IAA RSA state to a lesser degree than IAA

193   cluster 6. Overall, our analysis partitions the phenotypic space that we explored using the

194   systematic perturbation of hormone pathways and illustrates the tremendous impact of the

195   genotype in determining how root development responds to hormones. However, while this

196   analysis is highly useful for identifying classes of accessions

197   and visually illustrates the effects of hormones and how these effects differ, it was based on

198   capturing RSA using individual traits, some of which are dependent on each other and some of

199   them not. Moreover, hierarchical clustering gives insight into classes, rather than continuous

200   similarities. To gain a more integrative insight into the action of hormones, we conducted a

201   principal component analysis (PCA) on all our traits for all conditions (10 root traits across 192

202   accessions and four treatments). 99% of RSA variation (given by our 10 traits) could be captured

203   by five PCs, while 9 PCs are needed to explain the complete variation (Supplemental Table 5).

204   The first PC captures 62% of the total variation. Several traits contribute to it, including root

205   number (LR.No), lateral root density (LRD_P and LRD_R), total lateral root length (TLRL), and

206   length ratio (LRR). The second PC accounts for 26% of the variation and represents mainly

207   primary root length (P) and total root length (TRL) (Supplemental table 5). Comparable

208   contributions of the same root traits to each PC (PC loadings), as well as PC variability were

209   quantified for the same hormonal treatments of the reference accession Col-0 (Ristova et al.,

210   2013), demonstrating a highly reproducible pattern of the RSA traits and their relations to

211   hormonal perturbations. Overall, IAA treatment, and to a lesser extent ABA treatment, results in

212   a clear separation from the other three conditions when plotting the most informative PCs, PC1

213   and PC2, while CK and C are largely overlapping (Figure 4A). To identify accessions with

214   genotypes that alter from the norm of how RSA responds to hormone pathway perturbation, we

215   calculated the Euclidian distance to the average PC values in the RSA space defined by PC1 and

216   PC2 for each accession and each condition. This distance indicates the deviation of a particular

217   genotype to the average RSA response of all accessions (Supplemental Table 6). For each

218   accession, we then calculated the average Euclidian distance of all conditions (Supplemental

219   Table 6). This average represents a measure of how profoundly a genotype differs from the norm

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220   in altering its RSA in response to all hormonal perturbations that we had performed. Eight

221   accessions (Sh-0, Lu-1, Col-0, Sorbo, UKSE06-373, Kz-1, Or-0, Uk-1) were more than 2

222   standard deviations from the mean of all accessions in this regard (Figure 4A, Supplemental

223   Table 6), indicating that they show significant alterations of their hormonal response from the

224   average *Arabidopsis thaliana* accession. Again, the Col-0 reference accessions was among these

225   accessions, again highlighting the conclusion that much of what has been learned in studies on

226   Col-0 may not represent the species as a whole. Taken together, these data demonstrate that

227   hormone perturbations generally lead to specific patterns of developmental responses that shift

228   RSA from one configuration to another (i.e. IAA shifts RSA to shorter and more branched

229   configurations). However, these response patterns can be significantly altered by genotypic

230   variation. This genotypic variation must therefore be in the pathways that perceive or respond to

231   phytohormone cues. Overall, this demonstrates that natural genetic variation in phytohormone

232   pathways can be a major contributor in shaping the Arabidopsis root system.

233

234   **Genetic Variation leads to Changes in the Molecular Regulation of Hormone Pathways**

235   To test whether the phenotypic differences we observed are reflected at the molecular level, we

236   measured the expression of genes related to hormone signaling in accessions that represented

237   different PC1 values (Uk-1: high PC1; Tamm-2: low PC1; Col-0: intermediate PC1). We

238   included 8 genes of the *SHY2* network which are involved in auxin, cytokinin and abscisic acid

239   signaling, and 4 additional genes involved in auxin biosynthesis (*TAA1*) (Stepanova et al., 2008),

240   abscisic acid signaling (*ABA2*) (Leung et al., 1997), and cytokinin signaling (*ARR4, AHK4*)

241   (Salome et al., 2006, Yamada et al., 2001). We found that 8 of 12 genes were expressed at

242   different levels in these accessions, several of them in a pattern similar to the PC values (Figure

243   4B). Thus, the steady state expression level of important components of hormone pathways can

244   be vastly different in different accessions. To corroborate this finding, we also made use of a

245   public dataset of root transcriptome data of 7 accessions (Delker et al., 2010). Under control

246   conditions, there was also significant variation in expression of these key genes, providing

247   further evidence the expression levels of genes in hormonal pathways are highly dependent on

248   the genotype (Supplemental Figure 4). Overall, this demonstrates that there can be a wide variety

249   of transcriptional states in hormonal pathways relevant to RSA in different genotypes.

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8

Genome-wide Association (GWA) Mapping on RSA under Hormone Treatments

251

252 Our analysis of root responses to hormone pathway perturbation clearly showed that these

253 responses are under genetic control. To identify genetic variants that were associated with these

254 different responses, we performed genome wide association (GWA) mapping on all traits in all

255 conditions (10 traits * 4 conditions) using a mixed model algorithm (Kang et al., 2008, Yu et al.,

256 2006), that was previously shown to correct for population structure confounding (Seren et al.,

257 2012), and using SNP data from the 250K SNP chip (Atwell et al., 2010, Brachi et al., 2010,

258 Horton et al., 2012). We used a Benjamini Hochberg threshold (5% FDR) to select significant

259 associations. In total we identified 114 significant associations, corresponding to 441 genes

260 within a 20 kb window of the associated SNPs (Table 1, Supplemental table 7). We found

261 associations for each treatment, with ABA perturbation yielding the most at 98; CK had 5

262 significant associations and IAA only one. GWAS for the control condition without hormone

263 treatment added 9 associations (Table 1, Supplemental table 7). We observed only 3 cases where

264 GWAS identified the same SNP associated with different treatment conditions and the same trait

265 (Supplemental Figure 5).

266

267 Despite the large number of significant associations for the ABA pathway, the genes close to

268 these associations didn't contain obvious core ABA signaling related genes. To test whether this

269 absence of *bona fide* ABA candidate genes was statistically significant, we identified all genes

270 for which a role in the ABA pathway had been assigned based on reported experimental

271 evidence or mutant phenotypes (Supplemental table 8). There was only one gene that overlapped

272 between GWAS-based candidate gene list and the annotation-based ABA *bona fide* candidate

273 gene list. We then tested whether this overlap is expected by chance, we found that our observed

274 overlap was at the lower limit (towards our GWAS list being depleted of *bona fide* candidate

275 genes) of what was to be expected by chance (p<0.16; Supplemental Figure 6). This clearly

276 showed that *bona fide* candidate genes are not overrepresented in our list of GWAS candidates

277 and indicates that, for complex traits such as root responses to hormonal treatment, genes

278 targeted by natural variation are frequently not the genes found by traditional experimental

279 methods (such as forward genetic mutant screens).

280

281   To identify high confidence candidate genes whose variants underlie the associated traits, we

282   focused on two cases. The first case involved the trait LRR (ratio of total lateral root length and

283   primary root length) where a significant association on chromosome 3 was found under both

284   control (C) and cytokinin (CK) conditions (Supplemental Figure 7). Approximately 7KB from

285   the most significantly associated SNP (Chromosome 3; 6127399) is a gene with a known role in

286   the hormonal regulation of development, *JASMONATE-INSENSITIVE 3* (*JAI3,* AT3G17860)

287   (Chini et al., 2007). While the top SNP itself was located in the gene body of AT3G17910

288   (Figure 5B), the entire region surrounding this SNP contained many single-nucleotide

289   polymorphisms in a pattern consistent with the phenotypes of contrasting accessions

290   (Supplemental Figure 7A). When analyzing linkage disequilibrium (LD) in this region, we found

291   that SNPs in only two genes (AT3G17890 and AT3G17900) were in LD ($R^2>0.4$) with the SNP

292   that we had identified through GWAS. We tested whether the expression of any gene in this

293   region was correlated with the LRR trait. Only the three genes in LD showed expression levels

294   correlated with the LRR phenotype. Of these three genes, AT3G17910 showed the highest

295   correlation (Figure 5C, Supplemental Figure 7B). *JAI3,* the most likely candidate gene based on

296   its known role, did not show any correlation. To test whether one or more of these genes are

297   involved in regulating lateral root related traits, we obtained available T-DNA insertion mutant

298   lines for AT3G17890 (SALK_050958), AT3G17910 (SALK_045920) and *JAI3* (*jai3-1)* (Chini

299   et al., 2007). No insertion line in the genic region of AT3G17900 was available. The expression

300   levels of the respective genes were significantly reduced in the SALK lines that we obtained

301   (Supplemental Figure 7C). We examined the phenotypes of these three mutants under control

302   and cytokinin treatment conditions. While the *jai3* line showed an overall reduced root growth in

303   both conditions, the response to CK was altered for multiple traits in T-DNA mutants of the

304   other two genes (Figure 5D and 5E). In particular, alterations were observed in both mutants for

305   the branching zone (R) and lateral root number (LR.No) traits, as well as for primary root length

306   (P) and total root length (TRL) in one mutant (SALK_045920). While compared to Col-0, the

307   SALK_045920 (AT3G17910) line showed a decreased branching zone under control and an

308   increase of it upon CK treatment, the second line SALK_050958 (AT3G17890) showed a slight

309   decreased response upon CK treatment (Figure 5D). Moreover, expression levels of several

310   genes in hormonal pathways were significantly altered in the SALK_045920 (AT3G17910) line

311   and SALK_050958 (AT3G17890) (Supplemental figure 7D), with a much more pronounced

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312   perturbation of gene expression in the SALK_045920 line. Overall, this strongly suggests that

313   the yet uncharacterized genes AT3G17890 and AT3G17910 are involved in regulating aspects of

314   CK hormonal signaling that are relevant for shaping the same root traits in a complex manner.

315   We note that we can't evaluate the role of the third gene, AT3G17900, which is also a potential

316   candidate based on the LD pattern and the expression level correlation.

317   In contrast to the first case, a well-known candidate gene was identified for another trait. Here, a

318   significant SNP (chromosome 5; position 21621187) for lateral root number (LR.No) and root

319   density (LRD_R) (Figure 6A) under ABA treatment was located in the promoter region of

320   *CYTOKININ RESPONSE FACTOR3* (*CRF3*, AT5G53290) (Figure 6A, Supplemental figure 8A).

321   *CRF3* has been shown to be a key link between cytokinin signaling and auxin transport in root

322   development, acting downstream of cytokinin perception and transcriptionally regulating auxin

323   transport (PINs) (Simaskova et al., 2015). As this region was devoid of other genes and a SNP in

324   the coding region of *CRF3* was in LD ($R^2 > 0.4$), this was the only and best candidate gene. To

325   test whether we would obtain phenotypes that consistent with the GWAS phenotypes in our

326   conditions, we obtained a loss of function T-DNA line (SAIL_240_H09) for *CRF3* that has been

327   described previously (Simaskova et al., 2015). We found that this line had an overall reduced

328   root growth, affecting both root length and lateral root number, when grown under control and

329   abscisic acid (ABA) conditions (Figure 6C and 6D). While the most pronounced LR effect upon

330   ABA in the wild type is a strong reduction of the lateral root length (LRL and TLRL), these traits

331   were not affected by ABA in the *crf3* mutant line, leading to a highly significant interaction of

332   the *crf3* mutation and ABA treatment (Figure 6C and 6D). Moreover, expression levels of genes

333   related to auxin, cytokinin and ABA hormonal pathways were significantly altered in the *crf3*

334   line (Supplemental figure 8B). Taken together, this shows that *CRF3* not only links auxin and

335   cytokinin responses, but also impinges on ABA dependent root growth regulation.

336

337   **Natural Variation in Hormone Signaling Pathways and Local Adaptation**

338   As natural variation in pathways that affect root growth responses to phytohormones has a

339   tremendous impact on root system architecture related traits, it was tempting to speculate that the

340   variation we observed was relevant for local adaptation. Much of the local adaptation in

341   Arabidopsis is driven by climate related parameters (Fournier-Level et al., 2011). We therefore

342   first determined whether there is an overlap between the genetic variants associated with the root

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343   traits that we quantified in our study and the genetic variants associated with climate parameters

344   at the site of origin of the accessions. For this, we treated the climate parameters of every

345   accession's site of origin as a trait, and conducted the same population structure corrected

346   GWAS procedure that we had for our trait data on 35 climate variables capturing biologically

347   relevant climate aspects (Bioclim variables) (Hutchinson et al., 2009, Kriticos et al., 2012) for all

348   accessions of the RegMap panel (Horton et al., 2012). We then determined the overlap top SNPs

349   (0.5 top percentile) between each climate parameter GWAS and each GWAS from our RSA-

350   hormone study and computed the probability of this overlap occurring by chance. After

351   correction for multiple testing, out of the 1400 computed overlaps of phytohormone induced

352   RSA responses associated top SNPs and climate variable associated top SNPs, 15 overlaps

353   showed a significant (FDR < 5%) overlap (Supplemental Table 9). We then tested whether the

354   root traits and the climate traits were significantly correlated when accounting for population

355   structure and correcting for multiple testing. We found that out of these 15 trait combinations

356   that had a significant overlap of associated SNPs, 9 displayed a significant population structure

357   corrected trait correlation (FDR < 5%) and another 2 were marginally significant (FDR < 10%)

358   (Supplemental Table 9). Overall this suggests that the root traits that we measured in our study

359   are involved in local adaptation.

360   The most significant overlap between SNPs was found between ABA induced lateral root

361   density and "Mean moisture index of coldest quarter" (FDR < 0.2%), consistent with the well-

362   known role of ABA in response to water related responses. The trait correlation of this most

363   overlapping combination was negative (r= -0.189610096), suggesting that less lateral roots are

364   produced in an ABA dependent manner in accessions that are derived from areas in which the

365   soil contains high moisture in the coldest quarter of the year. As this trait correlation is

366   marginally significant when correcting for population structure (FDR < 7.5%), this suggests that

367   variation in genes that determine the impact of ABA on lateral root development is adaptive in

368   environments with contrasting soil moistures. Interestingly, none of the genes in proximity to the

369   19 overlapping SNPs is a *bona fide* ABA gene (Supplemental Table 10).

370   While the most significant overlap at the SNP level clearly suggested an important role of ABA

371   for local adaption for soil moisture, auxin and cytokinin treatments were also among the list of

372   root traits that were significantly overlapping with environmental parameters at the level of SNP

373   overlap and at least marginally significant correlated to the same climate trait (FDR < 8%).

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374   However, 7 out of the 11 combinations displaying significant overlaps at the level of SNPs and

375   traits did not associate with hormonal treatment, but with RSA traits under non-treated

376   conditions. Finally, only one of these overlapping traits is related to primary root growth.

377   However, as only 2 out of 10 traits that we had scored related specifically to the primary root,

378   this is still in the realm of expectation. Overall, these data clearly show that natural variation in

379   root traits is significantly associated with parameters that are highly relevant for local adaption in

380   *Arabidopsis thaliana* and that, in particular, ABA regulated lateral root traits seem to be relevant

381   for adaptation to soil moisture.

382

383   **DISCUSSION**

384

385   **Natural Variation of Hormone Signaling Pathways and Root Architecture**

386   In this study, we report the first comprehensive atlas of RSA responses to hormonal

387   perturbations in a large number of *Arabidopsis thaliana* natural accessions. Our results show

388   general patterns of phenotypic responses to the perturbation of specific hormonal pathways, as

389   well as significant natural variation in these responses across a set of accessions that captures a

390   large fraction of the genetic variation in Arabidopsis (Supplemental figure 1). We show that

391   specific hormonal pathways dominate a distinct subset of traits, as perturbations of specific

392   pathways can break correlations that exist between traits under control conditions (Figure 2E). In

393   our experimental conditions, auxin perturbation caused the most dominant effect on RSA by

394   strongly repressing root growth rate and stimulating lateral root growth. ABA treatment reduced

395   primary root length, as well as lateral root numbers (Figure 1B), and diminishing the correlation

396   between these two traits, thereby demonstrating its negative role in the emergence of lateral

397   roots. In contrast to the replication of these well described effects of auxin and abscisic acid (De

398   Smet et al., 2003, Evans et al., 1994), our cytokinin treatment conditions did not lead to the

399   previously observed strong inhibitory effects on primary and lateral root growth (Laplaze et al.,

400   2007, Li et al., 2006). We did however observe the stimulatory effect of CKs on root hair growth

401   which had been described before (Werner et al., 2001). A possible reason for the observed

402   cytokinin response in our experimental conditions might be the specific cytokinin chosen and the

403   concentration of nutrients in the medium, as we used kinetin and one-fifth-strength MS medium.

404   Regardless of the specifics of our treatments, our atlas of root growth responses revealed the

405   extent of variation in the responses to perturbation of hormone pathways. Our hierarchical

406   clustering results identified groups of accessions sharing similar or diverse responses to a

407   particular hormone perturbation (Figure 3). Importantly, these clusters can be very useful for

408   choosing parents for quantitative trait loci mapping (QTL) via linkage mapping approaches using

409   recombinant inbred lines (RILs). While current mapping populations are based on only a small

410   subset of accessions (Alonso-Blanco & Koornneef, 2000, Koornneef et al., 2004), they have

411   already been used to identify *BREVIS RADIX (BRX)*, a gene that is responsible for the hormone

412   related short-root phenontype of the UK-1 accession (Mouchel et al., 2004, Mouchel et al.,

413   2006). Our results provide an opportunity for choosing novel, previously unexplored accessions

414   with diverse root responses to hormones as parents for creating new populations to identify novel

415   alleles underlying hormone pathway dependent root developmental processes. Principal

416   component analysis (PCA) showed that hormones can shift RSA through the phenotypic space

417   (Figure 4A). In our conditions, auxin treatment led to the most pronounced phenotypic effect on

418   RSA compared to ABA and CK. Of course, we cannot exclude that other concentrations of

419   hormones and their interplay with nutrient concentrations could lead to even more pronounced

420   CK or ABA effects, but our findings corroborate the prominent role of auxin in root

421   development. Moreover, the PCA analysis allowed us to systematically identify outlier

422   accessions that behave clearly differently than the bulk of Arabidopsis accessions.

423   Importantly, accessions that are different with respect to their RSA response upon hormonal

424   treatments also show deviations in the expression patterns of hormone related genes in control

425   conditions (no hormone treatment). This shows that the varying responses we observed upon

426   hormonal stimuli are associated with different transcriptional states of the hormone signaling

427   networks. This conclusion is consistent with genome-scale observations in 7 accessions before

428   and upon auxin treatment, in which vastly different transcriptome responses were detected

429   (Delker et al., 2010). Importantly, such different transcriptional states that presumably underlie

430   altered responses to hormones can be caused quite indirectly, for instance by alterations of the

431   balance of another hormone pathway. This has been nicely illustrated by studies related to the

432   *BRX* gene that is causal for a large proportion of the root phenotype of the UK-1 accession.

433   There, the lack of auxin response in the *brx* mutant results from a root specific deficiency in

434   brassinosteroid production (Mouchel et al., 2006). Given the strong and widespread intraspecies

435 variation that we have observed here, it is not surprising that distinct differences in the wiring

436 and interaction of plant hormone pathway exist between species (Pacheco-Villalobos et al.,

437 2013)

438 Taken together our results show that the root growth responses of Arabidopsis accessions upon

439 the perturbation of hormone pathways display distinct patterns that depend on the hormone

440 shifting RSA towards distinct states, and that the extent of this shift depends on genetic factors.

441 This demonstrates that hormonal pathways are subject to natural genetic variation within a

442 species, and it is tempting to speculate that this might contribute to adaptation to diverse soil

443 environments.

444

445 **Reference accessions and the species**

446 Much progress in uncovering the genetic and molecular bases of traits has been made in

447 reference strains of model species, including in Arabidopsis, Drosophila, and yeast. Findings in

448 these reference strains have been frequently generalized for their respective species and often

449 even for a whole phylogenetic branch of species. Our data demonstrate that the response of a

450 reference strain can be quite different from a large proportion of the natural populations of the

451 species. Within our diverse panel of 192 Arabidopsis accessions, the reference strain Col-0

452 belongs to the accessions that are least representative of RSA responses upon perturbation of

453 hormone pathways (Supplemental Table 3, Figure 3 and Figure 4). This is not simply a

454 peculiarity of our investigation; for instance, in a study dissecting 107 diverse phenotypes

455 (Atwell et al., 2010), Col-0 is located in the 1% lower tail of the distribution of all accessions for

456 14% of the traits (Supplemental Figure 11). While these findings don't affect the validity of the

457 fundamental mechanisms for hormone responses that have mainly been discovered in Col-0, they

458 suggest that, at a certain level of detail, studies will uncover relations and mechanisms that are

459 only found in specific genotypes within a species. We note that we don't suggest that Col-0 is

460 always an outlier. In fact, for a small number of traits under some conditions in this study

461 (Supplemental table 3), and for root traits in other studies, it has been described to be rather

462 average (e.g. Rosas et al. 2013).

463

464 **Genome-wide Association (GWA) Mapping and candidate genes**

465 While phytohormone signaling pathways are fundamental mechanisms that are highly relevant

466   for plant growth and development, only a few, small-scale studies have addressed them in the

467   light of natural variation (Delker et al., 2010, Van Leeuwen et al., 2007). Consequently, these

468   studies were not able to map any of the causal variants underlying the remarkable natural

469   variation of responses to hormones. Using our data, we were able to search for common genetic

470   variants that associate with altered hormone responses using GWAS. Using the large number of

471   associations with ABA responses, we found that genes already implicated in the ABA pathway

472   were not enriched, and possibly even underrepresented, in our set of GWAS candidate genes

473   (Supplemental Figure 6). This supports the notion that genes that are targeted by natural

474   variation are frequently not the genes that are found by traditional means (such as forward

475   genetic mutant screens).

476

477   As more than 100 significant associations were detected, there were many good candidate genes

478   and we needed to focus on the most interesting ones. We chose to study two cases with known

479   candidate genes in regions associated with root traits upon hormone treatment. These turned out

480   to be a interesting cases with regard to candidate gene approaches. The first case was an

481   association for LRR that was detected under both control and CK treatment. We chose to

482   investigate this region because a gene, *JAI3* that had been described to be involved JA signaling

483   (Chini et al., 2007) was located within 20KB of the associated SNP (Figure 5B). As many

484   hormonal signaling pathways are tightly intertwined (Vanstraelen & Benkova, 2012), it was

485   tempting to assume *JAI3* was also involved in CK signaling. However, based on LD patterns,

486   other genes were even better candidates, leading us to treat *JAI3* as one of 5 candidate genes in

487   this region. Indeed, much like the LD pattern, gene expression analysis suggested that three other

488   genes, and not *JAI3*, were the prime candidates in this region, based on the correlation of their

489   expression pattern with the LRR trait (Figure 5C, Supplemental Figure 8B). The root traits

490   observed in the available T-DNA insertion lines suggest that while *JAI3* has an effect on root

491   length, this is not impacted by CK treatment and it is therefore not a likely candidate gene. In

492   contrast, at least two other genes, At3g17890 encoding an unknown protein and At3g17910

493   encoding a protein similar to a cytochrome c oxidase assembly factor in human, are within the

494   associated region and regulate root traits in response of CK (Figure 5D and 5E). These genes

495   (and a third gene for which we could not obtain insertion lines) were the prime candidates based

496   on LD and expression correlation with the trait and amongst each other (Supplemental Figure

16

497 8B). Consistently, both available lines showed an altered expression level of multiple hormone

498 related genes. While cytokinin related genes were among those (most notably ARR12), in

499 particular *PIN1* expression was altered strongly (Supplemental Figure 8D) in both lines and

500 several other genes involved in the *SHY2* network were altered in the second mutant line

501 (At3g17910). However, without further studies, it is difficult to clearly attribute the causality for

502 these expression changes to a perturbation of hormone signaling or altered root morphology in

503 these lines. Nevertheless, our data demonstrate that the yet uncharacterized genes At3g17890 and

504 At3g17910 are involved in cytokinin dependent root growth regulation. This shows that an

505 annotation based candidate gene approach can be misleading and that data-driven approaches

506 (e.g. LD, expression) seem to be more efficient in identifying genes with a function in the

507 GWAS trait of interest.

508

509 ***CRF3* is an integrator of multiple hormonal pathways**

510 While further analysis of *JAI3* revealed that this is almost certainly not the causal gene, our

511 second example showed that known genes should not be disfavored for consideration as

512 causative candidates. In this second case, *CRF3*, a gene implicated in cytokinin and auxin

513 signaling (Rashotte et al., 2006, Simaskova et al., 2015), was the sole candidate gene based on

514 both LD and the absence of other genes in the region (Figure 6A and 6B). A published T-DNA

515 insertion line (Simaskova et al., 2015) that led to loss of *CRF3* function displayed an ABA

516 dependent alteration of root and lateral root traits (Figure 6C and 6D). At the molecular level, we

517 observed a 3 to 4.5 fold increase in expression of *PIN1*, *PIN3*, and *PIN7* in whole roots of the

518 *crf3* line, as well as altered expression of other genes involved in hormonal pathways

519 (Supplemental Figure 8B). An opposite effect on *PIN1* and *PIN7* had been previously observed

520 in root tips in this same line (Simaskova et al., 2015), suggesting that the effect of *CRF3* strongly

521 depends on the tissue type (we used whole roots for our transcript measurements). Overall, our

522 results suggest the possible involvement of *CRF3* as an integral part of 3 major hormone

523 pathways, auxin and cytokinin, which were already known (Simaskova et al., 2015), and abscisic

524 acid, as revealed by our study. Moreover, *CRF3* could be an integrator of hormonal and

525 environmental stimuli, as it was recently shown that *CRF2* and *CRF3* regulate lateral root

526 development under cold stress (Jeon et al., 2016).

527

17

528 **Local Adaptation and Hormone Signaling**

529 Root traits are highly relevant for plant survival and productivity (Den Herder et al., 2010,

530 Lynch, 1995). Hormonal pathways are major factors in the regulation of root traits and root

531 system architecture (RSA). Here we showed that perturbation of hormonal pathways can shift

532 RSA through the phenotypic space but the extent of this regulation can strongly vary between

533 natural accessions of *Arabidopsis thaliana* (Fig. 4). As these accessions had been collected from

534 different parts of the world, ranging from northern Sweden to southern Spain (Supplemental

535 figure 1), we tested whether the variation in the root growth responses to hormones is associated

536 with the climate at the places from which the accessions were derived (Supplemental table 9).

537 Consistent with an important role of hormonal pathways for local adaptation, ABA regulated

538 lateral root density and soil moisture in the coldest quarter of the year were the most important

539 overlapping traits regarding the overlap of SNPs. The data indicate that less lateral roots are

540 produced in an ABA dependent manner in accessions that are derived from areas in which the

541 soil contains high moisture in the coldest quarter of the year. Support for the relevance of ABA

542 related genes for local adaptation comes from studies on signatures of selection of ABA related

543 genes when comparing closely related tomato species that grow in environments with contrasting

544 precipitation regimes (Fischer et al., 2011, Xia et al., 2010) Moreover, in a study in *Arabidopsis*

545 *thaliana*, ABA-responsive cis-regulatory elements (ABRE) showed significantly higher

546 frequencies of polymorphisms in genes that responded to drought in a genotype dependent

547 fashion than in drought responsive genes that responded similarly in all genotypes, suggesting

548 that natural genetic variation in ABA responsive transcriptional control contributes to genotype

549 specific drought responses (Lasky et al., 2014).

550 Despite 75% of all traits in our data were measured upon hormonal treatments, the majority of

551 significant overlaps at the level of SNPs that were supported by significant trait correlations did

552 not associate with hormonal treatments but with RSA traits under non-treated conditions. What

553 does this mean for the importance of hormonal pathways for local adaptation? It is difficult to

554 come up with a simple answer for this, as, also under control conditions, hormonal signaling

555 pathways are involved in shaping root traits. Certainly, in the case of the link between ABA and

556 Bio35, the data support an outstanding role for ABA for adapting to different soil moisture

557 regimes in the coldest quarter, as no control condition for the same root trait is significantly

558 associated with Bio35. The same holds true for TRL upon auxin treatment and Bio24 (Radiation

18

559   of wettest quarter [W/m^2]), as well as for P2 upon cytokinin treatment and Bio02 (Mean diurnal

560   temperature range (mean(period max-min)) [°C]). Overall, we can conclude that natural variation

561   in root traits is significantly associated with parameters that are highly relevant for local adaption

562   in *Arabidopsis thaliana* and that, in particular, ABA regulated lateral root traits seem to be

563   relevant for adaptation to soil moisture. Finally, we note that both SNP overlaps and trait

564   correlations clearly indicate that we are far from explaining a major proportion of adaptation to

565   climate variables, and that we expect numerous other processes and pathways to contribute to

566   local adaptation. We would hope that similar systematic studies in the future will lead to a more

567   complete picture that can then be tested in the wild and which might yield highly valuable

568   insights into which traits and which underlying pathways contribute to local adaptation.

569

570

571   **MATERIALS & METHODS**

572

573   **Plant Material and Growth Conditions**

574   192 *Arabidopsis thaliana* accessions were used form the Busch group collection at GMI, Vienna.

575   Names and numbers of all accessions are listed in Supplemental Table 1. Seeds were surface

576   sterilized in a desiccator using 100 mL of household bleach and 3.5 mL of 37% HCl for 1 h. The

577   seeds were stratified in water at 4°C in the dark for 3 days and were sown on square petri dishes

578   containing 57 mL of growth medium. The control growth medium (before transfer) consisted of

579   one-fifth-strength MS medium with MES buffer (Ducheva Biochemie), 1% Sucrose, 0.8% agar

580   (Ducheva Biochemie), and adjusted to pH=5.7. Plates were dried for 45 min in a sterile laminar

581   flow, before closing the cover and storing them at room temperature for three days before plating

582   the seeds. In each plate, four accessions were plated, two in each row, with five plant replicates

583   per accession (Supplemental Figure 2). Plates were placed vertically, and seeds were germinated

584   under long-day conditions (21°C, 16-h-light/8-h-dark cycle). Individual seven-day-old seedlings

585   were carefully transferred to the same position onto plates containing control medium

586   supplemented with ABA, abscisic acid (1µM), CK, kinetin (1µM) or IAA, indole-acetic acid (0.5

587   µM) as indicated per experiment (Supplemental Figure 2).

588   Before performing the experiment on all 192 accessions, we first tested the effect of different MS

589   (Murashige and Skoog) concentrations, since full-strength MS medium was found to inhibit root

19

590   growth (Dubrovsky et al., 2009) and was originally designed for tissue culture that includes very

591   high concentrations of mineral nutrients (Dubrovsky & Forde, 2012). We indeed observed

592   inhibition of root growth in the tested concentrations (Supplemental figure 9). Thus, we decided

593   to use the lowest concentration tested. Next, we tested the effect of placement in the upper vs.

594   lower row in the plate, because we intended to fit 4 accessions on one plate. Thus, it was

595   necessary to test if there is plate-position effect leading to differences in root growth between the

596   two rows. Our results show that there was no significant difference between the rows in the plate

597   in the tested accessions/concentrations (Supplemental Figure 10).

598

Phenotypic Analysis

600   After three days on treatment, plates were scanned with CCD flatbed scanners (EPSON

601   Perfection V600 Photo, Seiko Epson CO., Nagano, Japan) and images used to quantify root

602   parameters with FIJI (Schindelin et al., 2012) (Supplemental Figure 2). We quantified: primary

603   root length on day 10 (P), outgrowth of P on the treatment (P2), branching zone or the length of

604   P between first and last visible lateral root (R), average lateral root length (LRL), and visible

605   lateral root number (LR.No). Length was measured in millimeters (mm). Other traits were

606   calculated: lateral root density of P, LRD_P (LR.No/P), lateral root density of R, LRD_R

607   (LR.No/R), total lateral root length, TLRL (LR.No*LRL), total root length, TRL (TLRL+P), and

608   length ratio, LRR (TLRL/P). For detailed protocol see (Ristova & Busch, 2017)

609

Genome Wide Association Studies

611   We conducted GWA mapping on the mean trait values (in case of our root traits) and for each of

612   the 35 climate variables capturing biologically relevant climate aspects (Bioclim variables)

613   (Hutchinson et al., 2009, Kriticos et al., 2012) using a mixed model algorithm (Kang et al., 2008)

614   which has been shown to correct for population structure confounding (Seren et al., 2012) and

615   SNP data from the RegMap panel (Horton et al., 2012). SNPs with minor allele counts greater or

616   equal to 10 were taken into account. The significance of SNP associations was determined at 5%

617   FDR threshold computed by the Benjamini-Hochberg-Yekutieli method to correct for multiple

618   testing (Benjamini & Yekutieli, 2001)

619

Analyzing the overlap of ABA _bona fide_ genes and ABA GWAS candidates

20

621 To determine a list of ABA *bona fide* candidate genes, we used the GO SLIM annotation of the

622 TAIR10 release (ftp://ftp.arabidopsis.org/Ontologies/Gene_Ontology/ATH_GO_GOSLIM.txt;

623 creation date: 4/9/15). We then filtered for GO annotations that contained the key word "abscisic

624 acid" and were based on either IDA ("inferred by direct assay") or IMP ("inferred by mutant

625 phenotype"). The resulting list (Supplemental table 8) was subsequently used as the ABA *bona*

626 *fide* candidate gene list. We then used the hypergeometric distribution to compute the P-value for

627 the observed overlap of the GWAS based ABA candidate gene list and the as ABA *bona fide*

628 candidate gene list. This was done in R (V3.1.2) using the command "dhyper(1,361,33602-

629 361,276)" where 1 is the overlap of genes between the two lists, 361 is the number of genes in

630 the genome that were found in the GWAS, 33602-361 is the number of genes in the genome not

631 found in the GWAS, and 276 is the number of genes in the genome that were *bona fide* candidate

632 genes for ABA signaling.

633

634 **Computing the overlap of signals between GWASs**

635 To determine whether the top associations between hormone GWAS and GWAS for

636 environmental variables was higher than expected by chance, we first obtained the 0.5% most

637 significantly associated SNPs in each GWAS. To avoid biases caused by outlier accessions, only

638 SNPs with a minor allele count equal to or greater than 6 were included in the list of SNPs from

639 which the top SNPs were derived. For each possible combination of root trait and climate

640 variable GWAS, we computed the overlap of these top SNPs. We then used the hypergeometric

641 distribution to compute a P-value for the observed overlap. This was done in python using the

642 scipy.stats.hypergeom function. The list of all computed P-values for all possible combinations

643 was corrected for multiple testing using the p.adjust function in R applying the "fdr" correction

644 (Benjamini & Hochberg, 1995).

645

646 **Computing trait correlations**

647 For computing the trait correlation, we considered only significant combinations of root and

648 climate traits that we had identified using the SNP overlap analysis. We took only these lists into

649 account as we only had a clear hypothesis for them, and testing all possible combinations without

650 a clear hypothesis would have most likely resulted in an overly conservative penalization of P-

651 values by multiple testing corrections for the very large number of tests that would have needed

21

652   to be conducted. To calculate a population corrected association of root trait values and climate

653   variables, we used the lm function of R and fitted a linear model containing either the first two

654   eigenvectors that were obtained by performing a PCA on the SNP matrix from the RegMap

655   population (Horton et al., 2012) (reduced model) or a model to which we added the climate

656   variable (full model). We then performed an ANOVA to test whether the additional variable

657   (climate) accounted for a significant proportion of variance.

658

659   **Statistical Analysis of Root Traits**

660   A biological replicate for phenotyping purposes is an independent seedling of the same genotype.

661   For mutant trait quantification, each assay was repeated 3 times, with each repetition having at

662   least 10 plants per line and per conditions (Figure 5D,E, and Figure 6C,D). For RT-qPCR

663   experiments, a biological replicate consisted of bulked roots grown on the same plate and

664   technical replicates were cDNA samples that were split-up before RT-qPCR and independently

665   measured. RT-qPCR was done with at least 3-4 biological and 2 technical replicates.

666   Statistical analysis and figure generation was done using JMP12 (SAS) (individual license to

667   DR).   Hierarchical clustering was performed using the average linkage method, using

668   standardized mean values of the 10 root traits across the accessions and hormone treatments. The

669   smallest number of clusters for each condition was chosen based on the distance graph (JMP12).

670   Principal component analysis was performed on mean values of the 10 root traits across all

671   accessions and conditions.

672

673   **Gene Expression Analysis (RT-PCR)**

674   Total RNA extraction was performed using a commercial RNA isolation kit (RNeasy Mini Kit

675   Plus, QIAGEN) using whole roots of 10-day-old seedlings, bulking 7-10 roots from the same

676   accession/condition into one biological replicate. A qRT-PCR reaction was prepared using 2x

677   SensiMix^{TM} SYBR & Fluorescein Kit (PEQLAB LLC, Wilmington, DE, USA) and PCR was

678   conducted with a Roche Lightcycler® 96 (Roche) instrument. Relative quantifications were

679   performed for all the genes and the β-tubulin gene (AT5G62690) was used as an internal

680   reference. Gene specific primers used are listed in Supplemental Table 11. Previously described

681   primer pairs were used for *ARR1,* and *ARR12* (Moubayidin et al., 2010), *PIN3* and *PIN7* (Dello

682   Ioio et al., 2008), and *TAA1* (Cui et al., 2013).

22

683

Accession Numbers

Accession numbers for all 192 accessions of *Arabidopsis thaliana* used in this study can be found in Supplemental Table 1. T-DNA mutant lines used in this study: SALK_045920 (AT3G17910), SALK_050958 (AT3G17890), and SAIL_240_H09 (AT5G53290).

ACKNOWLEDGMENTS

Author Contribution

DR, Conception and design, Contributed unpublished essential data or reagents, Acquisition of data, Analysis and interpretation of data, Drafting or revising the article; KM, Acquisition of data; WB, Conception and design, Contributed unpublished essential data or reagents, Analysis and interpretation of data, Drafting or revising the article.
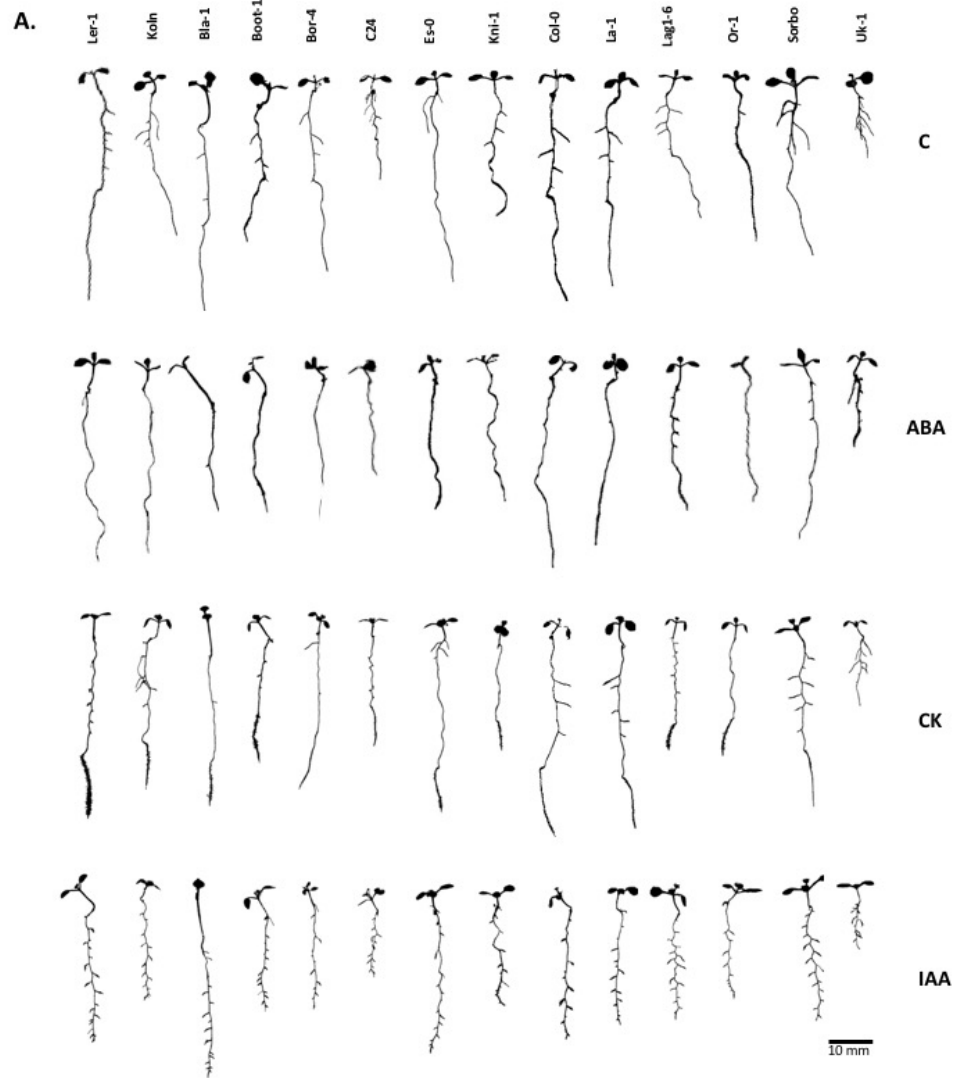
707 **Figures**

708

Figure 1. Representative root phenotypes of 14 Arabidopsis accessions on 3 hormone treatments and control. A) 192 Arabidopsis accessions, were grown on control conditions for 7 days followed by transfer to media supplemented with IAA, CK, and ABA, or no hormone (C, control). Plates were scanned on day 10, and root traits were quantified and segmented using FIJI. Here we show segmented seedlings of 14 accessions. **B)** Heatmap of (log2) fold-change for each trait and hormone treatment compared to control treatment across all 192 accessions and 4 conditions.
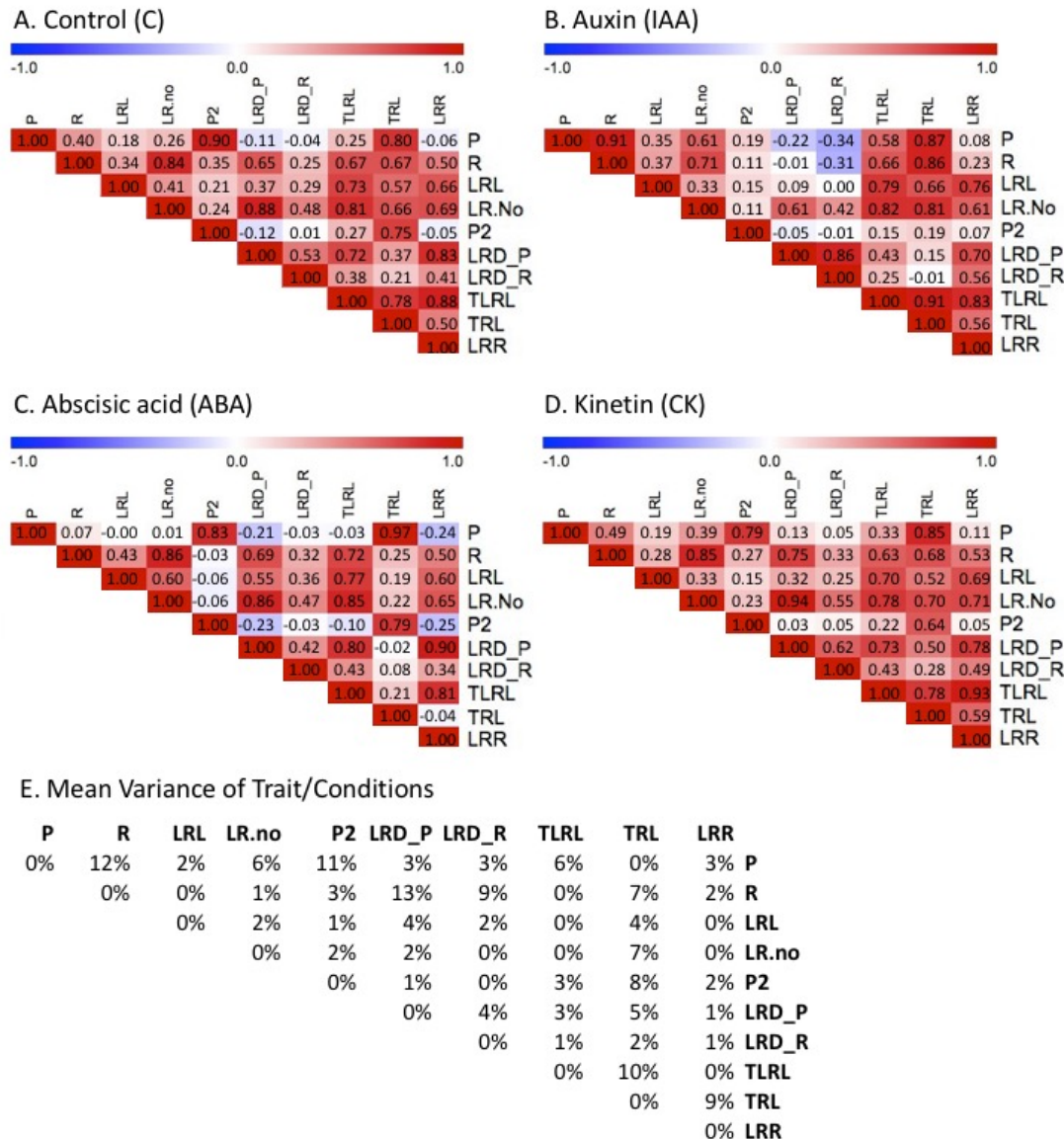
B.

Treat/Trait	P	R	LRL	LR.No	P2	LRD_P	LRD_R	TLRL	TRL	LRR
ABA	-0.24	-2.50	-2.80	-2.05	-0.43	-1.68	-1.61	-3.39	-0.45	-2.93
CK	0.01	0.46	-0.64	0.28	-0.59	0.23	-0.17	-0.36	-0.04	-0.41
IAA	-0.49	1.55	-0.79	2.20	-4.48	2.68	0.99	1.20	-0.05	1.67

Figure 2. Patterns of Root Trait Correlations. (A-D) Heatmaps of pairwise correlations (Pearson product-moment correlation coefficient) of 10 root traits in 192 accessions upon transfer on control (A), auxin,(B), abscisic acid (C), and cytokinin (D) medium. Colors gradient indicates negative (blue) or positive (red) correlation coefficients. (E) Mean variance of all traits in percent. Traits: primary root length (P), growth of P between day 7 and 10 (P2), branching zone of P (R), average lateral root length (LRL), and lateral root numbers (LR.no). Other traits are calculated by formulas: total LRL, TLRL (LR.L*LR.No), total root length, TRL (TLRL+P), density of P, LRD_P (LR.No/P), density of R, LRD_R (LR.No/R), and length ratio, LRR (TLRL/P).
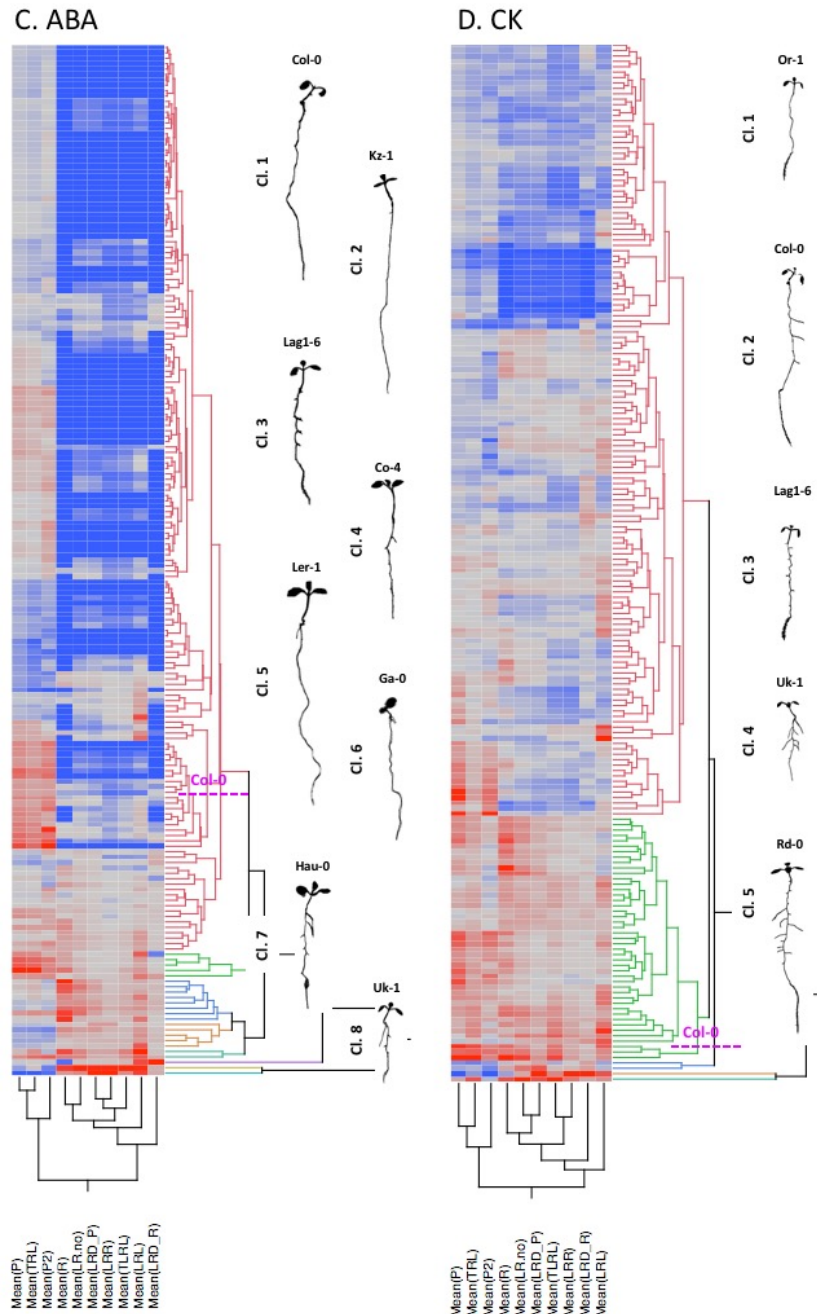
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Figure 3. Hierarchical Clustering (HC) of Arabidopsis accessions by Condition. HC was performed on mean root traits of 192 accessions, in each of the three hormones (ABA, CK, and IAA). On the right side of each dendrogram we show one representative accession from each cluster. **A)** HC of 192 accessions on C (control, no hormone added). **B)** HC of 192 accessions on IAA. **C)** HC of 190 accessions on ABA. D). HC of 192 accessions on cytokinin (CK). Cluster numbers and additional info in Supplemental Table 4.
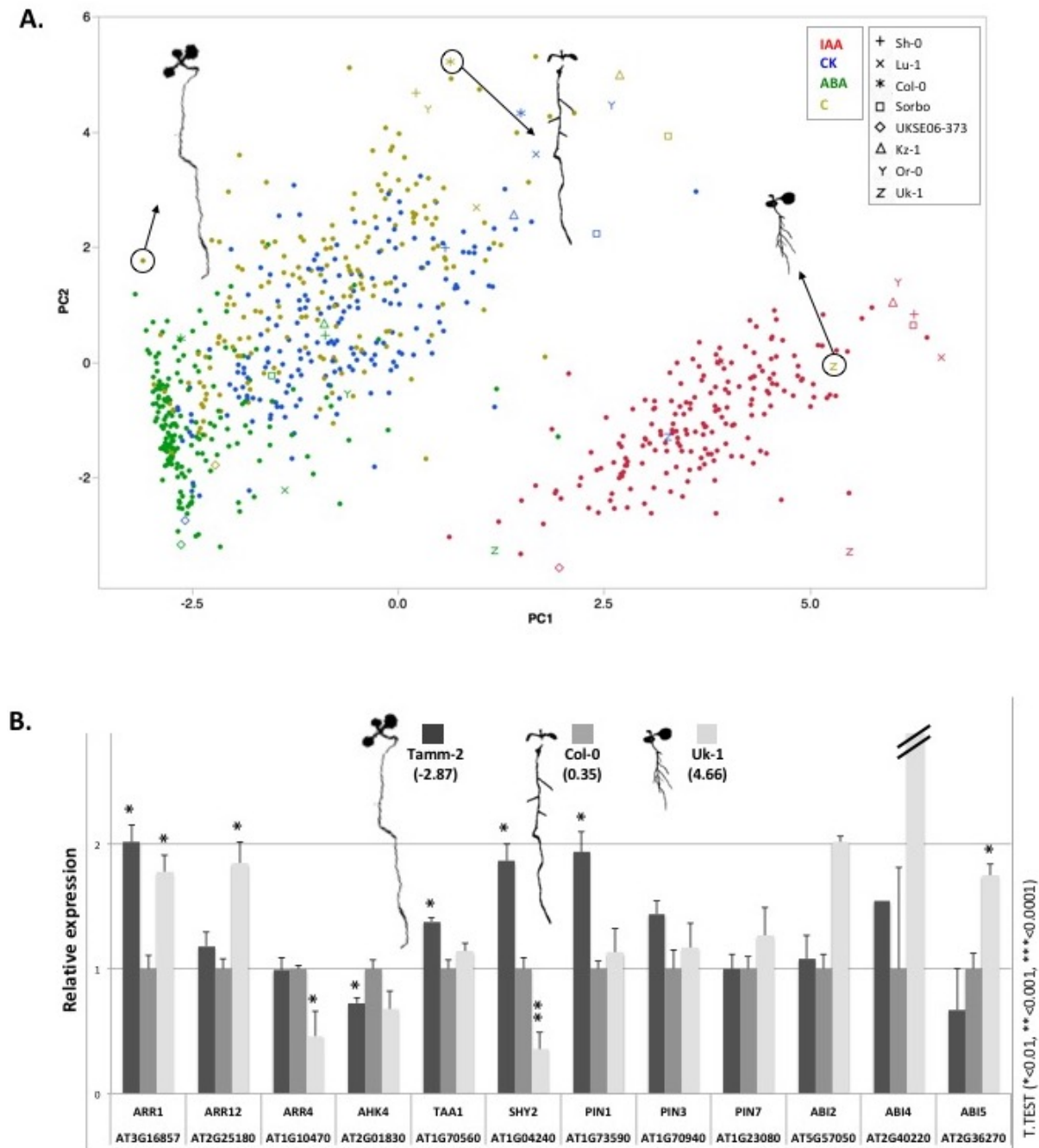
Figure 4. Multivariate Analysis of 192 *Arabidopsis* accessions on 4 conditions and gene expression analysis from extreme accessions. Mean values for 10 root traits across 192 Arabidopsis accessions and 4 conditions (IAA, CK, and ABA, and no hormone C) were used to perform a Principal Component Analysis (PCA). **A)** Biplots of the ten quantified root traits and 192 accessions under 4 conditions are shown for the first 2 principal components (PCs). Each accession in each condition is represented by a dot, and each condition has a different color.

741   Each extreme accession is displayed by a specific symbol that is colored according to the

742   treatment condition. **B)** Relative expression of 12 genes involved in IAA, CK, and ABA

743   hormonal signaling pathways in extreme accessions quantified by PC1: accession Tamm-2

744   (negative extreme in PC1), accession Uk-1 (positive extreme in PC1), and the reference

745   accession Col-0. Segmented seedling phenotypes of the 3 accessions are shown at the top,

746   including the PC1 value, and the position of each accession in control conditions is circled in A).

747   A Student's T-test was performed to determine the significance of gene expression changes in

748   the extreme accessions compared to the reference accession. Mean with s.e.m. is shown. Data

749   from 3-4 biological and 2 technical replicates. *: $P < 0.01$

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E.
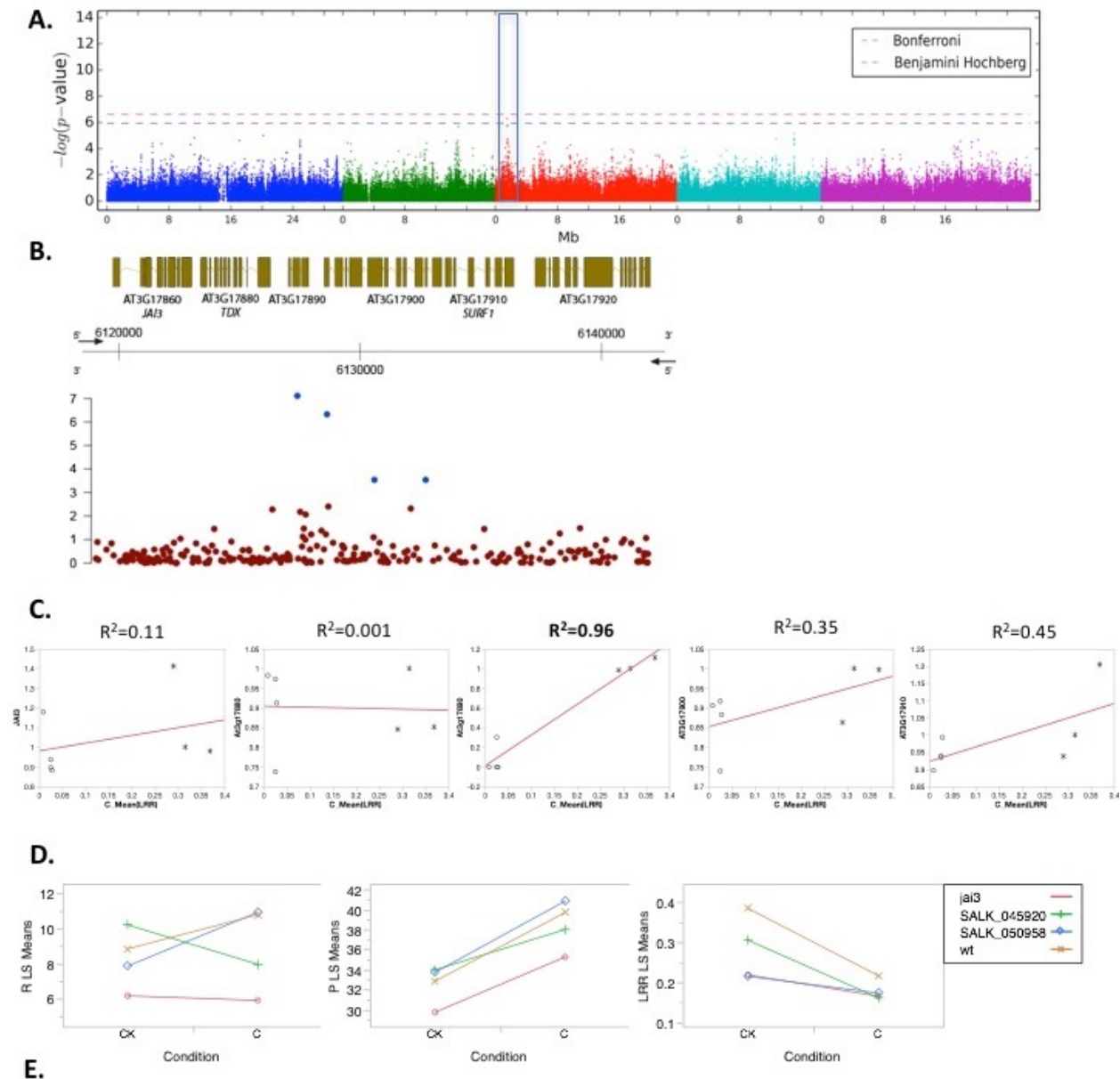
2-ANOVA				Prob>|t|				
Term/Traits	R	P	LRL	LR.No	LRD_P	TLRL	TRL	LRR
Genotype[jai3]	<.0001	<.0001	0.6238	0.0002	0.0133	0.003	<.0001	0.0324
Genotype[SALK_045920]	0.347	0.228	0.101	0.050	0.453	0.786	0.481	0.852
Genotype[SALK_050958]	0.109	<.0001	0.006	0.543	0.926	0.245	0.235	0.028
Condition[CK]	0.298	<.0001	0.007	0.360	0.0001	0.002	0.001	<.0001
Genotype[jai3]*Condition[CK]	0.419	0.662	0.187	0.615	0.346	0.202	0.474	0.171
Genotype[SALK_045920]*Condition[CK]	0.008	0.021	0.106	0.046	0.761	0.120	0.038	0.219
Genotype[SALK_050958]*Condition[CK]	0.016	0.101	0.088	0.044	0.903	0.120	0.073	0.065

Figure 5. Genome-wide association and mutant analysis. A) Manhattan plot of length ratio, LRR (total length of lateral roots/total primary root length) under CK treatment (a significant association is present for the same trait under control condition). B) The genomic region surrounding the significant GWA peak. Bottom, −log10 P values of association of the SNPs.

756   SNPs highlighted in blue are in in LD ($R^2 > 0.4$) with top SNP. Top, gene models in genomic

757   regions. The *x* axis represents the position on chromosome 3. C) Regression analysis of the

758   associated trait LRR (mean) and the relative expression of the 5 genes in the associated region in

759   several extreme accessions. D) Reaction norm plots of root branching zone (R, in mm), primary

760   root length (P, in mm), and length ratio (LRR) for available T-DNA lines for genes in the

761   associated region E) 2-way-ANOVA summary for all root traits quantified under control (C) and

762   cytokinin (CK) treatment (P-value=0.05). Mutant lines: *jai3-1* (At3g17860), SALK_050958

763   (At3g17890), SALK_045920(At3g17910); (N>30).

764

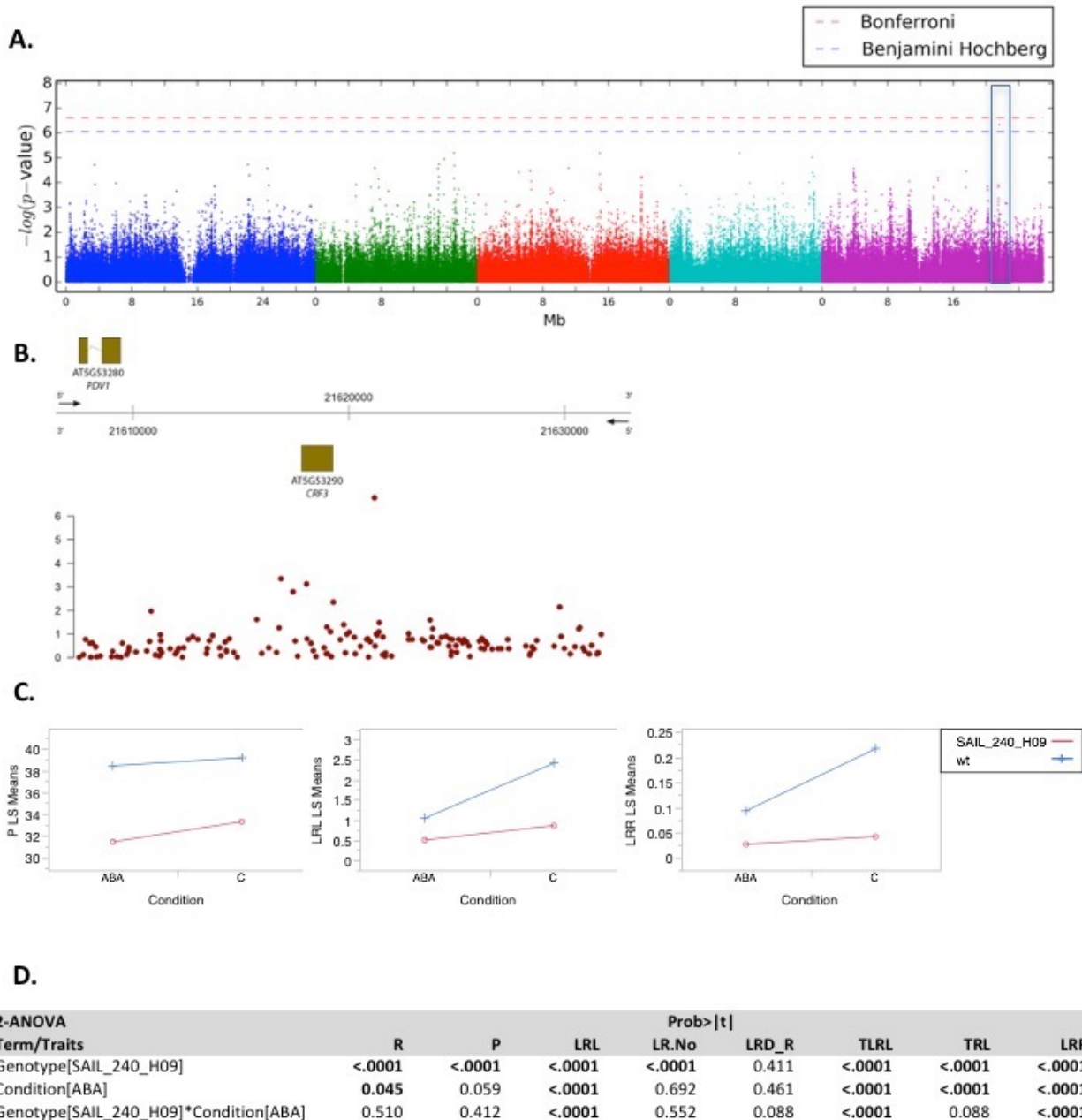| 2-ANOVA | | | | | Prob>\|t\| | | | |
Term/Traits	R	P	LRL	LR.No	LRD_R	TLRL	TRL	LRR
Genotype[SAIL_240_H09]	<.0001	<.0001	<.0001	<.0001	0.411	<.0001	<.0001	<.0001
Condition[ABA]	0.045	0.059	<.0001	0.692	0.461	<.0001	<.0001	<.0001
Genotype[SAIL_240_H09]*Condition[ABA]	0.510	0.412	<.0001	0.552	0.088	<.0001	0.088	<.0001

Figure 6. **Genome-wide association and mutant analysis of CRF3.** A) Manhattan plot of the significant genome-wide association for trait root density (LRD_R) (ch. 5: 21621187). B) The genomic region surrounding the significant GWA peak. Bottom, −log10 *P* values of association of the SNPs. Top, gene models in genomic regions. The *x* axis represents the position on chromosome 5. C) Reaction norm plots of primary root length (P, in mm), lateral root length (LRL, in mm), and length ratio (LRR) for the T-DNA line for CRF3 (SAIL_240_H09), the only gene in the associated region (20 kb window). D) 2-way-ANOVA summary for all root traits quantified under control (C) and abscisic acid (ABA) treatment (N>30).

774 **REFERENCES**
775

776 Ackerly DD, Dudley SA, Sultan SE*, et al.*, 2000. The evolution of plant ecophysiological traits:
777 recent advances and future directions new research addresses natural selection, genetic
778 constraints, and the adaptive evolution of plant ecophysiological traits. In: Bioscience, Pp.979-
779 995., ed.

780 Alonso-Blanco C, Koornneef M, 2000. Naturally occurring variation in Arabidopsis: an
781 underexploited resource for plant genetics. *Trends Plant Sci* 5, 22-9.

782 Atwell S, Huang YS, Vilhjalmsson BJ*, et al.*, 2010. Genome-wide association study of 107
783 phenotypes in Arabidopsis thaliana inbred lines. *Nature* 465, 627-31.

784 Benjamini YA, Hochberg Y, 1995. Controlling the false discovery rate: a practical and powerful
785 approach to multiple testing. Journal of the royal statistical society   *Series B (Methodological),*
786 *pp.289-300.*

787 Benjamini YA, Yekutieli D, 2001. The control of the false discovery rate in multiple testing
788 under dependency. In: Annals of Statistics P-, ed.

789 Brachi B, Faure N, Horton M*, et al.*, 2010. Linkage and association mapping of Arabidopsis
790 thaliana flowering time in nature. *PLoS Genet* 6, e1000940.

791 Chini A, Fonseca S, Fernandez G*, et al.*, 2007. The JAZ family of repressors is the missing link
792 in jasmonate signalling. *Nature* 448, 666-71.

793 Cui D, Zhao J, Jing Y*, et al.*, 2013. The arabidopsis IDD14, IDD15, and IDD16 cooperatively
794 regulate lateral organ morphogenesis and gravitropism by promoting auxin biosynthesis and
795 transport. *PLoS Genet* 9, e1003759.

796 De Smet I, Signora L, Beeckman T, Inze D, Foyer CH, Zhang H, 2003. An abscisic acid-
797 sensitive checkpoint in lateral root development of Arabidopsis. *Plant J* 33, 543-55.

798 Delker C, Poschl Y, Raschke A*, et al.*, 2010. Natural variation of transcriptional auxin response
799 networks in Arabidopsis thaliana. *Plant Cell* 22, 2184-200.

800 Dello Ioio R, Nakamura K, Moubayidin L*, et al.*, 2008. A Genetic Framework for the Control of
801 Cell Division and Differentiation in the Root Meristem. *Science* 322, 1380-4.

802 Den Herder G, Van Isterdael G, Beeckman T, De Smet I, 2010. The roots of a new green
803 revolution. *Trends in Plant Science* 15, 600-7.

33

804   Ding ZJ, Yan JY, Li CX, Li GX, Wu YR, Zheng SJ, 2015. Transcription factor WRKY46

805   modulates the development of Arabidopsis lateral roots in osmotic/salt stress conditions via

806   regulation of ABA signaling and auxin homeostasis. *Plant J* 84, 56-69.

807   Du H, Wu N, Fu J, *et al.*, 2012. A GH3 family member, OsGH3-2, modulates auxin and abscisic

808   acid levels and differentially affects drought and cold tolerance in rice. *Journal of Experimental*

809   *Botany* 63, 6467-80.

810   Dubrovsky JG, Forde BG, 2012. Quantitative analysis of lateral root development: pitfalls and

811   how to avoid them. *Plant Cell* 24, 4-14.

812   Dubrovsky JG, Soukup A, Napsucialy-Mendivil S, Jeknic Z, Ivanchenko MG, 2009. The lateral

813   root initiation index: an integrative measure of primordium formation. *Ann Bot* 103, 807-17.

814   Evans ML, Ishikawa H, Estelle MA, 1994. Responses of Arabidopsis Roots to Auxin Studied

815   with High Temporal Resolution - Comparison of Wild-Type and Auxin-Response Mutants.

816   *Planta* 194, 215-22.

817   Fischer I, Camus-Kulandaivelu L, Allal F, Stephan W, 2011. Adaptation to drought in two wild

818   tomato species: the evolution of the Asr gene family. *New Phytol* 190, 1032-44.

819   Fournier-Level A, Korte A, Cooper MD, Nordborg M, Schmitt J, Wilczek AM, 2011. A map of

820   local adaptation in Arabidopsis thaliana. *Science* 334, 86-9.

821   Gifford ML, Banta JA, Katari MS, *et al.*, 2013. Plasticity Regulators Modulate Specific Root

822   Traits in Discrete Nitrogen Environments. *Plos Genetics* 9.

823   Hong JH, Seah SW, Xu J, 2013. The root of ABA action in environmental stress response. *Plant*

824   *Cell Reports* 32, 971-83.

825   Horton MW, Hancock AM, Huang YS, *et al.*, 2012. Genome-wide patterns of genetic variation

826   in worldwide Arabidopsis thaliana accessions from the RegMap panel. *Nat Genet* 44, 212-6.

827   Hu YF, Zhou G, Na XF, *et al.*, 2013. Cadmium interferes with maintenance of auxin

828   homeostasis in Arabidopsis seedlings. *J Plant Physiol* 170, 965-75.

829   Hutchinson M, T. Xu, D. Houlder, H. Nix A, Mcmahon J, 2009. ANUCLIM 6.0 User's Guide

830   *Canberra: Australian National University, Fenner School of Environment and Society*.

831   Kang HM, Zaitlen NA, Wade CM, *et al.*, 2008. Efficient control of population structure in model

832   organism association mapping. *Genetics* 178, 1709-23.

833   Kang JY, Choi HI, Im MY, Kim SY, 2002. Arabidopsis basic leucine zipper proteins that

834   mediate stress-responsive abscisic acid signaling. *Plant Cell* 14, 343-57.

835   Koornneef M, Alonso-Blanco C, Vreugdenhil D, 2004. Naturally occurring genetic variation in

836   Arabidopsis thaliana. *Annu Rev Plant Biol* 55, 141-72.

837   Kriticos DJ, Webber BL, Leriche A*, et al.*, 2012. CliMond: global high-resolution historical and

838   future scenario climate surfaces for bioclimatic modelling. . *Methods in Ecology and Evolution,*

839   *3(1), pp.53-64.*

840   Krouk G, Lacombe B, Bielach A*, et al.*, 2010. Nitrate-Regulated Auxin Transport by NRT1.1

841   Defines a Mechanism for Nutrient Sensing in Plants. *Developmental Cell* 18, 927-37.

842   Kumar MN, Verslues PE, 2015. Stress physiology functions of the Arabidopsis histidine kinase

843   cytokinin receptors. *Physiol Plant* 154, 369-80.

844   Laplaze L, Benkova E, Casimiro I*, et al.*, 2007. Cytokinins act directly on lateral root founder

845   cells to inhibit root initiation. *Plant Cell* 19, 3889-900.

846   Lasky JR, Des Marais DL, Lowry DB*, et al.*, 2014. Natural variation in abiotic stress responsive

847   gene expression and local adaptation to climate in Arabidopsis thaliana. *Mol Biol Evol* 31, 2283-

848   96.

849   Leung J, Merlot S, Giraudat J, 1997. The Arabidopsis ABSCISIC ACID-INSENSITIVE2

850   (ABI2) and ABI1 genes encode homologous protein phosphatases 2C involved in abscisic acid

851   signal transduction. *Plant Cell* 9, 759-71.

852   Li X, Mo X, Shou H, Wu P, 2006. Cytokinin-mediated cell cycling arrest of pericycle founder

853   cells in lateral root initiation of Arabidopsis. *Plant Cell Physiol* 47, 1112-23.

854   Liu W, Li RJ, Han TT, Cai W, Fu ZW, Lu YT, 2015. Salt Stress Reduces Root Meristem Size by

855   Nitric Oxide-Mediated Modulation of Auxin Accumulation and Signaling in Arabidopsis. *Plant*

856   *Physiology* 168, 343-U607.

857   Lopez-Bucio J, Hernandez-Abreu E, Sanchez-Calderon L*, et al.*, 2005. An auxin transport

858   independent pathway is involved in phosphate stress-induced root architectural alterations in

859   arabidopsis. Identification of BIG as a mediator of auxin in pericycle cell activation. *Plant*

860   *Physiology* 137, 681-91.

861   Lynch J, 1995. Root Architecture and Plant Productivity. *Plant Physiology* 109, 7-13.

862   Malamy JE, 2005. Intrinsic and environmental response pathways that regulate root system

863   architecture. *Plant Cell and Environment* 28, 67-77.

864   Moubayidin L, Perilli S, Dello Ioio R, Di Mambro R, Costantino P, Sabatini S, 2010. The rate of

865   cell differentiation controls the Arabidopsis root meristem growth phase. *Curr Biol* 20, 1138-43.

866   Mouchel CF, Briggs GC, Hardtke CS, 2004. Natural genetic variation in Arabidopsis identifies

867   BREVIS RADIX, a novel regulator of cell proliferation and elongation in the root. *Genes Dev*

868   18, 700-14.

869   Mouchel CF, Osmont KS, Hardtke CS, 2006. BRX mediates feedback between brassinosteroid

870   levels and auxin signalling in root growth. *Nature* 443, 458-61.

871   Nacry P, Canivenc G, Muller B*, et al.*, 2005. A role for auxin redistribution in the responses of

872   the root system architecture to phosphate starvation in Arabidopsis. *Plant Physiol* 138, 2061-74.

873   Pacheco-Villalobos D, Sankar M, Ljung K, Hardtke CS, 2013. Disturbed local auxin

874   homeostasis enhances cellular anisotropy and reveals alternative wiring of auxin-ethylene

875   crosstalk in Brachypodium distachyon seminal roots. *PLoS Genet* 9, e1003564.

876   Perez-Torres CA, Lopez-Bucio J, Cruz-Ramirez A*, et al.*, 2008. Phosphate availability alters

877   lateral root development in Arabidopsis by modulating auxin sensitivity via a mechanism

878   involving the TIR1 auxin receptor. *Plant Cell* 20, 3258-72.

879   Rashotte AM, Mason MG, Hutchison CE, Ferreira FJ, Schaller GE, Kieber JJ, 2006. A subset of

880   Arabidopsis AP2 transcription factors mediates cytokinin responses in concert with a two-

881   component pathway. *Proc Natl Acad Sci U S A* 103, 11081-5.

882   Ristova D, Busch W, 2017. Genome-Wide Association Mapping of Root Traits in the Context of

883   Plant Hormone Research. *Methods Mol Biol* 1497, 47-55.

884   Ristova D, Rosas U, Krouk G, Ruffel S, Birnbaum KD, Coruzzi GM, 2013. RootScape: a

885   landmark-based system for rapid screening of root architecture in Arabidopsis. *Plant Physiol*

886   161, 1086-96.

887   Rosas U, Cibrian-Jaramillo A, Ristova D*, et al.*, 2013. Integration of responses within and across

888   Arabidopsis natural accessions uncovers loci controlling root systems architecture. *Proc Natl*

889   *Acad Sci U S A* 110, 15133-8.

890   Ruffel S, Krouk G, Ristova D, Shasha D, Birnbaum KD, Coruzzi GM, 2011. Nitrogen

891   economics of root foraging: Transitive closure of the nitrate-cytokinin relay and distinct systemic

892   signaling for N supply vs. demand. *Proceedings of the National Academy of Sciences of the*

893   *United States of America* 108, 18524-9.

894   Salome PA, To JPC, Kieber JJ, Mcclung CR, 2006. Arabidopsis response regulators ARR3 and

895   ARR4 play cytokinin-independent roles in the control of circadian period. *Plant Cell* 18, 55-69.

896   Satbhai SB, Ristova D, Busch W, 2015. Underground tuning: quantitative regulation of root

897   growth. *Journal of Experimental Botany* 66, 1099-112.

898   Schindelin J, Arganda-Carreras I, Frise E*, et al.*, 2012. Fiji: an open-source platform for

899   biological-image analysis. *Nat Methods* 9, 676-82.

900   Seren U, Vilhjalmsson BJ, Horton MW*, et al.*, 2012. GWAPP: a web application for genome-

901   wide association mapping in Arabidopsis. *Plant Cell* 24, 4793-805.

902   Simaskova M, O'brien JA, Khan M*, et al.*, 2015. Cytokinin response factors regulate PIN-

903   FORMED auxin transporters. *Nat Commun* 6, 8717.

904   Singh AP, Fridman Y, Friedlander-Shani L, Tarkowska D, Strnad M, Savaldi-Goldstein S, 2014.

905   Activity of the Brassinosteroid Transcription Factors BRASSINAZOLE RESISTANT1 and

906   BRASSINOSTEROID INSENSITIVE1-ETHYL METHANESULFONATE-

907   SUPPRESSOR1/BRASSINAZOLE RESISTANT2 Blocks Developmental Reprogramming in

908   Response to Low Phosphate Availability. *Plant Physiology* 166, 678-88.

909   Stepanova AN, Robertson-Hoyt J, Yun J*, et al.*, 2008. TAA1-mediated auxin biosynthesis is

910   essential for hormone crosstalk and plant development. *Cell* 133, 177-91.

911   Sun P, Tian QY, Chen J, Zhang WH, 2010. Aluminium-induced inhibition of root elongation in

912   Arabidopsis is mediated by ethylene and auxin. *Journal of Experimental Botany* 61, 347-56.

913   Van Leeuwen H, Kliebenstein DJ, West MA*, et al.*, 2007. Natural variation among Arabidopsis

914   thaliana accessions for transcriptome response to exogenous salicylic acid. In: The Plant Cell,

915   Pp.2099-2110., ed.

916   Vanstraelen M, Benkova E, 2012. Hormonal interactions in the regulation of plant development.

917   *Annu Rev Cell Dev Biol* 28, 463-87.

918   Vicente-Agullo F, Rigas S, Desbrosses G, Dolan L, Hatzopoulos P, Grabov A, 2004. Potassium

919   carrier TRH1 is required for auxin transport in Arabidopsis roots. *Plant Journal* 40, 523-35.

920   Vidal EA, Araus V, Lu C*, et al.*, 2010. Nitrate-responsive miR393/AFB3 regulatory module

921   controls root system architecture in Arabidopsis thaliana. *Proc Natl Acad Sci U S A* 107, 4477-

922   82.

923   Werner T, Motyka V, Strnad M, Schmulling T, 2001. Regulation of plant growth by cytokinin.

924   *Proc Natl Acad Sci U S A* 98, 10487-92.

925   Xia H, Camus-Kulandaivelu L, Stephan W, Tellier A, Zhang Z, 2010. Nucleotide diversity

926   patterns of local adaptation at drought-related candidate genes in wild tomatoes. *Mol Ecol* 19,

927   4144-54.

928   Yamada H, Suzuki T, Terada K*, et al.*, 2001. The Arabidopsis AHK4 histidine kinase is a

929   cytokinin-binding receptor that transduces cytokinin signals across the membrane. *Plant and Cell*

930   *Physiology* 42, 1017-23.

931   Yu J, Pressoir G, Briggs WH*, et al.*, 2006. A unified mixed-model method for association

932   mapping that accounts for multiple levels of relatedness. *Nat Genet* 38, 203-8.

933   Yuan HM, Xu HH, Liu WC, Lu YT, 2013. Copper regulates primary root elongation through

934   PIN1-mediated auxin redistribution. *Plant Cell Physiol* 54, 766-78.

935   Zhao YK, Wang T, Zhang WS, Li X, 2011. SOS3 mediates lateral root development under low

936   salt stress through regulation of auxin redistribution and maxima in Arabidopsis. *New*

937   *Phytologist* 189, 1122-34.

938

939

Table 1. Summary Information of Significantly Associated Regions obtained by GWA Mapping (BH threshold, 5% FDR).

Trait	Treat	Ch #	SNP position	GWA *P* value	Benjamini Hochberg (neg[log]Pval)
Mean(LRR)	C	3, 3, 5, 3 1, 5, 5, 1	18730847, 6127399, 20263253, 9654227, 20162056, 25855852, 6909572, 18708566	3.82E-09, 7.70E-08, 1.02E-07, 3.11E-07, 5.02E-07, 1.03E-06, 1.25E-06, 2.09E-06	5.65E+00
Mean (LRD_R)	C	1	16104248	6.63E-08	6.31E+00
Mean(LRL)	IAA	3	23029970	1.89E-07	6.31E+00
Mean(LRR)	CK	4, 3, 3, 3, 5	5502826, 6127399, 6128629, 1518618, 17211166	1.79E-08, 7.70E-08, 4.69E-07, 5.05E-07, 5.05E-07	5.94E+00
Mean(LR.no)	ABA	1, 5	3464235, 21621187	1.88E-07, 4.64E-07	6.05E+00
Mean(LRD_P)	ABA	3, 3, 3, 3, 3	18730847, 6432520, 10035920, 11140451, 6432784	2.00E-07, 2.77E-07, 3.85E-07, 5.23E-07, 9.74E-07	5.96E+00
Mean(LRD_R)	ABA	1, 5,3 ,1, 3, 1, 5, 5, 3, 1, 1, 4, 1, 1, 4, 5, 5, 1, 1, 4, 4, 2, 1, 5, 1	3476564, 21621187, 21283684, 26910129, 10995702, 3474640, 15450107, 21524212 21258706, 2318710, 3476243, 13282571, 5838544, 22271511, 13296141, 9498624, 9067032, 5821121, 11953256, 9368143, 3926453, 8276689, 17562670, 22270694, 3473536	1.23E-07, 1.65E-07, 2.44E-07, 3.43E-07, 3.53E-07, 7.12E-07, 7.27E-07, 8.41E-07, 8.83E-07, 1.01E-06, 1.09E-06, 1.12E-06, 1.20E-06, 1.87E-06, 2.01E-06, 2.17E-06, 2.17E-06, 2.41E-06, 2.45E-06, 2.50E-06, 3.71E-06, 3.80E-06, 3.98E-06, 4.03E-06, 4.07E-06	5.37E+00
Mean(LRR)	ABA	3, 5, 3, 3, 3, 3, 3, 3, 1, 1, 4, 1, 3, 5, 2, 3, 1, 1, 4, 3, 3, 1, 3, 3, 3, 4, 5, 2, 5, 3, 1, 1, 5, 3, 3, 5,	10035920, 14857304, 11140451, 6432520, 18730847, 18368482, 6432784, 9984934, 27053227, 6126365, 16527439, 27026361, 3036751, 21515990, 16222279, 9999583, 16504751, 25156407, 15708104, 9802078 9804462, 26583045, 6431861, 7945274, 9791884, 12837993, 25847727, 610291, 22270694, 9799941, 25162621, 10387202, 8248681, 10054595, 11153455, 8243957	6.21E-10, 3.51E-09, 1.08E-08, 1.17E-08, 1.39E-08, 1.61E-08, 3.02E-08, 5.82E-08, 7.51E-08, 7.60E-08, 7.70E-08, 1.66E-07, 1.86E-07, 3.24E-07, 3.73E-07, 4.54E-07, 5.75E-07, 6.83E-07, 8.70E-07, 9.05E-07, 9.05E-07, 9.11E-07, 1.04E-06, 1.08E-06, 1.17E-06, 1.28E-06, 1.33E-06, 1.39E-06, 1.39E-06, 1.48E-06, 1.60E-06, 2.27E-06, 2.39E-06, 2.39E-06, 2.41E-06, 2.43E-06,	5.47E+00

Mean(TLRL)	ABA	3, 1, 1, 3,	15049139, 2439854, 2439731, 21618856	2.56E-06, 2.82E-06, 2.82E-06, 3.29E-06,	5.51E+00
		3, 1, 5, 1,	10035920, 3464235, 8509007, 3476564,	2.20E-09, 8.78E-09, 3.05E-08, 3.98E-08,	
		5, 5, 5, 5,	8525033, 8512551, 8513042, 3454021,	4.00E-08, 5.28E-08, 7.41E-08, 8.37E-08,	
		1, 3, 1, 1,	3474640, 11140451, 3476243, 22084145,	1.87E-07, 2.05E-07, 3.87E-07, 4.90E-07,	
		1, 4, 5, 5,	22084211, 8096866, 14857304, 8524288,	4.90E-07, 5.65E-07, 6.84E-07, 1.23E-06,	
		1, 3, 5, 5,	6126365, 6431861, 21520565, 21525637,	1.24E-06, 1.24E-06, 1.45E-06, 1.45E-06,	
		3, 5, 2, 3,	6432784, 21524545, 13725657, 18730847,	1.47E-06, 1.86E-06, 2.13E-06, 2.22E-06,	
		1, 5	3474489, 21527231	2.48E-06, 2.90E-06,	