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1 **Supra-organismal regulation of strigolactone exudation**  
2 **and plant development in response to rhizospheric cues**  
3 **in rice**

4  
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15

16 **Keywords**

17 Strigolactones, neighbour detection, root exudates, rice, plant-plant interactions  
18  
19

## 20 SUMMARY

21 Plants have evolved elaborate mechanisms to detect neighbouring plants, which typically involve  
22 the perception of 'cues' inadvertently produced by the neighbour.<sup>1</sup> Strigolactones are hormonal  
23 signalling molecules<sup>2,3</sup> that are also exuded into the rhizosphere by most flowering plant species to  
24 promote arbuscular mycorrhizal symbioses.<sup>4</sup> Since flowering plants have an endogenous perception  
25 system for strigolactones,<sup>5</sup> strigolactones are obvious candidates to act as a cue for neighbour  
26 presence, but have not been shown to act as such. To test this hypothesis in rice plants, we  
27 quantified two major strigolactones of rice plants, orobanchol and 4-deoxyorobanchol, in root  
28 exudates by using LC-MS/MS (MRM), and examined feedback-regulation of strigolactone  
29 biosynthesis and changes in shoot branching phenotypes in rice plants grown at different densities  
30 in hydroponics and soil culture. We show that the presence of neighbouring plants, or greater root  
31 volume, results in rapidly-induced changes in strigolactone biosynthesis, sensitivity, and exudation,  
32 and the subsequent longer-term changes in shoot architecture. These changes require intact  
33 strigolactone biosynthesis in neighbouring plants and intact strigolactone signalling in focal plants.  
34 These results suggest that strigolactone biosynthesis and exudation in rice plants are driven by  
35 supra-organismal environmental strigolactone levels. Strigolactones thus act as a cue for neighbour  
36 presence in rice plants, but also seem to act as a more general root density sensing mechanism in  
37 flowering plants, that integrates soil volume and neighbour density, and allows plants to adapt to the  
38 limitations of the rhizosphere.

39

## 40 RESULTS

41

### 42 **Crowding induces decreased strigolactone biosynthesis and exudation**

43 To understand the effects of plant density-sensing on strigolactone biology, we used a hydroponic  
44 system to grow rice plants (*Oryza sativa* cv. Nipponbare) under phosphate (P) deficiency. Many soils  
45 are P-deficient, and even in fertile soils phosphorus concentration is lower than 10  $\mu\text{M}$ .<sup>6</sup> In addition,  
46 ca. 80% of applied phosphorus may be fixed in the soil through the interaction with other ions and  
47 unavailable to plants.<sup>7</sup> For example, when rice, maize, pea, and tomato plants were grown under  
48 'normal' nutrient conditions (160  $\mu\text{M}$  P in our test) in hydroponics, strigolactone levels in the growth  
49 media were quite low and often below detection limits.<sup>8,9</sup> However, we could detect and quantify  
50 strigolactones from plants grown under 'normal' nutrient conditions in soils, indicating that the plants  
51 were subjected to some level of P deficiency in particular in the rhizosphere where P can be rapidly  
52 depleted by plant root uptake.<sup>10</sup> Therefore, using P-depletion in our hydroponic work is likely more  
53 relevant to 'normal' soil conditions, than a 'normal' hydroponic condition, at least in terms of  
54 strigolactone exudation. After a 4-day period of P-depletion, 16-day old seedlings were placed into  
55 either a 1-, 2- or 3-plant culture in the same volume of hydroponate (180 mL). After 72 h, we  
56 measured levels of 4-deoxyorobanchol (4DO) and orobanchol (OB) in the hydroponate. Remarkably,

57 we found that levels of both 4DO and OB were essentially identical irrespective of the number of  
58 plants in the system; thus per plant exudation in a 2-plant culture was half that of a 1-plant culture,  
59 while exudation in a 3-plant culture was a third of that level (Figure 1A), indicating that the net  
60 strigolactone exudation in 1, 2- and 3-plant culture is constant (Figure S1A). As root growth biomass  
61 was not yet affected by density (Figure S1C, D), these results strongly suggest that plants can detect  
62 other plants and homeostatically adjust their strigolactone exudation before physiological responses  
63 including root growth become apparent. We measured levels of 4DO in the roots of the plants, and  
64 found no corresponding decrease in 4DO in the roots; if anything, there is a slight but non-significant  
65 increase (Figure 1B, Figure S1B). OB levels in root tissues were below the detection limit of our set-  
66 up. We examined expression of strigolactone biosynthesis genes in the roots by qRT-PCR, and  
67 found that expression of *D27*, *D17*, *D10* and *Os01g0701400* was reduced by 50–70% in plants  
68 grown in 3-plant culture compared to those in 1-plant culture (Figure 1C, D). However, expression  
69 of *Os01g070900* involving the conversion of carlactone to 4DO via carlactonoic acid (Figure 1D) was  
70 not significantly changed (Figure 1C). The decreased expression in strigolactone biosynthetic genes  
71 was accompanied by 3-fold increased expression of the *D14* SL receptor in plants grown in 3-plant  
72 culture, though there was no increased expression of the *D3* F-box or the *D53* target proteins (Figure  
73 1C, D). Thus, our results indicate that short exposure to neighbouring plants triggers a density-  
74 dependent reduction in strigolactone biosynthesis and exudation, with a balancing increase in  
75 strigolactone sensitivity.

76  
77 To assess how quickly plants respond to neighbouring plants, we repeated the experiment with a 7-  
78 day P-depletion period prior to transfer to either 1- or 3-plant cultures, and then sampled  
79 strigolactone levels every 24 h after transfer (Figure S1E, F). We found that per-plant exudation of  
80 4DO was already significantly decreased by 24 h, attaining the characteristic 3-fold reduction by 48  
81 h (Figure S1E), while OB levels were not reduced at 24 h, and did not attain the 3-fold reduction until  
82 72 h after transfer (Figure S1F). Thus, changes in strigolactone biosynthesis and exudation begin  
83 almost immediately after exposure to neighbouring plants, but take some time to equilibrate, and  
84 there are some differences in timescale on which this occurs for different strigolactone species.

85  
86 Our results raise the interesting question of whether changes in strigolactone exudation occur in  
87 response to the presence of neighbouring plants *per se* or in response to the overall density of roots  
88 (including their own) in the environment. To distinguish between these possibilities, we grew rice  
89 plants in our previously described hydroponic system, and after 7-day P-depletion transferred them  
90 to 1-plant cultures in either 180 mL or 360 mL hydroponic vessels. We observed that 4DO levels  
91 after 24 h were 2-fold higher in the 2-fold larger vessel (Figure S2A), strongly suggesting that plants  
92 exude strigolactone until a particular concentration in the growth media is reached. Therefore, plants  
93 appear to modulate strigolactone exudation in response to the density of roots, rather than presence  
94 of neighbours *per se*. Consistent with our previous results, 4DO levels in the roots of the plants were

95 not altered (Figure S2B), and the increase in strigolactone exudation in the 360 mL vessel was  
96 accompanied by a comparable increase in expression of core strigolactone biosynthesis genes  
97 (Figure S2C).

98

### 99 **Density-dependent changes in strigolactone exudation require strigolactone** 100 **perception**

101 Given that these strigolactone biosynthesis responses can be triggered in the absence of  
102 neighbouring plants, an intriguing possibility is that changes in strigolactone exudation associated  
103 with changes in root density are actually driven by the concentration of strigolactone in the  
104 environment, and not by a separate cue. To test this possibility, we tested the ability of *d14*  
105 strigolactone receptor mutants to respond to changes in plant density using our hydroponic system.  
106 We found that, unlike in wild-type plants, there was no statistically significant reduction in per-plant  
107 4DO or OB exudation in either 2- or 3-plant cultures relative to 1-plant cultures (Figure 2A). Thus,  
108 total strigolactone levels in the system were much higher in this experiment in the 2- and 3-plant  
109 cultures. Consistent with this, we observed a small increase in the levels of 4DO present in the root  
110 tissues of plants grown in 2- or 3-plants cultures (Figure 2B). In accordance with the failure to  
111 downregulate strigolactone exudation, we observed no reduction in the expression of core  
112 strigolactone biosynthesis genes in *d14* mutants (Figure 2C).

113

### 114 **Strigolactone biosynthesis and exudation is homeostatically regulated in** 115 **response to environmental strigolactone**

116 To test whether environmental strigolactones, including those produced by other plants, drive  
117 changes in strigolactone biosynthesis and exudation, we utilized combinatorial experiments in which  
118 we grew wild-type plants together with *d17* (CCD7) strigolactone biosynthesis mutants in 2-plant  
119 cultures (Figure 3A). Consistent with previous results, wild-type plants grown in 2-plant culture with  
120 other wild-type plants reduced their exudation of 4DO by 2-fold, 24 h after transfer (Figure 3B).  
121 However, wild-type plants grown in 2-plant culture with *d17* mutants did not significantly reduce their  
122 exudation of 4DO in the same time frame. This indicates that the lack of strigolactone exudation from  
123 *d17* mutants leads to a failure to down-regulate 4DO exudation in the wild-type plants, despite the  
124 increase in root density. We also utilized this set-up to demonstrate that plants can indeed take-up  
125 and perceive environmental strigolactone. As anticipated, we found no 4DO present in the roots of  
126 *d17* plants grown in 1-plant culture; however, 4DO was detected in the roots of *d17* plants co-cultured  
127 with wild-type plants (Figure 3C, Figure S3). 4DO levels in roots were not different between WT  
128 plants grown in 1-plant culture and WT plants co-cultured with *d17* mutants (Figure 3C). In  
129 accordance with these results, expression of strigolactone biosynthesis gene *D27* was higher in WT  
130 plants co-cultured with *d17* mutants than in WT plants co-cultured with other WT plants. By contrast,  
131 expression of *D27* gene was downregulated in WT plants co-cultured with *d14* mutants (Figure S4C).  
132 In general, rice *d14* mutants produce and exude more SLs than WT plants as they lack feedback

133 inhibition<sup>11-13</sup> (Figure S4A, B), and therefore it seems that strigolactones exuded by *d14* mutants  
134 negatively affect strigolactone biosynthesis in WT plants. Collectively these results show that rice  
135 plants can detect and respond to environmental strigolactone by changes in strigolactone  
136 biosynthesis and exudation, and that *d17* mutants are unable to trigger these changes in other plants  
137 because they cannot exude strigolactones. Furthermore, the expression of some genes especially  
138 *D3* and *D14* upregulated in *d17* mutants growing together with WT plants, though there were no  
139 statistically significant differences (Figure S4D), suggested that the shoot branching of SL  
140 biosynthesis mutants would be suppressed by co-culture with neighbouring plants exude  
141 strigolactones.

142

### 143 **Crowding in soil also reduces strigolactone exudation and affects the shoot** 144 **branching in WT plants**

145 Finally, we wanted to assess whether the detection of environmental strigolactone influenced the  
146 growth and development of plants, and also their relevance for soil-grown plants. The results were  
147 consistent with those observed in the hydroponic system, with a slight but not statistically significant  
148 reduction in per-plant exudation in the 3-plant culture of both 4DO and OB in WT plants (Shiokari  
149 background, which in general exude more strigolactones than the Nipponbare background WT  
150 plants), but not in *d14* and *d53* mutants (Figure 4A). Furthermore, we observed that shoot branching  
151 levels in 3-plant cultures tended to reduce compared to 1-plant cultures in WT plants, but not in  
152 strigolactone biosynthesis or perception mutants (Figure 4B). These differences in wild-type were  
153 not statistically significant due to low sample size, but in a companion manuscript, we show  
154 statistically significant differences in pea plant growth using a similar experimental design.<sup>14</sup> To  
155 confirm that these changes in shoot branching are mediated by the perception of strigolactone  
156 exuded by neighbouring plants, we grew combinations of *d10* (SL deficient) and *d14* (SL insensitive)  
157 plants together with *d17* or WT plants in soil and determined the number of shoot branches (Figure  
158 4C). We found that shoot branching of *d10* mutants was suppressed when they were grown together  
159 with WT plants, but not *d17* plants; conversely, *d14* showed no suppression when grown either WT  
160 or *d17* plants. Our data thus show that detection of environmental strigolactone can modulate plant  
161 development.

162 It is intriguing to notice that the rice plants in 3-plant soil culture exhibit less shoot branching as  
163 compared to that in 1-plant soil culture (Figure 4B) despite producing less SLs, 4DO and OB (Figure  
164 4A). These results are consistent with recent findings that canonical and non-canonical  
165 strigolactones are not interchangeable and perform different roles. For example, Ito et al.  
166 demonstrated that shoot branching phenotype of *Os900* knockout rice mutant which produces  
167 neither 4DO nor OB is the same to WT plants, suggesting 4DO and OB are not endogenous shoot  
168 branching inhibitors.<sup>15</sup> Mashiguchi et al. also show that these canonical strigolactones are not shoot  
169 branching inhibitors in rice plants.<sup>16</sup> Other reports suggest that non-canonical strigolactones are true  
170 hormones though their identities have not yet been clarified.<sup>17,18</sup>

171

## 172 DISCUSSION

173 Plant-plant interactions are non-transient in nature; plants cannot choose or change their neighbours,  
174 and as such may end up in extraordinarily long associations with members of other species.<sup>19</sup>  
175 Territorial conflicts between plants are not easily 'winnable', and although neighbouring plants may  
176 directly compete with each other for resources, these interactions may often be mutually detrimental  
177 to both parties.<sup>20</sup> The mechanisms by which plants detect each other typically rely on the presence  
178 of 'cues'; information inadvertently revealed by neighbouring plants. The most obvious example of  
179 this is the change in the abundance of red:far red wavelengths in light reflected by plants, which  
180 permits their detection by neighbouring plants.<sup>21</sup> The reflected light can trigger a range of growth  
181 responses in neighbouring plants, most notably the shade avoidance response.<sup>22</sup> Plants can also  
182 respond to the touch of neighbouring plants,<sup>23</sup> and to a range of chemicals present in the atmosphere  
183 (volatiles) and rhizosphere (exudates), which their neighbours release for other, functional  
184 reasons.<sup>24,25</sup>

185

186 Where tested, all groups of land plants (though not necessarily every species) exude strigolactones  
187 into the substrate.<sup>26</sup> Since exuded strigolactones promote the formation of associations with  
188 arbuscular mycorrhizal fungi (AMF), it is likely that this is their ancestral function.<sup>27</sup> Mosses do not  
189 form associations with AMF, but still exude strigolactones.<sup>28</sup> This allows neighbouring colonies to  
190 detect each other, and to avoid competition for space.<sup>28</sup> Thus, in mosses, strigolactones may act as  
191 active signals to neighbouring plants, in the absence of any other known functional role. In flowering  
192 plants, most species exude strigolactones to promote associations with AMF, and thus  
193 strigolactones could also act cues for the presence of neighbouring plants. In this study, we aimed  
194 to test the hypothesis that flowering plants use 'environmental' strigolactones as a cue for the  
195 presence of neighbouring plants. Collectively, our data suggest that plants can take up  
196 environmental strigolactones produced by other plants, and use this to regulate their own physiology  
197 and development as a homeostatic response to the environment. Strigolactone biosynthesis in the  
198 root system is under strong homeostatic control by strigolactone signalling,<sup>3,4,11-13</sup> and therefore  
199 offers a simple read-out of a plant's response to strigolactones. Our data thus show that  
200 strigolactones are a key molecule in neighbour detection and plant-to-plant communication in  
201 flowering plants, echoing previous work in the moss *Physcomitrella patens*.<sup>28</sup> This suggests that in  
202 flowering plants, strigolactones act as a 'cue' for neighbour detection; a functional molecule that  
203 inadvertently reveals the presence of a plant to its neighbours. In this context, the homeostatic  
204 downregulation of strigolactone biosynthesis in response to environmental strigolactones makes  
205 much sense. If the concentration of rhizospheric strigolactone is already high, this provides that  
206 plants with information that neighbouring plants are present, and increasing strigolactone exudation  
207 is unlikely to result in the recruitment more mycorrhizal partners, nor the capture of a higher

208 proportion of soil resources. Thus, individual plants can reduce their ‘expenditure’ in line with their  
209 likely reduced ‘profit’ from strigolactone exudation.

210

211 Our data also suggest a further possibility – that in addition their role in mycorrhizal signaling,  
212 strigolactones are also exuded for adaptive purposes relating to the sensing of soil volume. Plants  
213 show strong, pro-active responses to limitations in soil, and recent work suggests that plants  
214 measure the concentration of at least two different chemicals in the soil as a proxy for both available  
215 volume and neighbour density.<sup>29</sup> Our data show that plants can detect both their root volume and  
216 neighbour density, and precisely adjust their strigolactone biosynthesis exudation in response. Given  
217 our other data, the most obvious explanation is that they detect root density through strigolactone  
218 concentration: in a smaller volume strigolactone concentration rises more quickly, leading to earlier  
219 downregulation of exudation. Again, this simple mechanism would prevent ‘over-expenditure’ by the  
220 plant relative to the potential nutrient ‘profit’ that can be obtained from a smaller root volume. This  
221 suggests that strigolactone exudation may be doubly-functional, acting in both mycorrhizal  
222 recruitment and in root density sensing. In the context of root density sensing, it is likely that plants  
223 will derive benefit from detecting and responding to root density, *irrespective* of whose roots they  
224 are. In this sense, strigolactones are neither exactly a cue nor a signal, but rather a ‘universal density  
225 signal’. In essence, all plants will benefit from both exuding strigolactones and to responding to  
226 environmental strigolactones – whether it is a plant detecting its own roots, or its neighbours’ roots,  
227 or advertising its roots to its neighbours – because environmental strigolactones levels provide  
228 important information about overall root density in the environment. Strigolactones are chemically  
229 unstable and rapidly hydrolyzed especially in alkaline solutions. However, strigolactones have longer  
230 half-lives in the slightly acidic rhizosphere than in bulk soils, certainly long enough to exhibit their  
231 biological activities in the context of plant-plant interactions,<sup>18</sup> and as is indeed already demonstrated  
232 by the role of strigolactones in communication between plants and root parasitic weeds and AM fungi.  
233 Our work thus suggests that strigolactone exudation is an important tool for plants to adapt to the  
234 limitations of their soil environment, and to avoid unwinnable competitions in a crowded world.

235

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242

## 243 AUTHOR CONTRIBUTIONS

244 KaY, XX, and TN designed the study. KaY planned and carried out experiments and analyzed  
245 data. KaY, KoY and TB wrote the manuscript.

246

## 247 DECLARATION OF INTERESTS

248 The authors declare they have no competing interests.

249

250

## 251 MAIN FIGURE TITLES AND LEGENDS

252

### 253 **Figure 1. Crowding alters strigolactone biosynthesis and exudation**

254 (A) Quantification of 4-deoxyorobanchol (4DO) and orobanchol (OB) present in the hydroponate in  
255 1-, 2- or 3-plant cultures 72 h after transfer from 4-day P-depletion, expressed as pg/plant. Graph  
256 shows all individual data points.  $n = 7-9$  biologically independent samples. The different letters  
257 denote a statistically significant difference (ANOVA, Tukey-Kramer HSD test,  $p < 0.05$ ). See also  
258 Figure S1 and S2A.

259 (B) Quantification of 4DO present in the roots of plants in 1-, 2- or 3-plant cultures 72 h after transfer  
260 from 4-day P-starvation, expressed as pg/g fresh weight. Graph shows all individual data points.  $n$   
261 = 3-9 biologically independent samples. The same letter denotes no statistically significant  
262 difference (ANOVA, Tukey-Kramer HSD test,  $p < 0.05$ ). See also Figure S1 and S2B.

263 (C) Quantification of strigolactone-related gene expression in the roots of plants in 1-, 2- or 3-plant  
264 cultures 72 h after transfer from 4-day P-depletion. The upper and lower confines of the box indicate  
265 the interquartile range, the central line indicates the median, and the whiskers represent the  
266 maximum and minimum values.  $n = 3$  biologically independent samples. Expression levels for each  
267 gene normalized to 1 plant culture (=1). Asterisks indicate a statistically significant difference relative  
268 to 1-plant culture at the same time point (Student's  $t$ -test,  $p < 0.05$ ). See also Figure 2C.

269 (D) Diagram showing the biosynthesis and signaling pathways for strigolactone.

270

### 271 **Figure 2. Strigolactone perception is needed for altered exudation responses**

272 (A) Quantification of 4-deoxyorobanchol (4DO) and orobanchol (OB) present in the hydroponate in  
273 1-, 2- or 3-plant cultures of *d14* mutants 72 h after transfer from 4-day P-depletion, expressed as  
274 pg/plant. Graph shows all individual data points.  $n = 3$  biologically independent samples. The same  
275 letter denotes no statistically significant difference (ANOVA, Tukey-Kramer HSD test,  $p < 0.05$ ).

276 (B) Quantification of 4-deoxyorobanchol (4DO) present in the roots of plants in 1-, 2- or 3-plant  
277 cultures of *d14* mutants 72 h after transfer from 4-day P-starvation, expressed as pg/g fresh weight.  
278 Graph shows all individual data points.  $n = 3$  biologically independent samples. The same letter  
279 denotes no statistically significant difference (ANOVA, Tukey-Kramer HSD test,  $p < 0.05$ ).

280 (C) Quantification of strigolactone-related gene expression in the roots of plants in 1-, 2- or 3-plant  
281 cultures of *d14* mutants 72 h after transfer from 4-day P-starvation. The upper and lower confines of  
282 the box indicate the interquartile range, the central line indicates the median, and the whiskers  
283 represent the maximum and minimum values.  $n = 3$  biologically independent samples. Expression  
284 levels for each gene normalized to 1 plant culture (=1). No asterisk indicates no statistically  
285 significant difference relative to 1-plant culture at the same time point (Student's  $t$ -test,  $p < 0.05$ ).

286

287 **Figure 3. Strigolactone exudation is homeostatically regulated by**  
288 **environmental strigolactones**

289 (A) Cartoon showing experimental set-up.

290 (B) Quantification of 4-deoxyorobanchol (4DO) and orobanchol (OB) present in the hydroponate in  
291 1-plant cultures of WT rice, or 2-plant cultures of WT + WT or WT + *d17* mutants 24 h after transfer  
292 from 7-day P-depletion, expressed as pg per WT plant. Graph shows all individual data points.  $n =$   
293 3 biologically independent samples. The different letters denote a statistically significant difference  
294 (ANOVA, Tukey-Kramer HSD test,  $p < 0.05$ ). See also Figure S3.

295 (C) Quantification of 4DO present in the roots of WT or *d17* mutants grown either alone in 1-plant  
296 cultures, or in 2-plant cultures of WT + *d17*, 24 h after transfer from 7-day P-depletion, expressed as  
297 pg/g fresh weight. Graph shows all individual data points.  $n = 3$  biologically independent samples.  
298 The different letters denote a statistically significant difference (ANOVA, Tukey-Kramer HSD test,  $p$   
299  $< 0.05$ ). See also Figure S4.

300  
301 **Figure 4. Crowding in soil also reduces strigolactone exudation and inhibits**  
302 **shoot branching**

303 (A) Quantification of 4-deoxyorobanchol (4DO) and orobanchol (OB) present in the soil of 1- or 3-  
304 plant cultures after 10 days of P-depletion, expressed as pg/plant (4DO) or MS-peak area/plant (OB).  
305 No asterisk indicates no statistically significant difference relative to 1-plant culture at the same time  
306 point (Student's *t*-test,  $p < 0.05$ ).

307 (B, C) Determination of shoot branching of plants grown in soil after transferred to each condition for  
308 10 days. The error bars indicate the SEM ( $n = 3-8$  biologically independent samples). No asterisk  
309 indicates no statistically significant difference, and an asterisk indicates a statistically significant  
310 difference (Student's *t*-test,  $p < 0.05$ ).

311

312

313 **STAR★METHODS**

314

315 **RESOURCE AVAILABILITY**

316

317 **Lead contact**

318

319 Further information and requests for resources and reagents should be directed to and will be  
320 fulfilled by the Lead Contact, Kaori Yoneyama (yoneyama.kaori.wx@ehime-u.ac.jp).

321

322 **Material availability**

323

324 This study did not generate new unique reagents.

325

326 **Data and code availability**

327

328 ● All data reported in this paper will be shared by the lead contact upon request.

329 ● This paper does not report original code.

330 ● Any additional information required to reanalyze the data reported in this paper is available from  
331 the lead contact upon request.

332

## 333 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

334

335 Rice seeds (*Oryza sativa* cv. Nipponbare) were obtained from a local supplier. Seeds of rice *dwarf*  
336 mutants (*d10-2*, *d14-2*, *d17-2*) in the cv Nipponbare background, *O. sativa* cv. Shiokari, Norin 8, and  
337 *d53* in the cv Norin 8 background were provided from Prof. Junko Kyojuka (Tohoku University), as  
338 detailed in the Key resource table. Plants were grown in controlled growth conditions, as described  
339 in Method details.

340

## 341 **METHOD DETAILS**

342

### 343 **Hydroponic experiment**

344 Rice seeds were surface-sterilized in 70% ethanol for 3 min and thoroughly rinsed with sterile distilled  
345 water. Then, the seeds were germinated in sterile distilled water for 2 days at 25–28°C in the dark.  
346 Germinated seeds were sown in a strainer (28 x 23 x 9 cm, W x L x H) lined with a sheet of gauze  
347 moistened by placing it on a slightly larger container (28.5 x 23.5 x 11 cm, W x L x H) containing tap  
348 water for 7 days, 1/2 Tadano-Tanaka (TT) media<sup>30</sup> (1.43 mM NH<sub>4</sub>NO<sub>3</sub>, 1 mM NaNO<sub>3</sub>, 0.16 mM  
349 NaH<sub>2</sub>PO<sub>4</sub>, 1 mM K<sub>2</sub>SO<sub>4</sub>, 1 mM CaCl<sub>2</sub>, 1mM MgSO<sub>4</sub>, 20 mM MnSO<sub>4</sub>, 50 mM H<sub>3</sub>BO<sub>3</sub>, 3 mM ZnSO<sub>4</sub>,  
350 0.2 mM CuSO<sub>4</sub>, 0.05 mM (NH<sub>4</sub>)<sub>6</sub>/Mo<sub>7</sub>O<sub>24</sub>, 40 mM FeSO<sub>4</sub>) for 7 days, and phosphate free 1/2 TT  
351 media for 10 days in a plant growth shelf (NK system, #LS-1520PFD-4-10L) under 16-h light (28°C,  
352 approximately 240 μmol m<sup>-2</sup> s<sup>-1</sup>)/8-h dark (25°C) regime. One μM 2-(*N*-morpholino)ethanesulfonate  
353 (MES) was used as a buffer in the hydroponic media and pH of media was set to 6.0. Media was  
354 disposed of and replaced every 2–3 days. For test experiments, the equal sized plants were selected  
355 and transferred into plastic cups (9.5 cm in diameter, 17 cm deep, ca. 200 ml in volume or 19 cm in  
356 diameter, 17 cm deep, ca. 400 ml in volume) containing 180 or 360 mL of distilled water to remove  
357 the effects of nutrients depending on plant number or volume of media. To float plants, disk shaped  
358 styrofoam (1.8 cm thick) having holes were put on the cup and plants were set in the hole with  
359 sponge belt (1cm cube).<sup>31</sup>

360

### 361 **Soil experiment**

362 To examine shoot branching phenotype and SL exudation from plants grown in soils, rice plants  
363 were first grown hydroponically as in the hydroponic experiment. After growing in 1/2 TT media for  
364 8 days, seedlings were transferred to each condition (1 seedling /pot or 3 seedlings/pot, co-culture  
365 with WT or *d* mutant) in pots (i.d. 10 cm, 10 cm deep) filled with vermiculite (300 g) and watered  
366 twice a week with 150 mL/pot of a mixture of 1/2 TT media lacking phosphate and distilled water for  
367 10 days. To provide each rice seedling with similar amounts of available nutrients per plant, mixtures

368 of 1/2 TT and distilled water were applied at 50 mL and 100 mL, 100 mL and 50 mL, and 150 mL  
369 and 0 mL to pots containing 1, 2, and 3 seedlings, respectively. Plants were grown under the same  
370 environmental conditions of hydroponics. The number of shoot branching was counted as one which  
371 the tiller was over 1 cm.

372

### 373 **Strigolactone extraction**

374 The root exudates released into hydroponic media (ca. 100 mL) were collected, 500 pg of  $d_6$ -4DO  
375 dissolved in acetonitrile was added as an internal standard, and then immediately extracted with 100  
376 mL of ethyl acetate by using separatory funnel (200 mL). In the case of soil culture, 200 mL of tap  
377 water was poured onto the soil surface evenly and root exudates (ca. 200 mL) eluted from the holes  
378 of pot bottom were collected, which were then extracted with 200 mL of ethyl acetate. The upper  
379 phase (ethyl acetate solution) was dried over anhydrous  $MgSO_4$  and concentrated *in vacuo*. For  
380 extracting strigolactones from root tissues, the harvested fresh root tissues (ca. 1 g) were soaked in  
381 ethyl acetate in the dark at 4°C for 2 days. After removal of plant residues by filtration, ethyl acetate  
382 extract was washed with 0.2 M  $K_2HOPO_4$  by using separatory funnel, dried over anhydrous  $MgSO_4$ ,  
383 and concentrated *in vacuo*. These samples were transferred to vials and kept at 4°C until analysis.<sup>31</sup>

384

### 385 **Strigolactone analysis**

386 Rice (*O. sativa* cv Nipponbare) plants were shown to produce and exude mainly orobanchol and 4-  
387 deoxyorobanchol among known strigolactones,<sup>32</sup> thus we analyzed these two strigolactones by  
388 using ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-  
389 MS/MS). For this, the Acquity UPLC System (Waters) coupled to a Xevo TQD triple-quadrupole  
390 mass spectrometer (Waters MS Technologies) with electrospray (ESI) interface was used.  
391 Chromatographic separation was achieved using an ODS column (ACQUITY UPLC, BEH C<sub>18</sub>, 2.1  
392 × 100 mm, 1.7 μm; Waters) with a water-methanol gradient containing 4% 50 mM ammonium  
393 acetate to promote ionization. Separation started at 35% methanol, followed by a 2 min gradient to  
394 55% methanol, followed by a 13 min gradient to 95%, kept 96% methanol for 2 min to wash  
395 column and then back to 35% methanol for 3 min. The column was equilibrated at this solvent  
396 composition for 5 min before the next run. Total runs time was 25 min. The column oven  
397 temperature was maintained at 40°C with a flow-rate of 0.2 mL min<sup>-1</sup> (sample injection volume of 1  
398 μL).

399 For mass spectrometry, the nebulizer and desolvation gas flows were 50 L h<sup>-1</sup> and 1200 L h<sup>-1</sup>,  
400 respectively. The capillary voltage was set at 0.5 kV, the source temperature at 120°C and the  
401 desolvation has temperature at 550°C. Fragmentation was performed by collision induced  
402 dissociation with argon at 3~5 × 10<sup>-3</sup> mbar. The collision energy was optimized for each compound.  
403 Multiple reaction monitoring (MRM) was used to detect each strigolactone. MRM transitions for  
404 orobanchol eluting 4.8 min were monitored for  $m/z$  347/97 at a collision energy (CE) of 22 V and  
405  $m/z$  347/233 at CE of 10 V with a cone voltage of 30 V. The MRM transitions of  $m/z$  331/97 at a CE

406 of 20 V and  $m/z$  331/217 at 22 V with a cone voltage of 25 V were used for the detection of 4DO  
407 eluting at 8.0 min. The MRM transition of  $m/z$  353/97 at a CE of 20 V with a cone voltage of 25 V  
408 was used for  $d_6$ -4DO eluting at 7.9 min. Data acquisition and analysis were performed using  
409 MASSLYNX 4.1 software (Waters).

410

### 411 **Gene expression analyses**

412 Total RNA was extracted from the roots (>100 mg) of RNeasy Plant Mini Kit (QIAGEN) according to  
413 manufacturer's instructions and quantified with a spectrophotometer Nano Drop One C (Thermo  
414 Fisher Scientific, #ND-ONEC-W). One  $\mu$ g of total RNA was used to synthesize the cDNA with the  
415 PrimeScript RT Reagent Kit with gDNA eraser (Takara Bio, Japan). Real-time PCR was performed  
416 by  $\Delta\Delta$ CT method on a StepOnePlus real-time PCR system (Thermo Fisher Scientific,  
417 #StepOnePlus-01) with THUNDERBIRD SYBR qPCR kit (Toyobo, Japan). The PCR program was  
418 as following: an initial DNA denaturation at 95°C for 20 s; 40 cycles including a denaturation step at  
419 95°C for 3 s, an annealing step at 60°C for 30 s and an extension step at 95°C for 15 s; and a melting  
420 curve from 60°C to 95°C. *OsTFIIE* was selected as an internal reference gene in this study, based  
421 on its use in previous studies.<sup>32</sup> The specific primers used for qRT-PCR are listed in Supplementary  
422 Table 1.

423

## 424 **QUANTIFICATION AND STATICTICAL ANALYSIS**

425 Initial data was stored and organized in Microsoft Excel, and then subjected to ANOVA utilizing  
426 JMP software, version 5.0 (SAS Institute INC., Cary, NC, USA).

427

## 428 **SUPPLEMENTAL ITEM TITLES**

429

### 430 **Table S1. List of primer for real-time PCR, related to STAR Methods.**

431

432

## 433 **REFERENCES**

434

- 435 1. Murphy, G.P., and Dudley, S.A. (2007). Above- and below-ground competition cues elicit  
436 independent responses. *J. Ecol.* *95*, 261–272.
- 437 2. Gomez-Roldan, V., Fermas, S., Brewer, P.B., Puech-Pagès, V., Dun, E.A., Pillot, J.-P.,  
438 Letisse, F., Matusova, R., Danoun, S., Portais, J.-C., et al. (2008). Strigolactone inhibition  
439 of shoot branching. *Nature* *455*, 189–194.
- 440 3. Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N.,  
441 Magome, H., Kamiya, Y., Shirasu, K., Yoneyama, K., et al. (2008). Inhibition of shoot  
442 branching by new terpenoid plant hormones. *Nature* *455*, 195–200.
- 443 4. Akiyama, K., Matsuzaki, K., and Hayashi, H. (2005). Plant sesquiterpenes induce hyphal  
444 branching in arbuscular mycorrhizal fungi. *Nature* *435*, 824–827.
- 445 5. Machin, D.C., Hamon-Josse, M., and Bennett, T. (2020). Fellowship of the rings: a saga of  
446 strigolactones and other small signals. *New Phytol.* *225*, 621–636.
- 447 6. Bialeski, R.L. (1973). Phosphate pools, phosphate transport, and phosphate availability.  
448 *Annu. Rev. Plant Physiol.* *24*, 225–252.

- 449 7. Raghothama, K.G. (1999). Phosphate acquisition. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* *50*, 665–693.
- 450
- 451 8. Shindo, M., Shimomura, K., Yamaguchi, S., and Umehara, M. (2018). Upregulation of  
452 *DWARF27* is associated with increased strigolactone levels under sulfur deficiency in rice.  
453 *Plant Direct* *2*, e00050.
- 454 9. Yoneyama, K., Akiyama, K., Brewer, P.B., Mori, N., Kawada, M., Haruta, S., Nishiwaki, H.,  
455 Yamauchi, S., Xie, X., Umehara, M., et al. (2020). Hydroxyl carlactone derivatives are  
456 predominant strigolactones in *Arabidopsis*. *Plant Direct* *4*, e00219.
- 457 10. Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., and Zhang, F. (2011).  
458 Phosphorus dynamics: From soil to plant. *Plant Physiol.* *156*, 997–1005.
- 459 11. Mashiguchi, K., Sasaki, E., Shimada, Y., Nagae, M., Ueno, K., Nakano, T., Yoneyama, K.,  
460 Suzuki, Y., and Asami, T. (2009). Feedback-regulation of strigolactone biosynthetic genes  
461 and strigolactone-regulated genes in *Arabidopsis*. *Bioscience, Biotechnology and*  
462 *Biochemistry* *73*, 2460–2465.
- 463 12. Dun, E.A., de Saint Germain, A., Rameau, C., and Beveridge, C.A. (2013). Dynamics of  
464 strigolactone function and shoot branching responses in *Pisum sativum*. *Mol. Plant* *6*, 128–  
465 140.
- 466 13. Hayward, A., Stirnberg, P., Beveridge, C., and Leyser, O. (2009). Interactions between  
467 auxin and strigolactone in shoot branching control. *Plant Physiol.* *151*, 400–412.
- 468 14. Wheeldon, C.D., Hamon-Josse, M., Lund, H., Yoneyama, K., and Bennet, T. (2022).  
469 Environmental strigolactone level drives early growth responses to neighbouring plants and  
470 soil volume. companion paper.
- 471 15. Ito, S., Braguy, J., Wang, J.Y., Yoda, A., Fiorilli, V., Takahashi, I., Jamil, M., Felemban, A.,  
472 Miyazaki, S., Mazzarella, T., et al. (2022). Canonical strigolactones are not the tillering-  
473 inhibitory hormone but rhizospheric signals in rice. *bioRxiv*. 10.1101/2022.04.05.487102.
- 474 16. Mashiguchi, K., Seto, Y., Onozuka, Y., Suzuki, S., Takemoto, K., Wang, Y., Dong, L.,  
475 Asami, K., Noda, R., Kisugi, T., et al. (2022). A carlactonoic acid methyltransferase that  
476 contributes to the inhibition of shoot branching in *Arabidopsis*. *Proc. Nat. Acad. Sci. U.S.A.*  
477 *119*, e2111565119.
- 478 17. Wakabayashi, T., Hamana, M., Mori, A., Akiyama, R., Ueno, K., Osakabe, K., Osakabe, Y.,  
479 Suzuki, H., Takikawa, H., Mizutani, M., and Sugimoto, Y. (2019). Direct conversion of  
480 carlactonoic acid to orobanchol by cytochrome P450 CYP722C in strigolactone  
481 biosynthesis. *Sci. Adv.* *5*, eaax9067.
- 482 18. Yoneyama, K., Xie, X., Yoneyama, K., Kisugi, T., Nomura, T., Nakatani, Y., Akiyama, K.,  
483 and McErlean, C.S.P. (2018). Which are the major players, canonical or non-canonical  
484 strigolactones? *J. Exp. Bot.* *69*, 2231–2239.
- 485 19. Bilas, R.D., Bretman, A., and Bennett, T. (2021). Friends, neighbours and enemies: an  
486 overview of the communal and social biology of plants. *Plant Cell Environ.* *44*, 997–1013.
- 487 20. Pierik, R., Mommer, L., Voesenek, L.A.C.J., and Robinson, D. (2013). Molecular  
488 mechanisms of plant competition: neighbour detection and response strategies. *Func. Ecol.*  
489 *27*, 841–853.
- 490 21. Roig-Villanova, I., and Martínez-García, J.F. (2016). Plant responses to vegetation  
491 proximity: A whole life avoiding shade. *Front. Plant Sci.* *7*, 236.
- 492 22. Ballare, C.L., and Pierik, R. (2017). The shade-avoidance syndrome: multiple signals and  
493 ecological consequences. *Plant Cell Environ.* *40*, 2530–2543.
- 494 23. de Wit, M., Kegge, W., Evers, J.B., Vergeer-van Eijk, M.H., Gankema, P., Voesenek, L.A.,  
495 and Pierik, R. (2012). Plant neighbor detection through touching leaf tips precedes  
496 phytochrome signals. *Proc. Nat. Acad. Sci. U.S.A.* *109*, 14705–14710.
- 497 24. Ninkovic, V., Rensing, M., Dahlin, I., and Markovic, D. (2019). Who is my neighbor? Volatile  
498 cues in plant interactions. *Plant Signal. Behav.* *14*, 1634993.
- 499 25. Kong, C.-H., Zhang, S.-Z., Li, Y.-H., Xia, Z.-C., Yang, X.-F., Meiners, S.J., and Wang, P.  
500 (2018). Plant neighbor detection and allelochemical response are driven by root-secreted  
501 signaling chemicals. *Nat. Commun.* *9*, 3867.
- 502 26. Delaux, P.-M., Xie, X., Timme, R.E., Puech-Pages, V., Dunand, C., Lecompte, E.,  
503 Delwiche, C.F., Yoneyama, K., Becard, G., and Sejalón-Delmas, N. (2012). Origin of  
504 strigolactones in the green lineage. *New Phytol.* *195*, 857–871.

- 505 27. Walker, C.H., Siu-Ting, K., Taylor, A., O'Connell, M.J., and Bennett, T. (2019).  
506 Strigolactone synthesis is ancestral in land plants, but canonical strigolactone signalling is a  
507 flowering plant innovation. *BMC Biol.* *17*, 70.
- 508 28. Proust, H., Hoffmann, B., Xie, X., Yoneyama, K., Schaefer, D.G., Yoneyama, K., Nogué, F.,  
509 and Rameau, C. (2011). Strigolactones regulate protonema branching and act as a quorum  
510 sensing-like signal in the moss *Physcomitrella patens*. *Development* *138*, 1531–1539.
- 511 29. Wheeldon, C.D., Walker, C.H., Hamon-Josse, M., and Bennett, T. (2020). Wheat plants  
512 sense substrate volume and root density to proactively modulate shoot growth. *Plant Cell*  
513 *Environ.*
- 514 30. Tadano, T., and Tanaka, A. (1980). The effect of low phosphate concentrations in culture  
515 medium on early growth of several crop plant (in Japanese, translated by the authors). *Jpn.*  
516 *J. Soil Sci. Plant Nutr.* *51*, 399–404.
- 517 31. Xie, X., Yoneyama, K., Nomura, T., and Yoneyama, K. (2021). Evaluation and  
518 quantification of natural strigolactones from root exudates. In *Strigolactones*, C. Prandi, and  
519 F. Cardinale, eds. (Humana), pp. 3–12.
- 520 32. Yoneyama, K., Xie, X., Nomura, T., and Yoneyama, K. (2020). Do phosphate and cytokinin  
521 interact to regulate strigolactone biosynthesis or act independently? *Front. Plant Sci.* *11*,  
522 438.
- 523 33. Ueno, K., Hanada, A., Yamaguchi, S., and Asami, T. (2010). Preparation of multideuterated 5-  
524 deoxystrigol for use as an internal standard for quantitative LC/MS. *J. Label. Compd. Radiopharm.*  
525 *53*, 763-766.
- 526 34. Arite, T., Iwata, H., Ohshima, K., Maekawa, M., Nakajima, M., Kojima, M., Sakakibara, H., and  
527 Kyoizuka, J. (2007). *DWARF10*, an *RMS1/MAX4/DAD1* ortholog, controls lateral bud outgrowth in  
528 rice. *Plant J.* *51*, 1019–1029.
- 529 35. Kameoka, H., Dun, E.A., Lopez-Obando, M., Brewer, P.B., de Saint Germain, A., Rameau, C.,  
530 Beveridge, C.A., and Kyoizuka, J. (2016). Phloem transport of the receptor *DWARF14* protein is  
531 required for full function of strigolactones. *Plant Physiol.* *172*, 1844–1852.
- 532 36. Kobae, Y., Kameoka, H., Sugimura, Y., Saito, K., Ohtomo, R., Fujiwara, T., and Kyoizuka, J.  
533 (2018). Strigolactone biosynthesis genes of rice are required for the punctual entry of arbuscular  
534 mycorrhizal fungi into the roots. *Plant Cell Physiol.* *59*, 544–553.
- 535 37. Zhou, F., Lin, Q., Zhu, L., Ren, Y., Zhou, K., Shabek, N., Wu, F., Mao, H., Dong, W., Gan, L., et al.  
536 (2013). D14-SCF<sup>D3</sup>-dependent degradation of D53 regulates strigolactone signalling. *Nature* *504*,  
537 406–410.  
538

## Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Biological samples</b>		
<i>Oryza sativa</i> (japonica, c. v. Nipponbare)	Nouken, Japan	N/A
<b>Chemicals, peptides, and recombinant proteins</b>		
2-( <i>N</i> -morpholino)ethanesulfonate (MES)	TCI	4432-31-9
NH <sub>4</sub> NO <sub>3</sub>	FUJIFILM Wako	017-03235
NaNO <sub>3</sub>	FUJIFILM Wako	195-02545
NaH <sub>2</sub> PO <sub>4</sub> /2H <sub>2</sub> O	FUJIFILM Wako	192-02815
K <sub>2</sub> SO <sub>4</sub>	FUJIFILM Wako	169-04485
CaCl <sub>2</sub> /2H <sub>2</sub> O	FUJIFILM Wako	033-25035
MgSO <sub>4</sub> /7H <sub>2</sub> O	FUJIFILM Wako	131-00405
MnSO <sub>4</sub> /5H <sub>2</sub> O	FUJIFILM Wako	130-13182
H <sub>3</sub> BO <sub>3</sub>	FUJIFILM Wako	021-02195
ZnSO <sub>4</sub> /7H <sub>2</sub> O	FUJIFILM Wako	264-00402
CuSO <sub>4</sub> /5H <sub>2</sub> O	FUJIFILM Wako	039-04412
(NH <sub>4</sub> ) <sub>6</sub> /Mo <sub>7</sub> O <sub>24</sub> /4H <sub>2</sub> O	FUJIFILM Wako	019-03212
FeSO <sub>4</sub> /7H <sub>2</sub> O	FUJIFILM Wako	094-01082
Ethyl acetate	FUJIFILM Wako	056-00367
Anhydrous MgSO <sub>4</sub>	FUJIFILM Wako	132-00435
Methanol	FUJIFILM Wako	134-14523
Ammonium acetate	Millipore Sigma	73594-25G-F
<i>d</i> <sub>6</sub> -4-deoxyorobanchol	Provided from Prof. Tadao Asami (Tokyo Univ., Japan) <sup>33</sup>	N/A
<b>Critical commercial assays</b>		
RNAeasy Plant Mini Kit	QIAGEN	# 74904
PrimeScript RT Reagent Kit with gDNA eraser	Takara Bio	# RR047B
THUNDERBIRD SYBR qPCR kit	Toyobo	# QPS-201
<b>Experimental models: Organisms/strains</b>		
<i>Oryza sativa</i> : dwarf 10-2	<sup>34</sup>	N/A
<i>Oryza sativa</i> : dwarf 14-2	<sup>35</sup>	N/A
<i>Oryza sativa</i> : dwarf 17-2	<sup>36</sup>	N/A
<i>Oryza sativa</i> : Shiokari	Rice Genome Resource Center (RGRC), Tsukuba, Japan	N/A
<i>Oryza sativa</i> : Norin 8	Rice Genome Resource Center (RGRC), Tsukuba, Japan	N/A
<i>Oryza sativa</i> : dwarf 53	<sup>37</sup>	N/A
<b>Oligonucleotides</b>		
Real-time PCR primer sets	See Table S1	

Figure 1

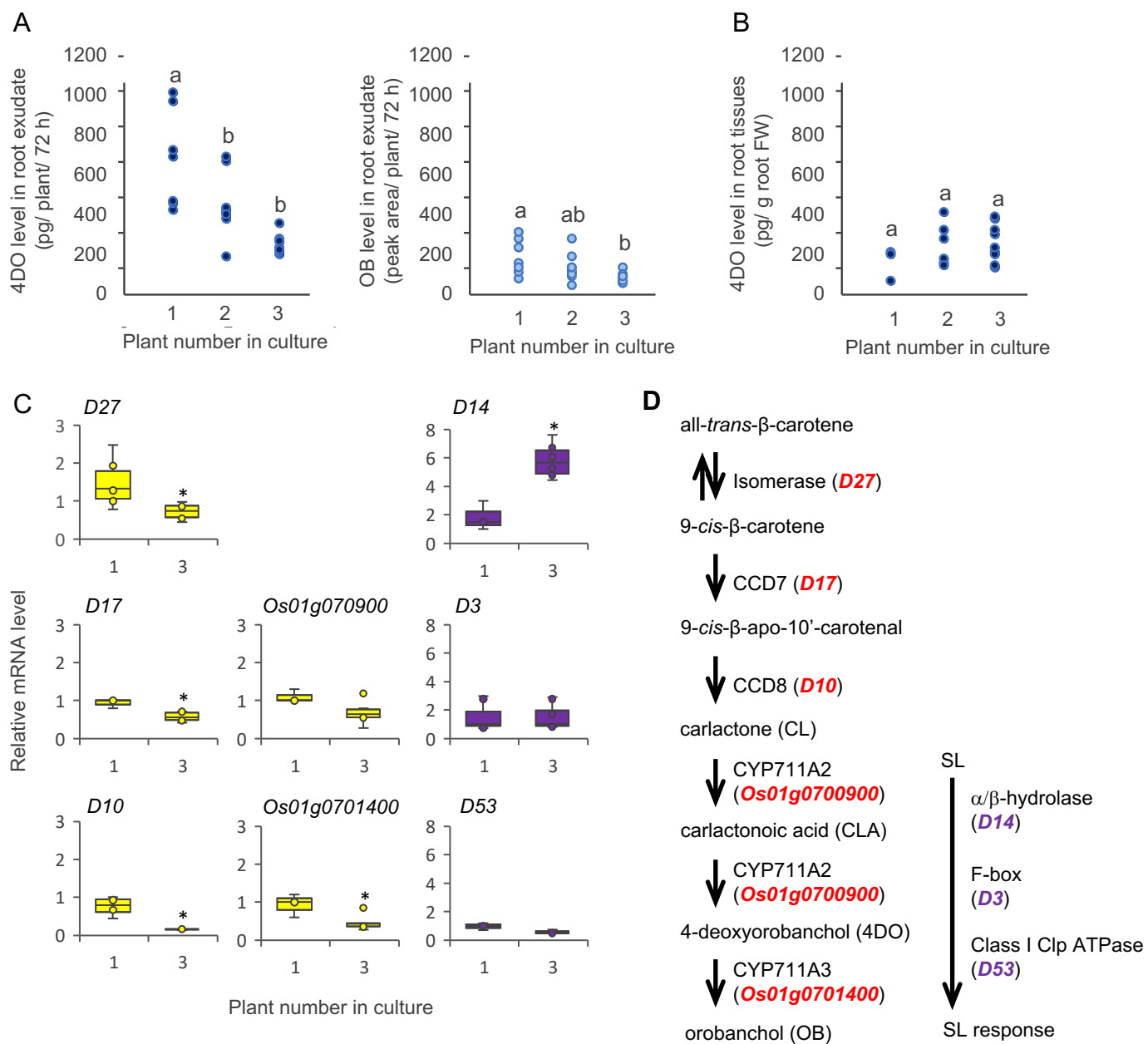


Figure 2

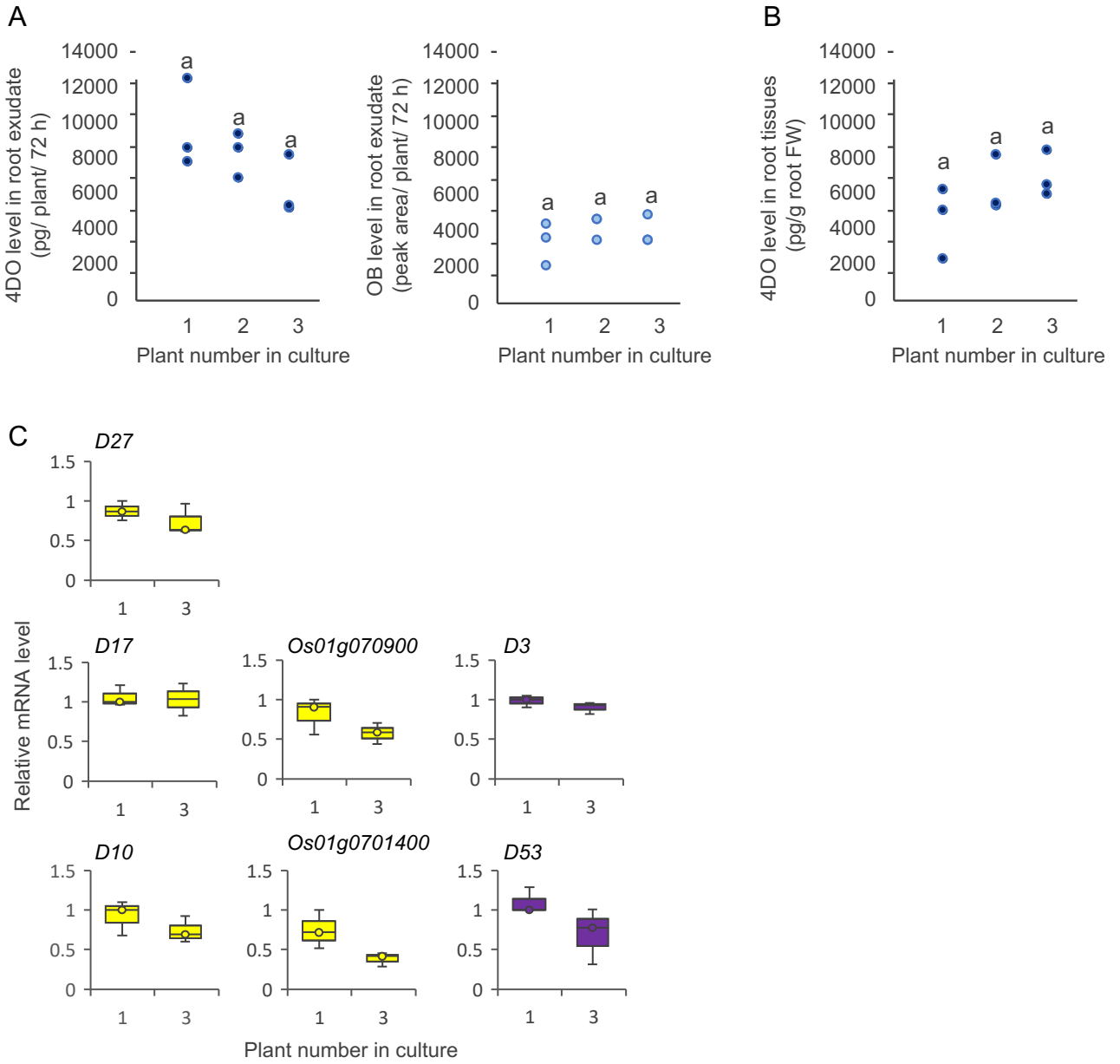
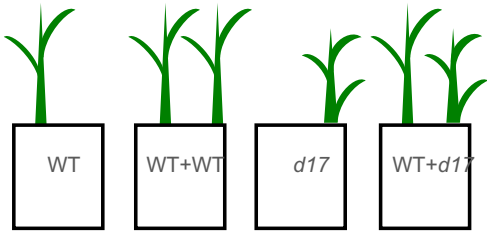
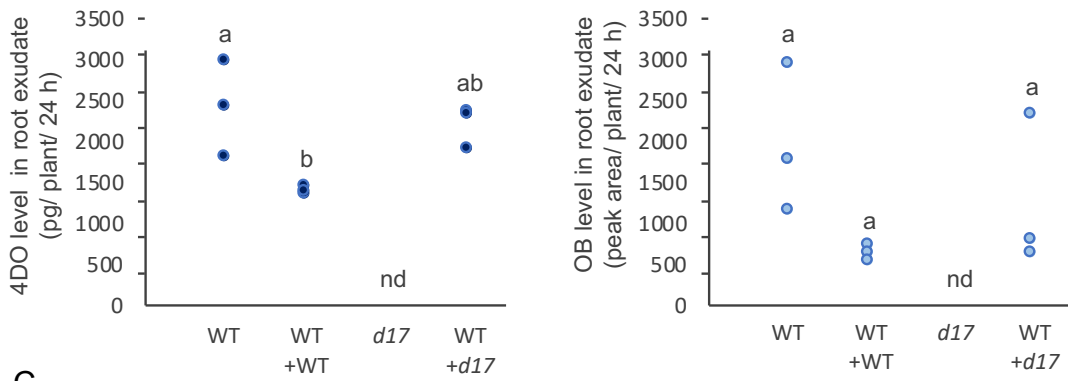


Figure 3

A



B



C

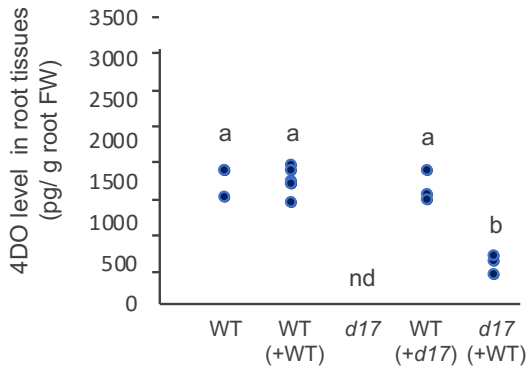


Figure 4

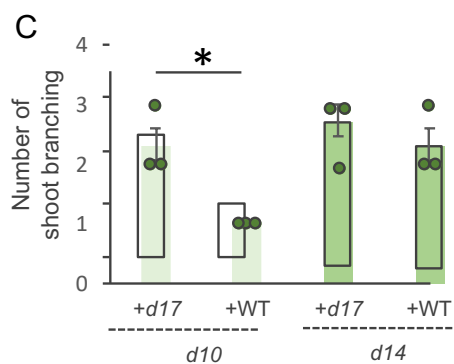
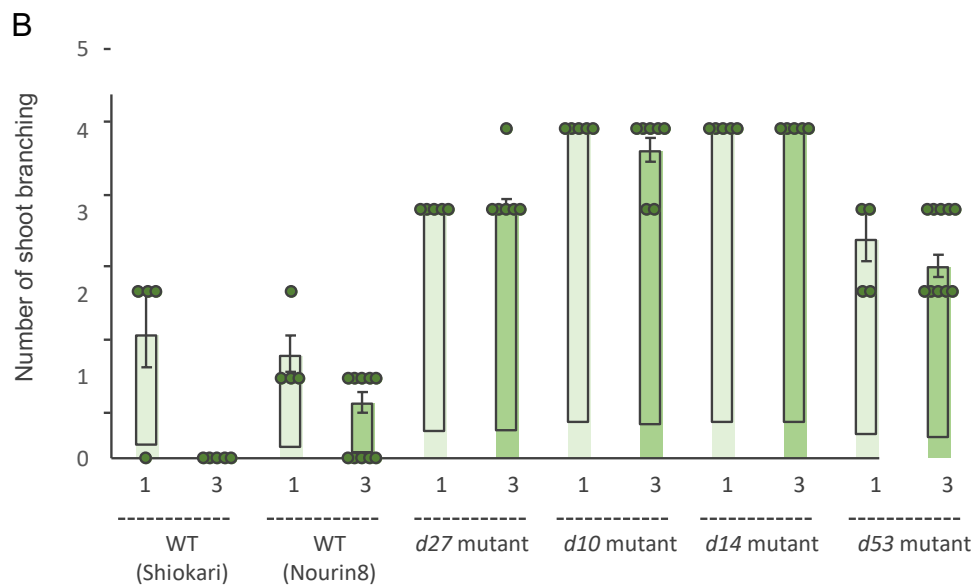
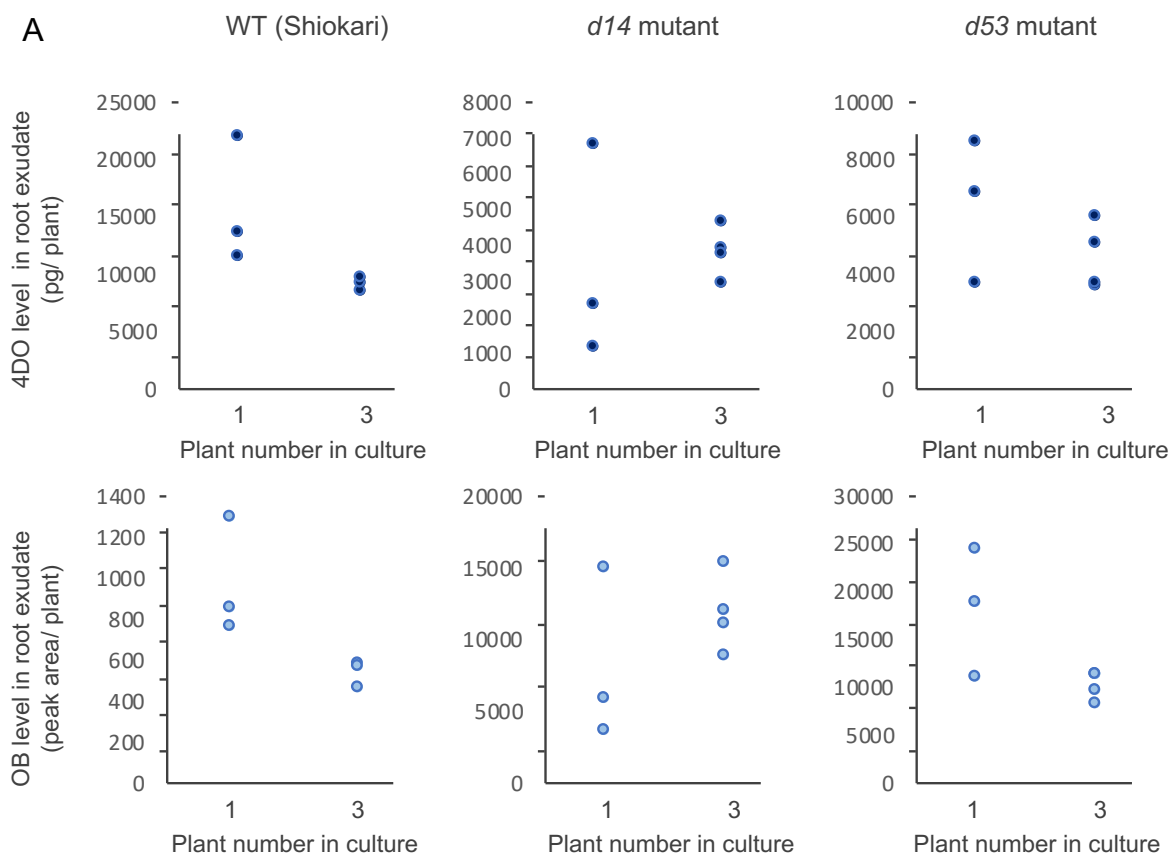


Figure 1

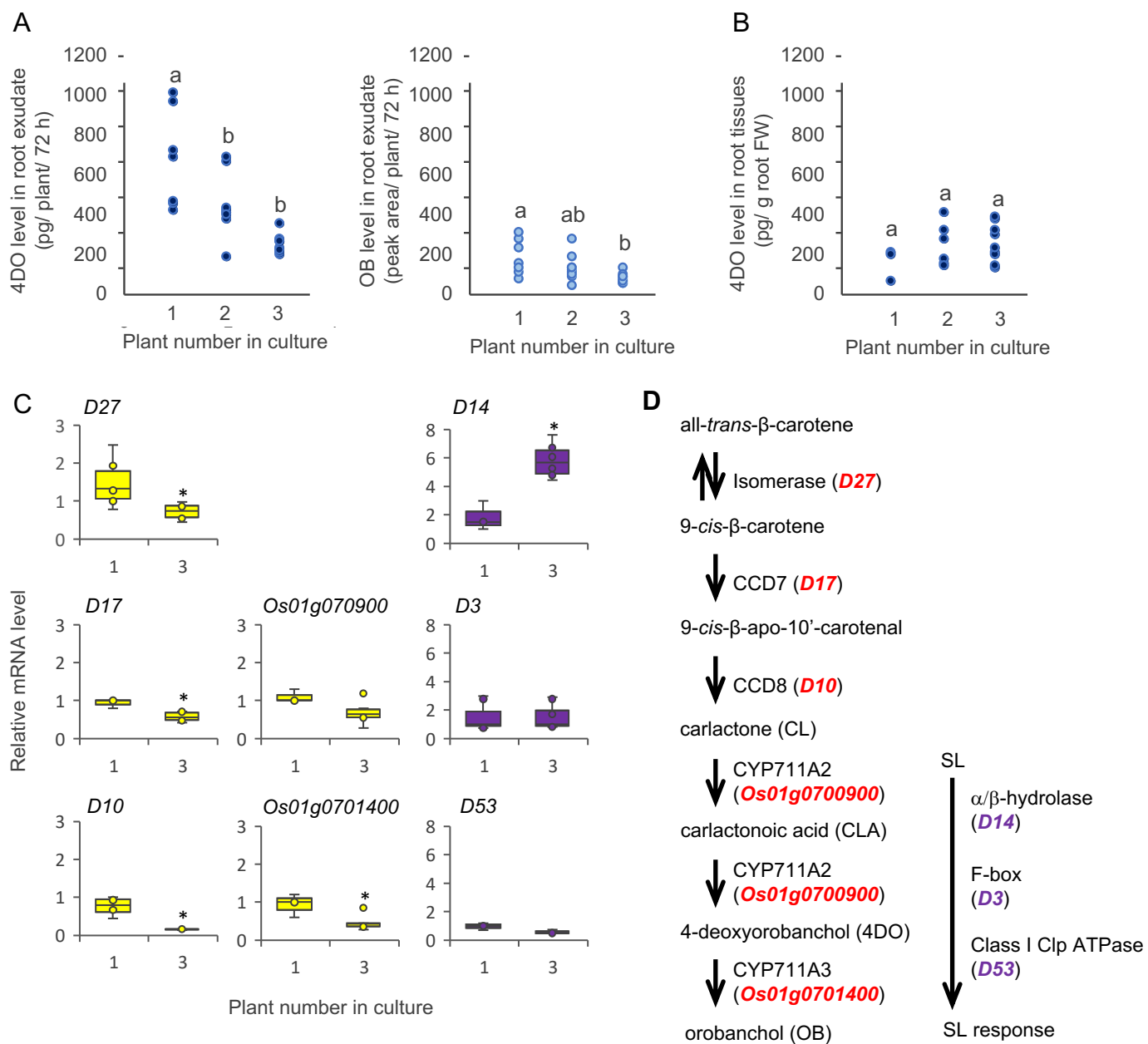


Figure 2

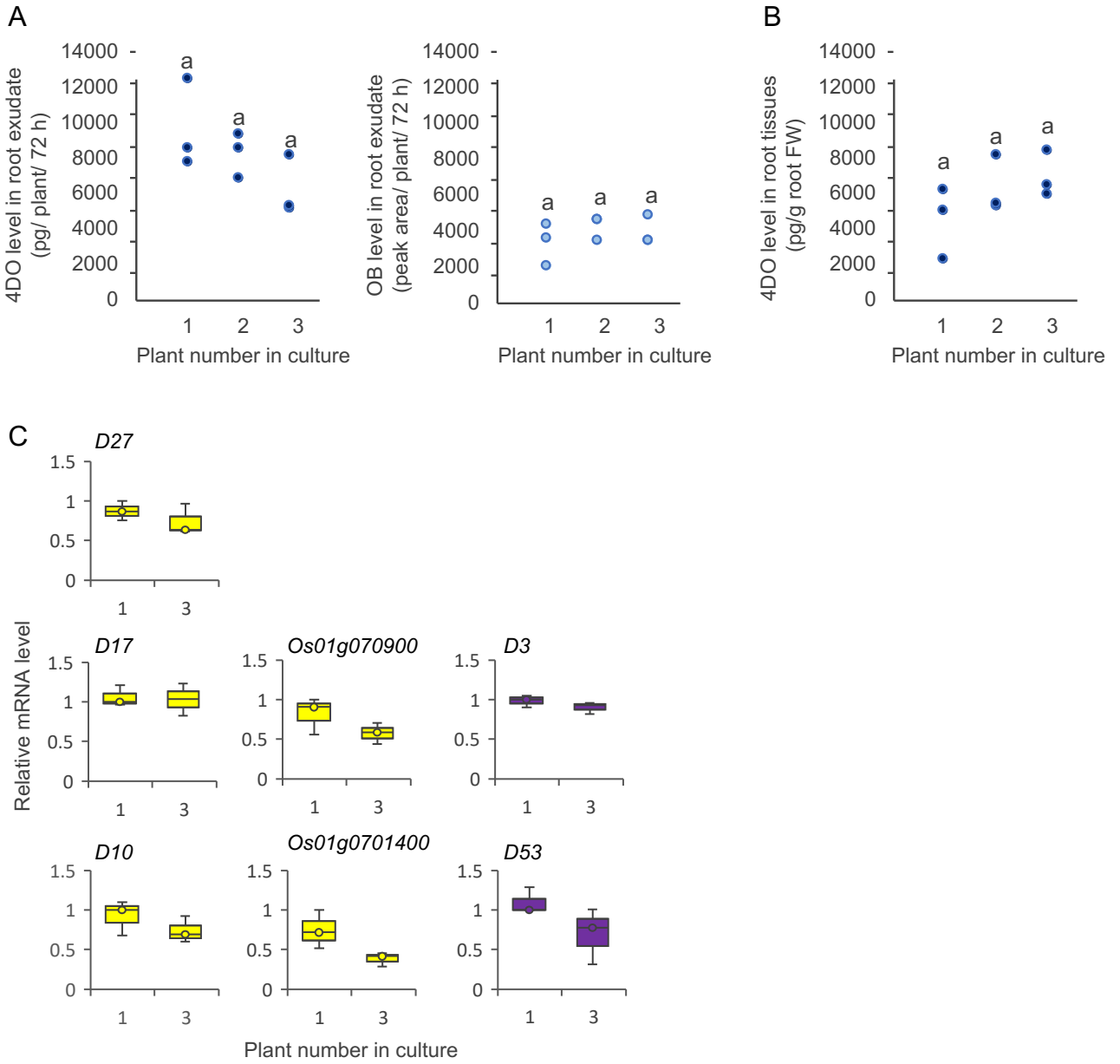
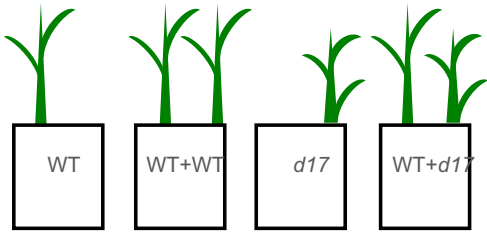
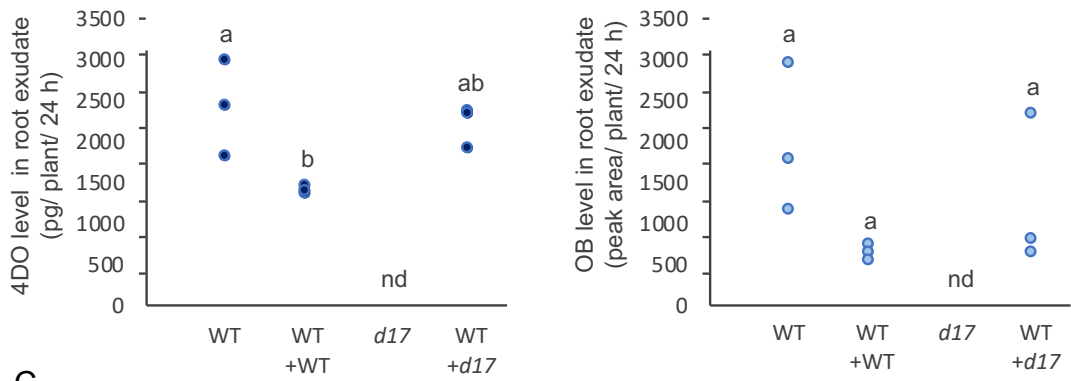


Figure 3

A



B



C

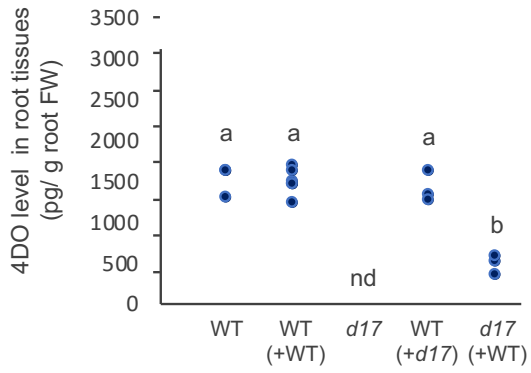
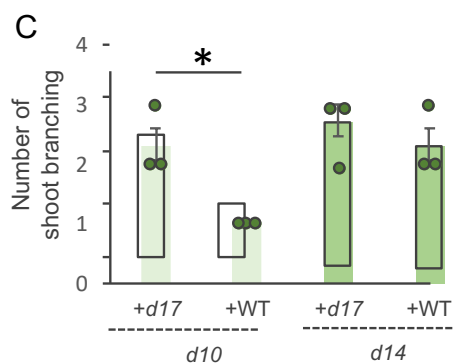
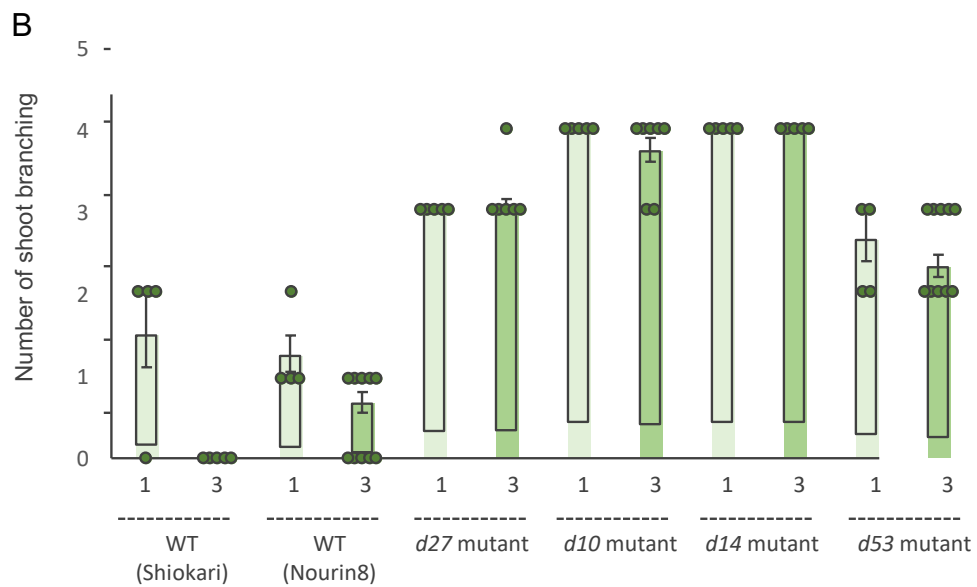
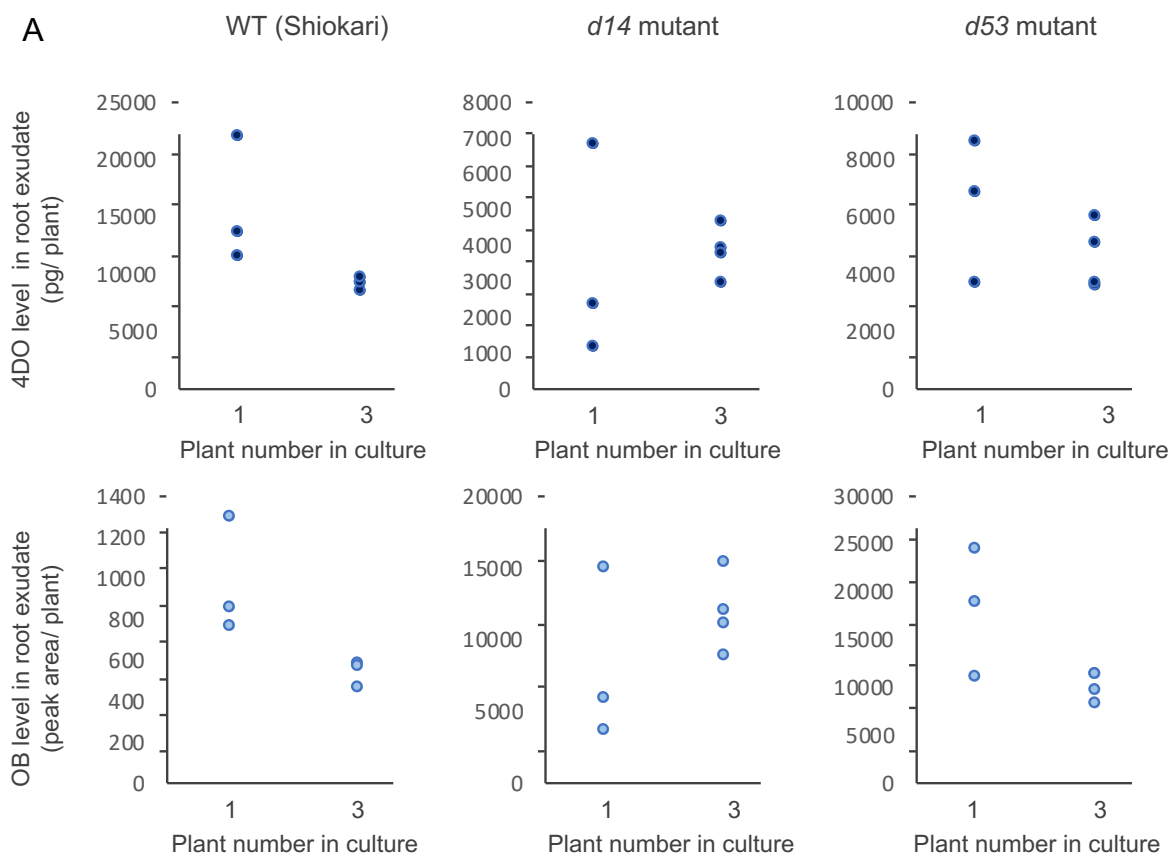
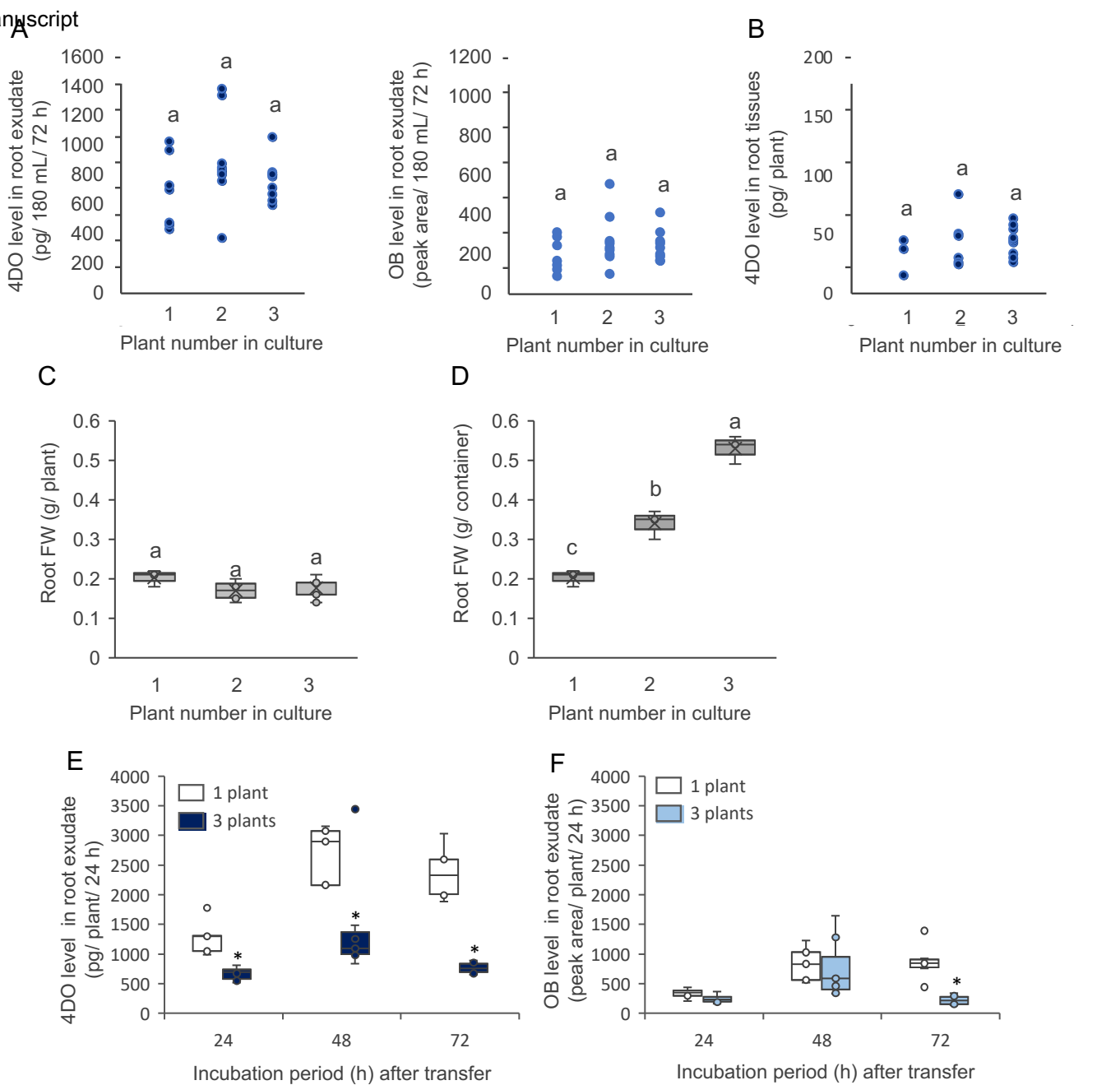


Figure 4





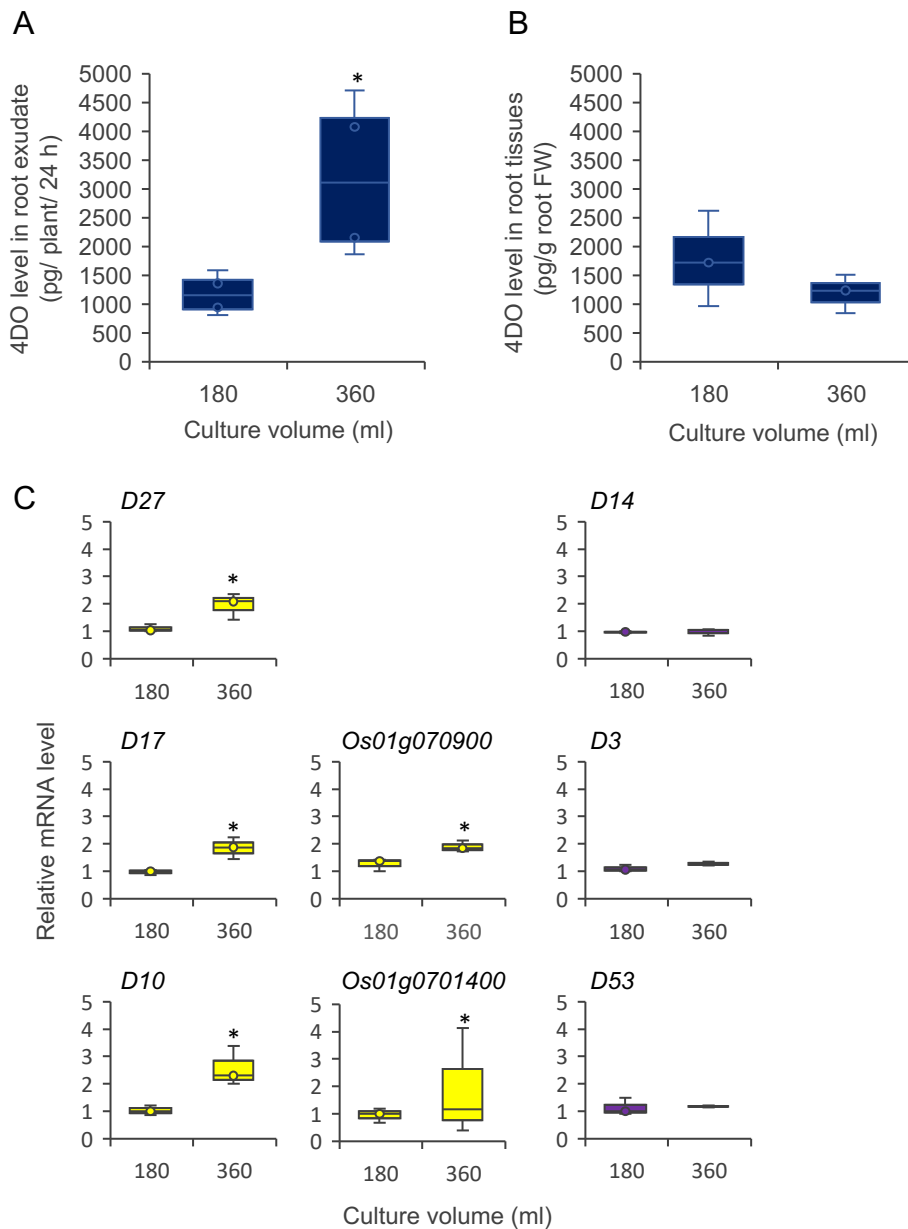
**Figure S1. The net strigolactone exudation in 1, 2- and 3-plant culture is constant and changes in strigolactone exudation in response to neighbouring plants occur rapidly, related to Figure 1.**

(A) Quantification of 4-deoxyorobanchol (4DO) and orobanchol (OB) present in the hydroponate in 1- or 3-plant cultures 72 h after transfer from 4-day P-depletion, expressed as pg/180 mL of media. Graph shows all individual data points.  $n = 7-9$  biologically independent samples. The same letter denotes no statistically significant difference (ANOVA, Tukey-Kramer HSD test,  $p < 0.05$ ).

(B) Quantification of 4DO present in the roots of plants in 1-, 2- or 3-plant cultures 72 h after transfer from 4-day P-depletion, expressed as pg/plant. Graph shows all individual data points.  $n = 3-9$  biologically independent samples. The same letter denotes no statistically significant difference (ANOVA, Tukey-Kramer HSD test,  $p < 0.05$ ).

(C, D) Measurements of root fresh weights expressed as g of plants or container. The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values.  $n = 3$  biologically independent samples. The same letter denotes no statistically significant difference and different letters denote a statistically significant difference (ANOVA, Tukey-Kramer HSD test,  $p < 0.05$ ).

(E, F) Quantification of 4DO and OB present in the hydroponate in 1- or 3-plant cultures 24, 48 and 72 h after transfer from 7-day P-starvation, expressed as pg/plant (4DO) or MS-peak area/plant (OB). The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values.  $n = 5-7$  biologically independent samples. Asterisks indicate a statistically significant difference relative to 1-plant culture at the same time point (Student's  $t$ -test,  $p < 0.05$ ).

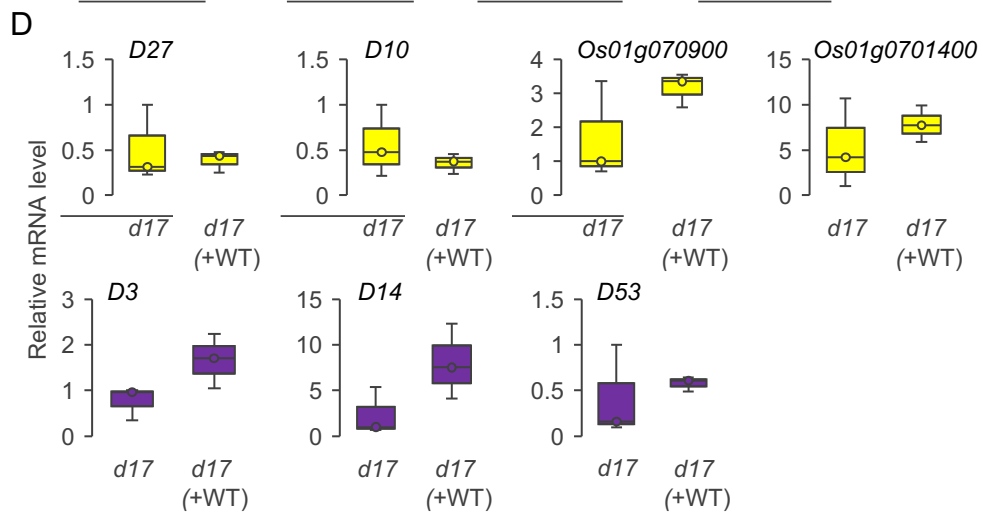
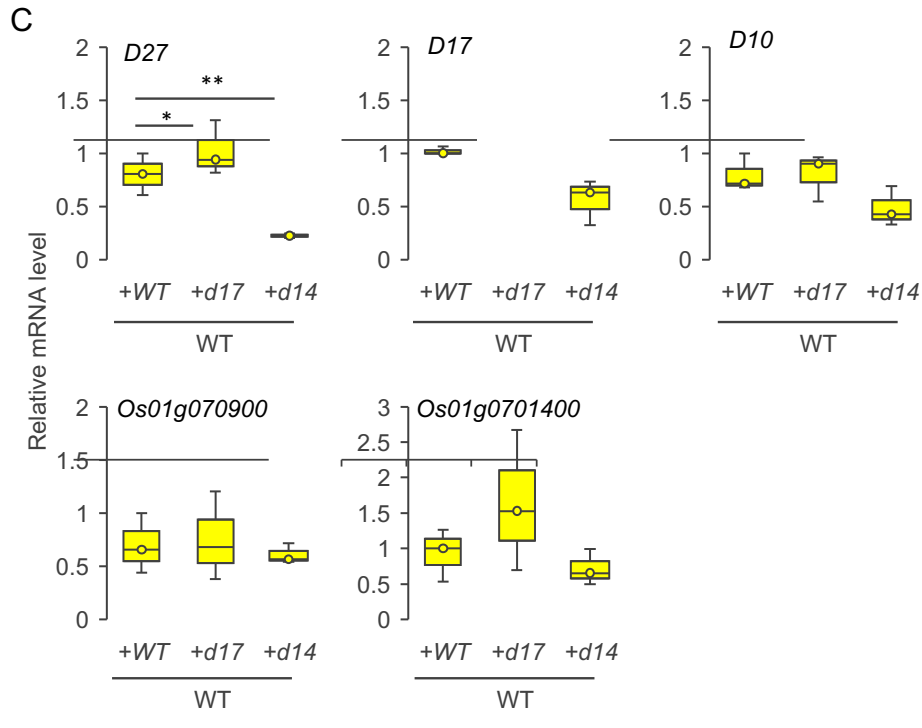
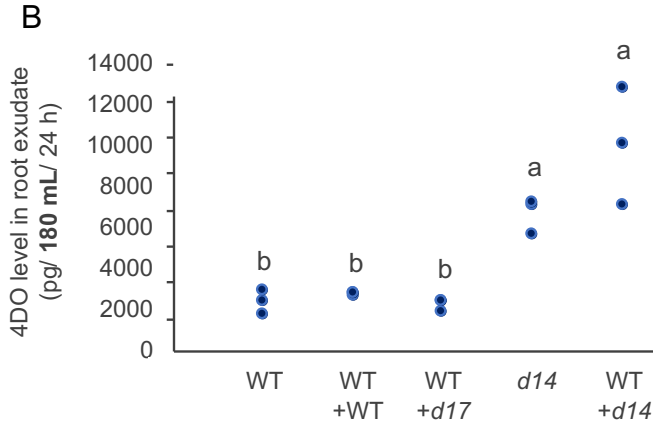
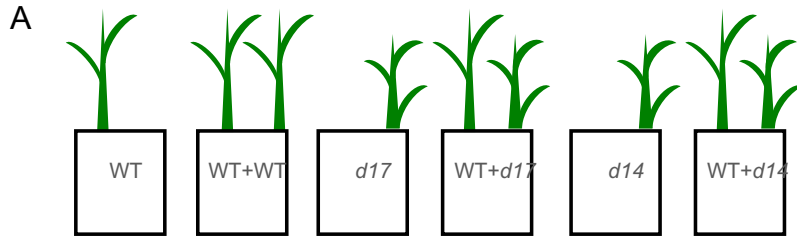


**Figure S2. Strigolactone exudation responds to root density, related to Figure 1.**

(A) Quantification of 4-deoxyorobanchol (4DO) present in the hydroponate in 1-plant cultures 24 h after transfer from 7-day P-depletion to 180 mL or 360 mL hydroponic vessels, expressed as pg/plant. The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values.  $n = 4$  biologically independent samples. Asterisks indicate a statistically significant difference relative to 180 mL culture at the same time point (Student's  $t$ -test,  $p < 0.05$ ).

(B) Quantification of 4DO present in the roots of plants 1-plant cultures 24 h after transfer from 7-day P-depletion to 180 mL or 360 mL hydroponic vessels, expressed as pg/g fresh weight. The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values.  $n = 3$  biologically independent samples. No asterisk indicates no statistically significant difference relative to 180 mL culture at the same time point (Student's  $t$ -test,  $p < 0.05$ ).

(C) Quantification of strigolactone-related gene expression in the roots of plants in 1-plant cultures 24 h after transfer from 7-day P-starvation to 180 mL or 360 mL hydroponic vessels. The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values.  $n = 3$  biologically independent samples. Asterisks indicate a statistically significant difference relative to 180 mL culture at the same time point (Student's  $t$ -test,  $p < 0.05$ ).

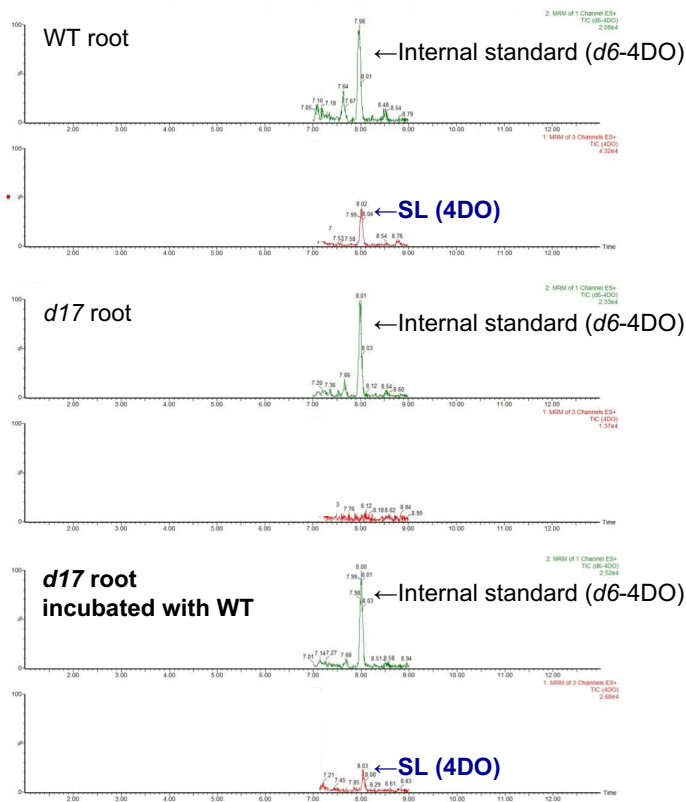


**Figure S3. Strigolactone exudation is homeostatically regulated by environmental strigolactones, related to Figure 3.**

(A) Cartoon showing experimental set-up.

(B) Quantification of 4-deoxyorobanchol (4DO) present in the hydroponate in 1-plant cultures of WT or *d14*, 2-plant cultures of WT+WT, WT+*d17* mutants, or WT+*d14* mutants 24 h after transfer from 7-day P-depletion, expressed as pg per 180 mL media. Graph shows all individual data points.  $n = 3$  biologically independent samples. The different letters denote statistically significant difference (ANOVA, Tukey-Kramer HSD test,  $p < 0.05$ ).

(C, D) Quantification of strigolactone-related gene expression in the roots of WT plants grown in 2-plant cultures with either WT, *d17*, or *d14* plants, and 1-plant cultures of *d17* plants or *d14* plants co-cultured with WT plants 24 h after transfer from 7-day P-depletion. The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values.  $n = 3$  biologically independent samples. Asterisks indicate a statistically significant difference (Student's *t*-test,  $p < 0.05$ ).



**Figure S4. Strigolactone was detected from *d17* roots co-cultured with WT, related to Figure 3.** Detection of  $d_6$ -4-deoxyorobanchol ( $d_6$ -4DO, internal standard) and 4-deoxyorobanchol (4DO) in root tissues of WT, *d17*, and *d17* grown with WT. Multiple reaction monitoring chromatograms of  $d_6$ -4DO (green) and 4DO (red) by LC-MS/MS are shown.

Primer name	5' to 3' sequence
D27-F	AGATGACCCTGCATTGAAGCA
D27-R	GCAATTCACACCATGTTCTGC
D10-F	CTGTACAAGTTCGAGTGGCACC
D10-R	CCTGTCCGTCTCCTCGTAC
D17-F	CCTCGTCCAGAAGCGTGAG
D17-R	TAGTGGGTGTCGGTGAAGGC
CYP711A2-F	CGTGAACCTCACGCTCGG
CYP711A2-R	TTCATTGCAGCCGTCGG
CYP711A3-F	TGCATTGAGTGCGTGTCCA
CYP711A3-R	GAAGCCGAGAGCGAGATCG
D3-F	CCCAACCTCCGCAAGCT
D3-R	GACGCAATCGCTGAACCG
D14-F	GCCTCTCCCCGTTCTTG
D14-R	TGCTGTATCTCCTCCAGCTCG
D53-F	CCAAGCAGTTTGAAGCGAC
D53-R	CCGCAAGTTTATCAAAGTCAA
OsTFIIE-F	GTGCAGCCCAAGGCTAAG
OsTFIIE-R	CGTCGAATAAGCGTAGAGCA

**Table S1. List of primer for real-time PCR, related to STAR Methods.**