The multiple plant response to high ammonium conditions

The *Lotus japonicus* AMT1; 3 protein acts as a putative transceptor

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Plant evolved a complex profile of responses to cope with changes of nutrient availability in the soil. These are based on a stringent control of expression and/or activity of proteins involved in nutrients transport and assimilation. Furthermore, a sensing and signaling system for scanning the concentration of substrates in the rooted area and for transmitting this information to the plant machinery controlling root development can be extremely useful for an efficient plant response. Ammonium represents for plants either a preferential nitrogen source or the trigger for toxicity symptoms depending by its concentration. We propose a role for the high affinity *Lotus japonicus* ammonium transporter *Lj*AMT1;3 as an intracellular ammonium sensor to achieve a convenient modulation of the root development in conditions of potentially toxic external ammonium concentration.

Plants are sessile organisms that cope with large variations in soil, apoplastic and intracellular concentrations of mineral nutrients. This justifies the presence of a large number of proteins involved in nutrients uptake, intercellular and/or intracellular transport and sophisticated regulatory arrays to ensure a condition-dependent activity.

Plants can extract and use various forms of nitrogen (N) from soil, most importantly the inorganic ions ammonium (NH_4^+) and nitrate (NO_3^-). NH_4^+ assimilation requires less energy than that of nitrate,[1] but only a few species perform well when NH_4^+ is the only or predominant source of N, whereas by contrast, most species develop toxicity symptoms in these conditions.[2-7] The recent cloning of the genes for a large number of transport proteins and the availability of knockout mutants in *Arabidopsis thaliana* allowed the dissection of the ammonium transport process in greater detail and the understanding of how this might be modulated to respond to the environment commitments. The high-affinity ammonium transporters (AMT1) isolated in different plant species show a regulation of the expression at the transcriptional level. They are mainly induced in roots of plants deprived of N and downregulated after ammonium re-supply (**Fig. 1B**).[8,9] Recently, a post-translational regulation of the AMT activity has been described for the *A. thaliana* AMT1;1 protein.[10,11] Phosphorylation of the conserved T460, triggered by ammonium in a time- and concentration-dependent manner leads to allosteric inactivation of the *At*AMT1;1 trimeric complex. This modification triggers the tuning of the uptake capacity, allowing the quick inactivation of transport in a potentially toxic environment (**Fig. 1A**).

The investigation carried out by Rogato et al. allowed the characterization of an additional mechanism of plant response to potential cytotoxic ammonium external concentrations.[12] In a high ammonium range of concentration, a specific short root phenotype in wild-type *Lotus*
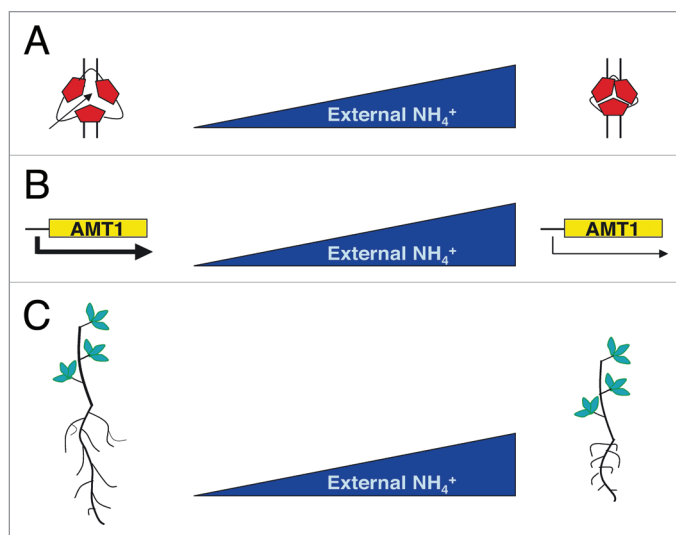
Figure 1. Chronology of the repertoire of plant protective responses to high ammonium conditions. (A) Regulation of ammonium uptake through the conformational control of the trimeric *At*AMT1;1 protein.[10,11] (B) Transcriptional downregulation of *AMT1* genes expression.[8,9] (C) Block of primary and secondary roots elongation.[12,16]

japonicus plants was reported (**Fig. 1C**). Primary and secondary roots elongation rates are drastically reduced in plants grown in a ≥10 mM ammonium condition when compared to plants grown in lower ammonium concentration or equivalent high nitrate/glutamine conditions. This is a specific root response that is not associated to any shoot phenotype or pleiotropic stress related symptoms. In addition, ammonium acts as a signal rather than as a nutrient and the effect is exerted locally on Lotus roots. This protective plant response might be needed to avoid energy waste in a potentially unfavorable environmental condition. Such a mechanism might be complementary to those underlying the ammonium transport regulation through the control of the AMT1 activity (**Fig. 1A**) and expression (**Fig. 1B**). As an alternative, the root developmental response might occur only in plants with a reduced capability to regulate the ammonium influx rate.[6]

The modulation of the root developmental program is a typical plant response to the changes of nutrients availability in the rhizosphere.[13-15] A specific ammonium-dependent local inhibition of primary and secondary roots elongation has been very recently characterized also in *A. thaliana* where the effect is exerted mainly on root cell elongation and is associated

to an increased efflux rate in the root elongation zone.[16] This is consistent with the proposed mechanism explaining the NH_4^+-induced toxicity as consequence of an NH_4^+ futile cycle at the plasma membrane of root cells.[6]

This scenario entails the existence of a plant root derived mechanism for sensing the ammonium external concentration and for transducing this signal to the plant machinery driving primary and secondary roots growth. The results reported in Rogato et al. identify a potential ammonium sensor involved in this root developmental response.[12] First, the high affinity ammonium transporter *LjAMT1;3* is the only member of the AMT1 family to be induced in the high ammonium conditions, where the root phenotype is observed. Second, its overexpression in independent transgenic lines phenocopy the wild-type phenotype. Interestingly, the phenotype of the overexpressing plants is not related to the increased ammonium uptake, suggesting an additional signaling function of the AMT1;3 protein that is independent by its uptake activity. These features have been already described for different transporter proteins such as the *Saccaromyces cerevisiae* and *Candida albicans* methylammonium permease (Mep2), required for pseudo-hyphal differentiation in N starvation

conditions, and the *A. thaliana* nitrate transporter (NRT1.1), controlling the secondary roots elongation in response to local patches of nitrate.[17-19] In both cases a direct mutagenesis approach demonstrated the uncoupling of the transport and signaling functions, confirming their role as transceptors.[18,19]

The *Lj*AMT1;3, spatial profile of expression, confined to the root stele, makes unlikely its contribution to high-affinity ammonium uptake in roots. Nutrients such as ammonium are mostly absorbed at the epidermis (rhizodermis) and move symplastically through the cortex to the stele. However, nutrients may also enter the symplast later in cortical and endodermal cells, but the casparian strip provides a major barrier for further apoplastic movement. Yuang et al. proposed a model for a coordinated transport activity of the different AMT1 members in the Arabidopsis roots where *At*AMT1 members, with different transport capacities and different root cell layers location, work in a synergic way by taking up ammonium from the soil and apoplastic space to allow NH_4^+ assimilation (**Fig. 2**).[20] The model shown in **Figure 2** to couple external ammonium concentration and short root phenotype proposes that in the presence of increasing external ammonium supply, a linear increase of the amount of un-assimilated ion reaching the xylematic tube occurs[21,22] and *Lj*AMT1;3 acts as an internal sensor, leading to the induction of its own expression. Therefore, *Lj*AMT1;3 could perceive the intracellular ammonium transported to the vascular tube, that would reflect the external concentration (**Fig. 2**). In this model the ammonium acts as an intracellular signal,[23,24] whereas in another report the apoplastic ammonium was proposed as the signal triggering the uptake regulation.[11]

A likely connection can be hypothesized between the nutrient signaling governing root development and hormones-induced regulatory arrays. In the case of nitrate signaling the high affinity transporter NRT1.1 is itself a facilitator of the auxin uptake involved in secondary roots elongation,[25] while the correlation between auxin and ammonium signaling pathways is not clear yet and needs further investigation.[16,26]

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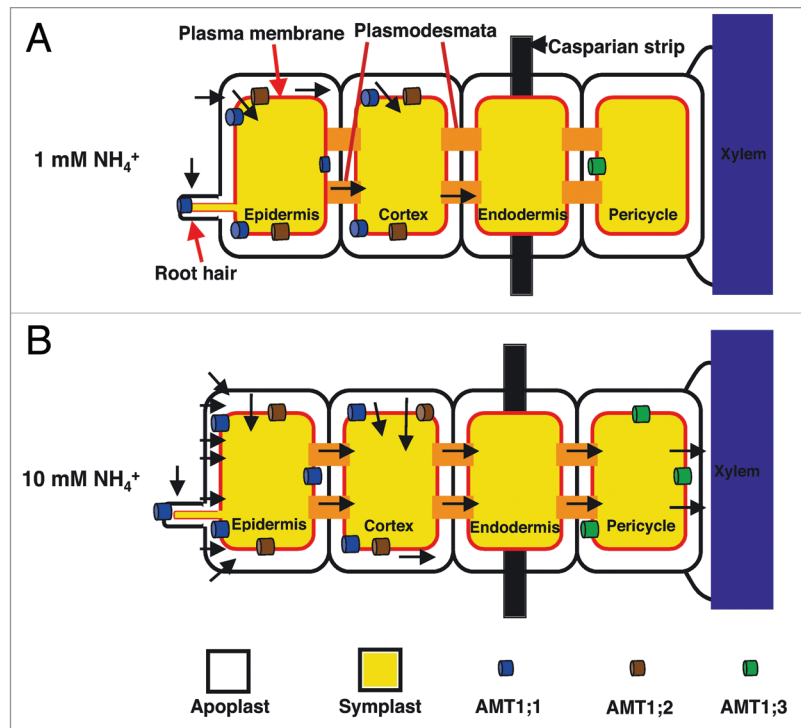
Figure 2. Model summarizing the putative action of *Lj*AMT1;3 as intracellular ammonium sensor. (A and B) represent moderate and high ammonium external concentrations. The root cell layers, symplastic/apoplastic ammonium routes and AMT members are represented.