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SHORT COMMUNICATION

Arabidopsis MYB28 and MYB29 transcription factors are involved in ammonium-mediated alterations of root-system architecture

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ABSTRACT

Ammonium (NH_4^+) is known to produce alterations in root-system architecture, notably, by inhibiting primary root elongation and stimulating lateral root branching. This stimulation is associated with higher auxin transport promoted by apoplast acidification. Recently, we showed that MYB28 and MYB29 transcription factors play a role in ammonium tolerance, since its double mutant (*myb28myb29*) is highly hypersensitive toward ammonium nutrition in relation to altered Fe homeostasis. In the present work, we observed that primary root elongation was lower in the mutant with respect to wild-type plants under ammonium nutrition. Moreover, ammonium-induced lateral root branching was impaired in *myb28myb29* in a Fe-supply dependent manner. Further research is required to decipher the link between MYB28 and MYB29 functions and the signaling pathway leading to root-system architecture modification by NH_4^+ supply.

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Plants shape their root-system architecture (RSA) in order to adapt to soil heterogeneity and therefore, to optimize nutrient uptake. Ammonium (NH_4^+) is a major inorganic nitrogen (N) source readily available in most natural and agricultural soils for plant uptake. However, a high concentration of NH_4^+ in the soil is a cause of stress in most plant species. NH_4^+ competes with the uptake of other essential cations, leads to changes in hormonal homeostasis and may inhibit photosynthesis, produce oxidative stress and generate osmotic unbalance. These alterations commonly entail visible symptoms such as the general loss of biomass and leaf chlorosis.[1–5] Furthermore, NH_4^+ provokes changes in RSA by 1) inhibiting primary root (PR) and lateral roots (LR) elongation, through the repression of cell growth and proliferation, and 2) stimulating root branching.[4,6]

Reduced PR length is a common marker of ammonium stress. This inhibition responds to a number of different ammonium-related processes. Among others, NH_4^+ stress is known to generate the overproduction of reactive oxygen species (ROS), and this increase could have a negative impact on root meristem cells proliferation.[7] Indeed, Xie *et al.*[8] showed ammonium-induced root shortening alleviation in the rice overexpressor *OsSE5/HO1 (heme-heme oxygenase 1)*, which has a higher ROS-detoxifying capacity. Besides, Arabidopsis *vtc1-1* mutant, which is deficient in GDP-mannose pyrophosphorylase (GMPase), shows a dramatic PR inhibition under NH_4^+ supply. Still unclear, the reasons underlying *vtc1-1* phenotype appear associated with disturbed protein N-glycosylation, perturbed auxin and ethylene homeostases and pH alterations associated with NH_4^+ uptake.[9–11]

LR branching is induced under local low-NH_4^+ concentrations in an AMT1;3 NH_4^+ transporter-dependent manner.[12] Very recently, Meier *et al.*[13] demonstrated that ammonium-induced LR branching is a consequence of root apoplast acidification mediated by NH_4^+ uptake via AMTs. This acidification increases the protonation of indole-3-acetic acid and thus, its transport into the epidermal cell to finally promote LR formation.[13]

In a recent work, we reported that Arabidopsis double mutant for MYB28 and MYB29 transcription factors (*myb28-myb29*) is highly hypersensitive to ammonium nutrition.[14]MYB28 and MYB29 are master regulators of aliphatic glucosinolate (GSL) biosynthesis.[15] The hypersensitivity of *myb28myb29* to ammonium nutrition was associated with a clear leaf chlorosis and reduced biomass. These symptoms appeared independent of the absence of aliphatic GSLs but were related to a defective Fe homeostasis.[14] In the present work, we analyzed whether RSA was also affected in *myb28-myb29* in response to NH_4^+. To do so, wild-type Col-0 (WT) and *myb28myb29* plants grown as described in Coleto *et al.*[14] were scanned using an EPSON expression 10000 XL scanner. PR length was analyzed using ImageJ software and LR density calculated by dividing PR length by the number of LRs.

As expected, ammonium nutrition provoked PR elongation inhibition with respect to control plants grown with nitrate, being this inhibition stronger when the concentration of NH_4^+ supplied was higher (Figure 1a, b). In agreement with the reported *myb28myb29*

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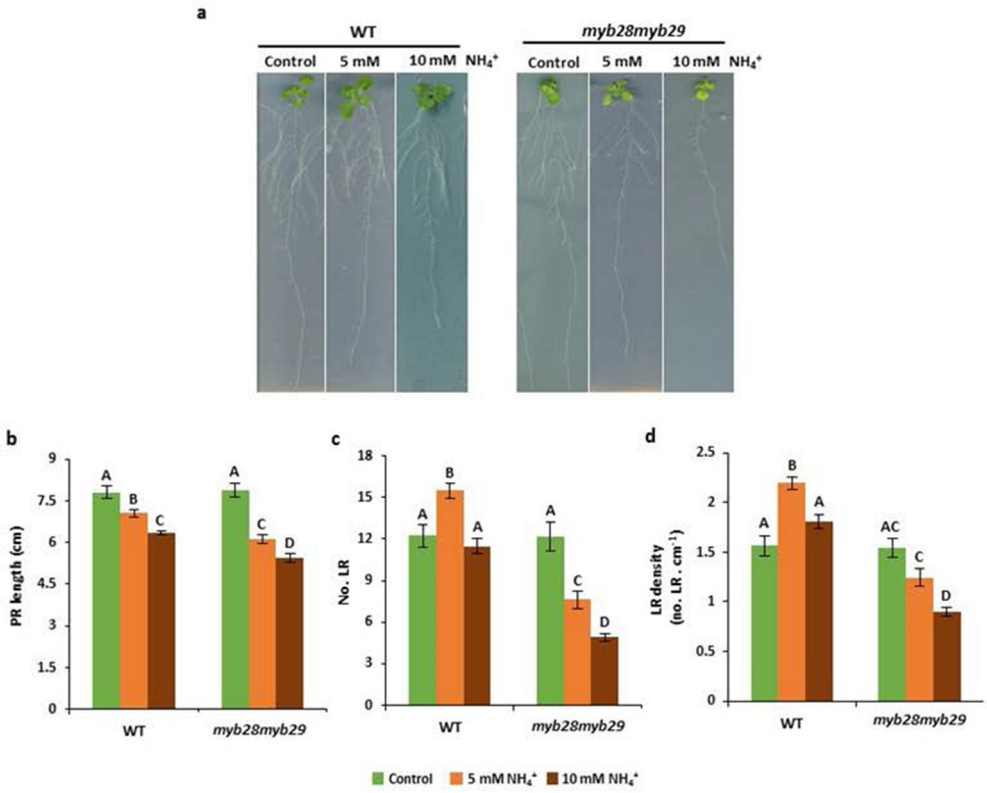

Figure 1. Root-system architecture is affected in *myb28myb29* under ammonium nutrition. (a) Representative images, (b) PR length, (c) number of LR and (d) LR density of WT and *myb28myb29* plants grown with 5 mM NO_3^- (control), 5 mM NH_4^+, or 10 mM NH_4^+ for 15 days. Values represent mean ± SE (n = 20). Different letters indicate significant differences between treatments and genotypes (one-way ANOVA followed by Duncan´s *post hoc* test, $p \leq 0.05$).

sensitivity, PR length in the mutant was shorter with respect to WT plants (Figure 1a,b). Regarding LR number and density, moderate ammonium stress (5 mM NH_4^+) provoked the reported stimulation of LR emergence in WT plants. However, the stimulation was not observed under severe ammonium stress (10 mM NH_4^+) (Figure 1a,c,d). Apart from the stress severity, this absence of stimulation could be due to the repressive effect that Gln levels derived from NH_4^+ assimilation have on LR formation.[9]

In *myb28myb29*, ammonium-induced stimulation of LR number and density did not occur (Figure 1a,c,d); therefore, opening the question of whether MYB28, MYB29 function might be somehow associated with pH-related auxin transport. In the same line, as reported for *myb28myb29* hypersensitivity,[14] the observed effect on RSA was also independent of the lack of aliphatic GSLs, since *myc234* mutant, which is also devoid of aliphatic GSLs,[16] did not display any RSA difference with respect to WT plants (data not shown). In addition, in Coleto *et al.*,[14] we also reported that when *myb28myb29* was grown with a higher Fe supply (200 µM respect to 100 µM) its hypersensitivity to ammonium was restored. In this work, we also show that the differences in RSA observed between WT and *myb28myb29* disappeared when growing the plants with 200 µM Fe supply (Figure 2). Besides *MYB28* and *MYB29*, the disruption of other genes including *GMPase*[10] and *AMT1;3*,[12] as described above, and others such as *GSA-1/ARG1* (GRAVITROPISM-SENSITIVE-TO-AMMONIUM-1), that encodes a DnaJ-like protein, and *AMOT1* (AMMONIUM TOLERANCE 1) that encodes an EIN3 transcription factor, provokes alteration in RSA in response to ammonium nutrition. In particular, root gravitropism is affected in *gsa-1* mutant and a lower inhibition of lateral root formation by ammonium stress was reported in *amot1*[17,18]. In these works, the potential association with Fe supply or GLS metabolism was not studied and thus, at present, it is difficult to predict a potential link between the function of MYB28 and MYB29 and these genes. Future studies will be of great interest to analyze the potential interconnection among these genes in RSA response to ammonium.

Altogether, our data indicate that the disruption of Fe homeostasis, as a consequence of the functional lack of MYB28 and MYB29 transcription factors, may also be involved in ammonium-mediated changes in RSA.

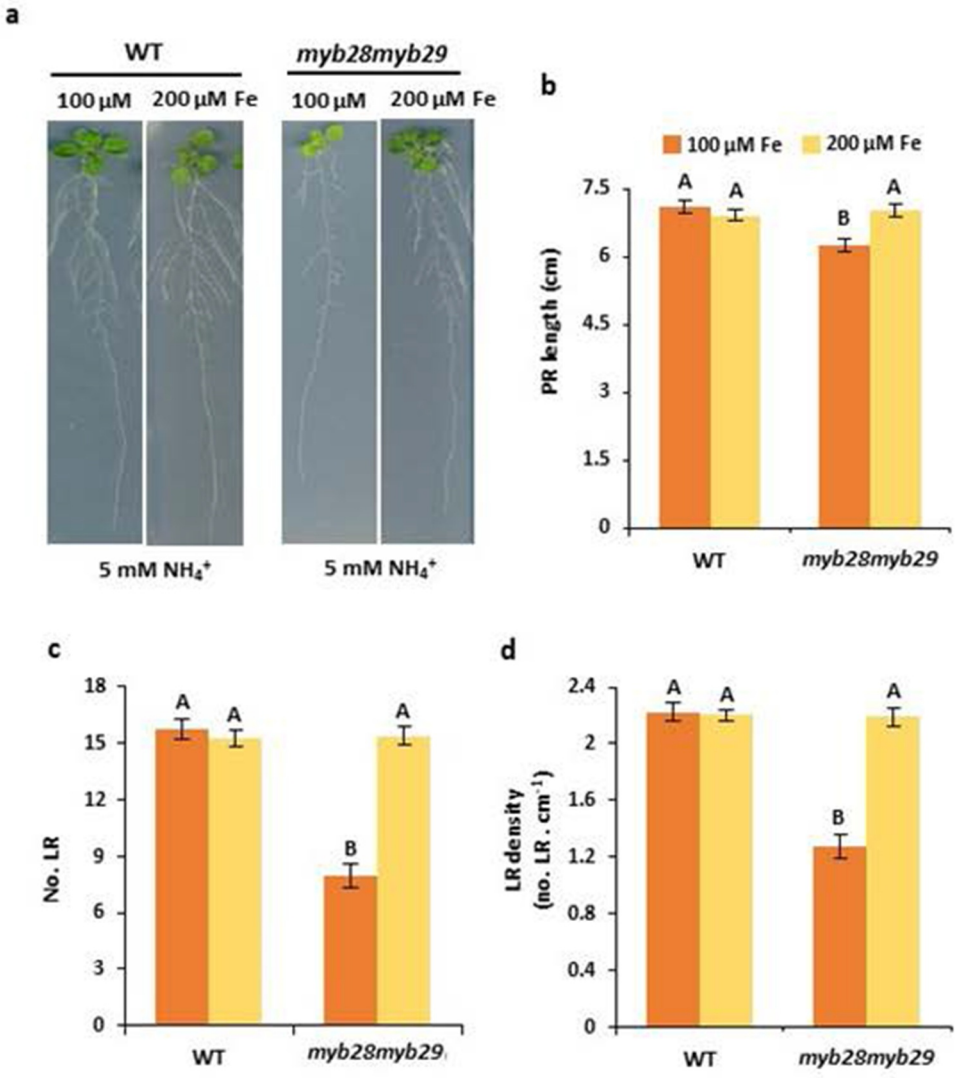

Figure 2. *Myb28myb29* root-system architecture is restored by higher Fe supply. (a) Representative images, (b) PR length (c) number of LR and (d) LR density of WT and *myb28myb29* plants grown with 5 mM NH_4^+ and 100 µM or 200 µM Fe supply for 15 days. Values represent mean ± SE (n = 20). Different letters indicate significant differences between treatments and genotypes (one-way ANOVA followed by Duncan´s *post hoc* test, $p \leq 0.05$).

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Disclosure of potential conflicts of interest

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