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1 *Short Communication*

2 ***Brachypodium distachyon tar2l^{hypo}* mutant shows reduced root developmental**
3 **response to symbiotic signal but increased arbuscular mycorrhiza**

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9 **Key Words:** lateral root formation, lipo-chitooligosaccharide, auxin, arbuscular mycorrhizal

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15

16 **Abstract**

17 Auxin is a major phytohormone that controls root development. A role for auxin is also

18 emerging in the control of plant-microbe interactions, including for the establishment of root

19 endosymbiosis between plants and arbuscular mycorrhizal fungi (AMF). Auxin perception is

20 important both for root colonization by AMF and for arbuscule formation. AMF produce

21 symbiotic signals called lipo-chitooligosaccharides (LCOs) that can modify auxin homeostasis

22 and promote lateral root formation (LRF). Since *Brachypodium distachyon* (Brachypodium)

23 has a different auxin sensitivity compared to other plant species, we wondered whether this

24 would interfere with the effect of auxin in arbuscular mycorrhizal (AM) symbiosis. Here we

25 tested whether *tar2l^{hypo}* a Brachypodium mutant with an increase in endogenous auxin content

26 is affected in LRF stimulation by LCOs and in AM symbiosis. We found that, in contrast to
27 control plants, LCO treatment inhibited LRF of the *tar2l^{hypo}* mutant. However, the level of AMF
28 colonization and the abundance of arbuscules were increased in *tar2l^{hypo}* compared to control
29 plants, suggesting that auxin also plays a positive role on both AMF colonization and arbuscule
30 formation in *Brachypodium*.

31

32 **Results and discussion**

33 Auxin is a phytohormone with a major effect on plant root development (Overvoorde et al.,
34 2010). More recently, auxin has been shown to play an important role for the establishment of
35 root endosymbioses such as arbuscular mycorrhizal (AM) or nitrogen-fixing symbioses
36 (Etemadi et al., 2014; Champion et al., 2015; Kohlen et al., 2018). During AM symbiosis, auxin
37 signaling is activated in root cortical cells in which arbuscular mycorrhizal fungi (AMF) form
38 branched exchange structures called arbuscules. Moreover, exogenous auxin application or
39 down-regulation of auxin perception affects arbuscule formation (Etemadi et al., 2014). Since
40 lateral roots (LR) are also preferred sites for AMF colonization (Gutjahr and Paszkowski,
41 2013), auxin may also affect AM symbiosis through the control of lateral root formation (LRF).
42 The lipo-chitoooligosaccharides (LCOs) signal molecules produced by rhizobial bacteria
43 (Dénarié et al., 1996) and AMF (Maillet et al., 2011) induce LRF in various plant species,
44 including dicots and monocots (Olah et al., 2005; Maillet et al., 2011; Sun et al., 2015; Buendia
45 et al., 2019). We recently showed that this probably occurs through modification of auxin
46 homeostasis, leading to an increase of the auxin content in the part of the root containing the
47 LR initiation zone (Buendia et al., 2019). Whereas exogenous auxin treatment with auxin
48 analogs like Naphthalene-Acetic acid (NAA) increased LRF in most plant species including the
49 monocots rice (Zhao et al., 2015) and maize (Jansen et al., 2013), it failed to promote LRF in
50 *Brachypodium distachyon* (*Brachypodium*), and by contrast, tended to inhibit LRF even at low

51 concentrations. Only exogenous treatment with low concentrations (10^{-9} M) of the auxin
52 precursor Indole-3-Butyric Acid (IBA) promotes LRF in *Brachypodium* (Buendia et al., 2019).
53 Similarly, whereas LCOs act synergistically with exogenous auxin for stimulation of LRF in
54 the model legume *Medicago truncatula* (Herrbach et al., 2017), combination of 10^{-9} M IBA and
55 10^{-7} M LCO fails to stimulate LRF in *Brachypodium* (Buendia et al., 2019). This suggests that
56 *Brachypodium* is highly sensitive to exogenous auxin treatment for LRF, probably because
57 endogenous auxin content is close to the optimal concentration required for LRF. Indeed,
58 *Brachypodium* shows some specificities for auxin production and accumulation such as a
59 different ethylene-auxin crosstalk wiring controlling auxin biosynthesis gene expression and a
60 different *AUX1* mutant phenotype compared to *Arabidopsis* (Pacheco-Villalobos et al., 2013;
61 van der Schuren et al., 2018). These differences in auxin sensitivity and homeostasis compared
62 to other plant species raise the question of the role of auxin during AM symbiosis in
63 *Brachypodium*.

64

65 **Brachypodium roots with higher endogenous auxin content display less lateral roots in**
66 **response to exogenous auxin or LCO treatments**

67 In order to answer this question, we used *tar2l^{hypo}*, an hypomorphic mutant of *TAR2L*, a gene
68 involved in auxin biosynthesis in *Brachypodium* (Pacheco-Villalobos et al., 2013). This mutant
69 shows reduced transcript level of *TAR2L* but a higher endogenous IAA content in primary root
70 as well as a modified root architecture with higher number of LRs (Pacheco-Villalobos et al.,
71 2013). Similarly, in our conditions, *tar2l^{hypo}* had more LR than the control in non-treated
72 condition (Fig. 1A and B). We tested the effect of IBA or LCO on LRF in the wild type control
73 (Fig. 1A), plants of the Bd21 genotype regenerated from embryogenic calli. Similarly to what
74 was observed with the Bd21.3 genotype (Buendia et al., 2019), 10^{-9} M IBA and 10^{-7} M LCO
75 significantly increased LRF by 37 % and 32%, respectively (from a LR mean number of 13.1
76 in the control condition to 17.9 or 17.3 upon IBA and LCO treatments respectively). By

77 contrast, in the *tar2^{hypo}* mutant (Fig. 1B), both 10⁻⁹M IBA and 10⁻⁷M LCO treatments
78 significantly reduced LRF by 31% and 25% (from a LR mean number of 25 in the control
79 condition to 17.3 or 18.8 upon IBA and LCO treatments respectively). These results confirm
80 our initial hypothesis that Brachypodium is highly sensitive to exogenous auxin treatment likely
81 because the endogenous auxin concentration in wild type plants is close to the optimum
82 concentration required to stimulate LRF, and that any higher auxin content inhibits LRF
83 (Buendia et al., 2019). The negative effect of LCO on the number of LR in the *tar2^{hypo}* mutant
84 also fits with the mechanism we suggested whereby LCO application can enhance endogenous
85 auxin level in roots (Buendia et al., 2019), that might lead to a negative effect on LRF in
86 *tar2^{hypo}*.

87

88 **Brachypodium roots with higher endogenous auxin content show increased AMF** 89 **colonization and arbuscule formation**

90 In parallel, we inoculated the *tar2^{hypo}* mutant and the wild type control with 200 spores of the
91 AMF species *Rhizophagus irregularis* (DAOM197198, Agronutrition, Carbonne, France), and
92 analyzed fungal colonization by two different methods. We first performed RT-qPCR in order
93 to quantify at 3 week post inoculation (wpi) the amount of AMF in the root system and the
94 amount of arbuscules. We did so by measuring a fungal housekeeping gene (*RiGAPDH*) as in
95 (Buendia et al., 2016) and plant genes whose expressions are highly induced in AMF colonized
96 plants (*Bradi2g51930* and *Bradi2g45520*). *Bradi2g51930* codes for a LysM-domain containing
97 protein (ortholog of the rice *AM3* marker gene induced during AMF colonization (Gutjahr et
98 al., 2008)) and *Bradi2g45520* codes for a phosphate transporter specifically expressed in
99 arbuscule-containing cells (Hong et al., 2012). We observed a significantly higher expression
100 of *RiGAPDH* and *Bradi2g51930* in the mutant (Fig. 2A) suggesting that *tar2^{hypo}* is more
101 colonized than the control. In addition, we observed significantly higher expression of
102 *Bradi2g45520*, suggesting that there were more arbuscules formed in the mutant than in the

103 control. To better understand the effect of the *tar2l^{hypo}* mutation on AMF colonization, in an
104 independent experiment, we used the mycolcalc method (Trouvelot et al., 1986) to score by
105 microcopy root systems at 3wpi. We observed that *tar2l^{hypo}* had a significantly higher i)
106 frequency of AMF colonization, ii) AMF colonization intensity and iii) arbuscule abundance
107 (Fig. 2B and 2C) compared to the wild type control. Altogether, this shows that the *tar2l^{hypo}*
108 mutation leads to both an increase in AMF colonization and in arbuscule formation.

109

110 This is likely due to the increase in root endogenous auxin content since similar effects were
111 observed after treatments with exogenous auxin or interference with auxin signaling in tomato
112 and *M. truncatula* (Etemadi et al., 2014). Interestingly both AMF colonization and arbuscule
113 formation were increased in the Brachypodium *tar2l^{hypo}* mutant as in tomato and *M. truncatula*
114 after exogenous auxin treatment while only arbuscule formation was increased after exogenous
115 auxin treatment in rice (Etemadi et al., 2014). This role of auxin on AMF colonization might
116 occur directly by auxin promoting penetration and/or spreading of AMF in roots and/or
117 indirectly by controlling root architecture. Indeed, we cannot rule out that the increased AMF
118 colonization of *tar2l^{hypo}* roots is linked to the increased LR number or to other developmental
119 phenotypes that might result from the mutation in *TAR2L*. Our results open interesting questions
120 on the possible combined effect of LCOs and auxin on arbuscule formation *in planta*.

121

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128

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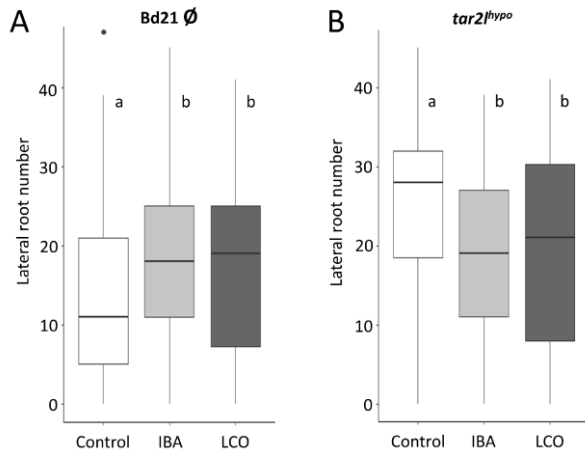
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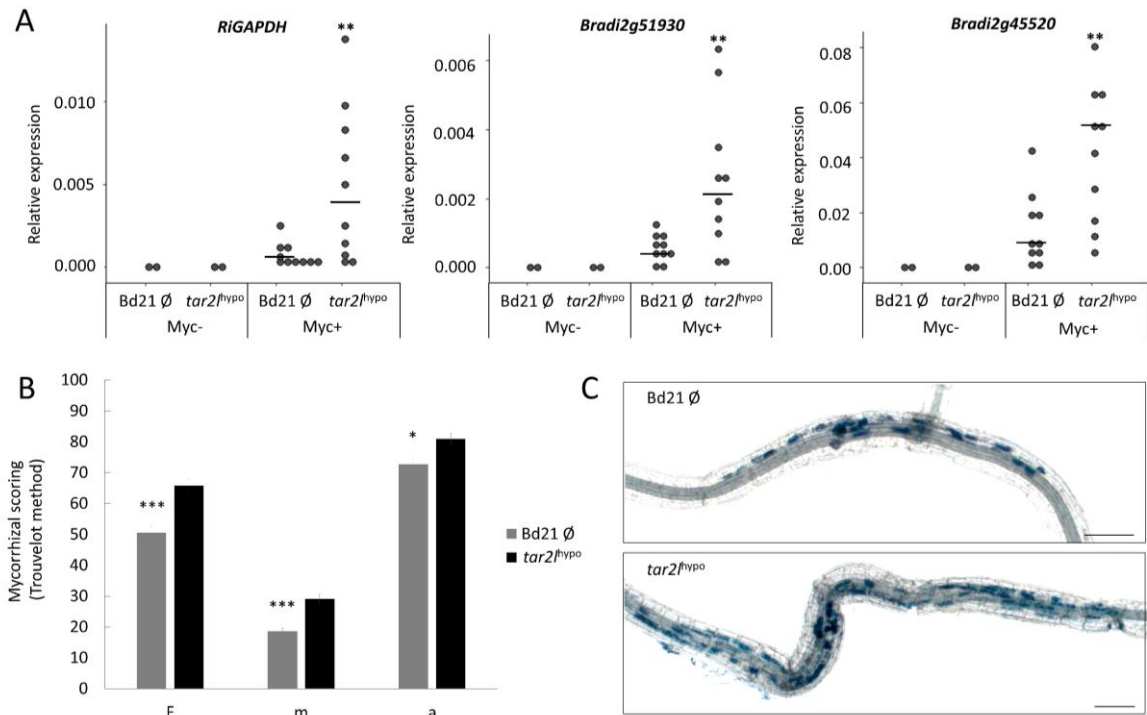


192

193 **Figure 1**

194 **IBA and LCO stimulate lateral root formation in *Brachypodium* control plants and inhibit**
 195 **lateral root formation in the auxin overproducer mutant *tar2l^{hypo}*.**

196 Effect of IBA (10^{-9} M) and LCO-V(C18:1,NMe,S) (10^{-7} M) on *Brachypodium* emerged lateral
 197 root number observed at 10 day post-transplantation in wild type control plants (A) or *tar2l^{hypo}*
 198 plants (B). Non-parametric Kruskal-Wallis followed by a post-hoc Van-Werden test was used
 199 for statistical analyses. Different letters represent statistically different categories. Data are from
 200 3 biological replicates (60 to 90 individuals in total).



201

202 **Figure 2**

203 **The auxin overproducer mutant *tar2^l^{hypo}* is more colonized by AMF than the wild type**
 204 **control.**

205 A) Relative expression of a fungal housekeeping gene (*RiGAPDH*) and plant AM marker genes
 206 (*Bradi2g51930* and *Bradi2g45520*) in wild type control plants or *tar2^l^{hypo}* plants, in absence
 207 (Myc-) or presence (Myc+) of AMF. RNA extraction (from entire root systems collected at 3
 208 wpi) and qRT-PCR were performed as described in Buendia et al., 2016. Expression was
 209 normalized to the plant housekeeping gene *BdEF1α* expression. Dotplots show the distribution
 210 of 2 to 10 pools of five plants, from 1 (Myc-) or 3 (Myc+) biological replicates. The median is
 211 shown for the Myc+ samples. ** = t- test $p < 0.01$. B) Frequency of AMF colonization (% of the
 212 root system), intensity of AMF colonization (arbitrary unit, AU) and arbuscule abundance (AU)
 213 in wild type control plants and *tar2^l^{hypo}* plants. 18 individuals of each genotype were grown in
 214 50 ml tubes filled with attapulgite as described in Buendia et al., 2016. Thirty root pieces were
 215 randomly collected from each plant at 3 wpi, stained and phenotyped according to the mycocalc
 216 method (Trouvelot et al., 1986). F= frequency of colonization in the root system; m = intensity
 217 of colonization in the root fragments; a = arbuscule abundance in the root fragments. Error bars
 218 indicate standard error of the mean. * = T-test p value < 0.05 ; *** = T-test p value < 0.001 . C).
 219 Bright field images of control (upper panel) and *tar2^l^{hypo}* (lower panel) roots stained with ink
 220 as described in (Vierheilig et al., 1998) . AMF stained in blue. Bars, 100 μ m.