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Roles of abscisic acid and auxin in shoot-supplied ammonium inhibition of root system development

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A plastic root system is a prerequisite for successful plant acclimation to variable environments. The normally functioning root system is the result of a complex interaction of root-borne signals and shoot-derived regulators. We recently demonstrated that AUX1, a well-studied component of auxin transport, mediates shoot-supplied ammonium (SSA) inhibition of lateral root (LR) formation in Arabidopsis. By contrast, the response did not involve ABA pathways, via which several other abiotic stresses affect LR formation. We proposed that SSA regulates LR emergence by interrupting AUX1-mediated auxin transport from shoot to root. Here, by analyzing both ABA- and auxin-related mutants, we show that AUX1 is also required for SSA-mediated suppression of primary root growth. Ammonium content in shoots was furthermore shown to increase linearly with shoot-, but not root-supplied, ammonium, suggesting it may represent the internal trigger for SSA inhibition of root development. Taken together, our data identify AUX1-mediated auxin transport as a key transmission step in the sensing of excessive ammonium exposure and its inhibitory effect on root development.

Analysis of the Internal Trigger for SSA Inhibition of Root Development

Different ammonium concentrations often have opposite effects on many aspects of plant development, including seed germination, primary root growth and lateral root (LR) elongation.[1,2] In addition, the effect of ammonium on LR formation has been shown to be dependent on whether ammonium exposure occurs in the roots or the shoots. While excess root-supplied ammonium increases LR formation,[3] we reported in a recent study[4] that shoot-supplied ammonium (SSA) reduces it. Our work demonstrated that (1) SSA inhibits LR emergence but not LR initiation in LR formation; (2) SSA-mediated inhibition is not related to altered ABA signaling, as is the case with exposure to high nitrate and osmotic stresses; (3) SSA-mediated inhibition reduces cellular auxin response in both roots and shoots; (4) SSA-mediated inhibition reduces auxin transport from shoots to roots but not basipetal auxin transport in roots; (5) the auxin importer AUX1, but not auxin exporter PIN1 and PIN2 or auxin acceptor TIR1, is required for SSA-mediated inhibition of LR formation; and (6) SSA decreases the expression of AUX1 in vascular tissues rather than in LR cap cells. These results raise interesting questions about the nature of the internal trigger for reduced auxin signaling in shoots and roots under SSA conditions. Accumulation of ammonium in shoots is considered to be a key index of ammonium toxicity.[5,6] Here, our data show that ammonium content in shoots increased linearly with increased shoot-, but not with root-supplied, ammonium (**Fig. 1**). However, the ammonium content in roots was only ~10% of that in shoots under the same concentration of SSA (data not shown). Hence, the greatly increased ammonium content in shoots may be the intrinsic trigger that leads to the reduced auxin response and LR formation under SSA conditions.

Shoot-Derived Signals for LR Formation under Abiotic Stress

If the ammonium content in shoots is the trigger, how is the signal transmitted? Many shoot-derived signals influence root development. It has been reported that shoot-derived auxin is necessary for LR formation.[7-10] Generally, ABA accumulation is regarded as a negative regulator in LR formation,[11] especially under stresses such as osmotic stress and high nitrate.[12-15] Recently, it was found that the uptake of sucrose in shoots promotes LR development in Arabidopsis in culture,[15] whereas it is reduced by the accumulation of ABA in shoots, which, in turn, is induced by osmotic stress.[15] Additionally, light-signaling component HY5 mediates ABA inhibition of LR growth in Arabidopsis.[16] Given that accumulation of ABA is a common response when plants experience abiotic stresses, it is reasonable to hypothesize that ABA pathways are major mediators through which abiotic stresses act on LR formation. Thus, the discovery that SSA inhibits LR formation by interfering with shoot-derived auxin, but not with ABA pathways,[4]

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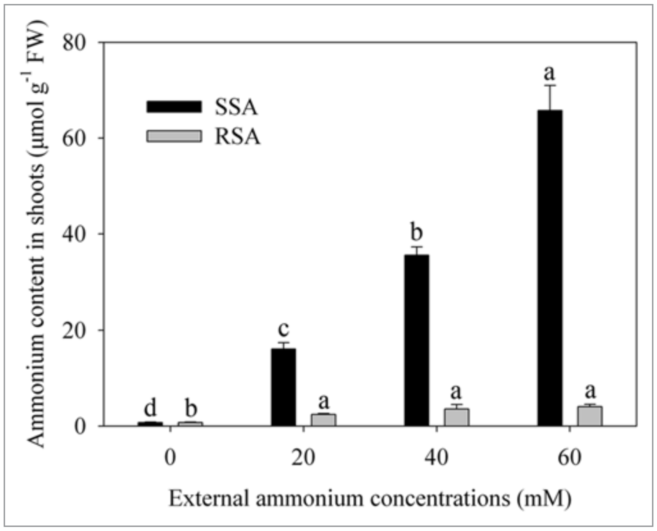

Figure 1. The increased ammonium content in shoots. Seedlings at five days after germination (DAG) were transferred to the shoot-supplied ammonium (SSA) and the root-supplied ammonium (RSA) treatment medium for an additional six days as described in references 3 and 4. 30 mg fresh weight of shoots was extracted with 1 ml formic acid for the ammonium content assay with by HPLC after derivatization with o-phthaldialdehyde.[25] Data represent means ± SE, n = 4. Letters indicate significantly different means between ammonium treatments within a given condition (one-way analysis of variance with Tukey-B multiple comparison test, $p < 0.05$).

is counterintuitive. Shoot-derived auxin signaling suggests a novel pathway for LR development in response to environmental stresses.

The Role of Auxin Importer AUX1 in SSA Inhibition of LR Emergence

Auxin transport has been well studied for its role in promoting LR formation.[17,18] Experimental evidence supports the role of AUX1 in the regulation of LR position, initiation and emergence.[19-22] Expression patterns of AUX1 in LR cap and vascular tissues suggest its function in basipetal and acropetal auxin transport, respectively.[19,21,23] SSA was shown to reduce the expression of AUX1 in vascular tissues but not in LR cap cells in roots.[4] This indicates the two functions of AUX1 can be regulated independently under changing environmental conditions. Mutants with defects in AUX1 function displayed insensitivity to SSA inhibition of LR formation and acropetal auxin transport in roots,[4] which suggests AUX1 is essential for SSA inhibition of acropetal auxin transport and LR emergence. It is, thus, likely that AUX1-mediated auxin transport represents the key transmission step in the effect of SSA on LR development. However, another possibility, that AUX1 may be important for ammonium entry into shoot cells, cannot be excluded and warrants further experiments.

Primary Root Growth of ABA or Auxin-Related Mutants in Response to SSA

In contrast to their ability to regulate LR formation, shoot-derived signals are much less known for their role in primary root

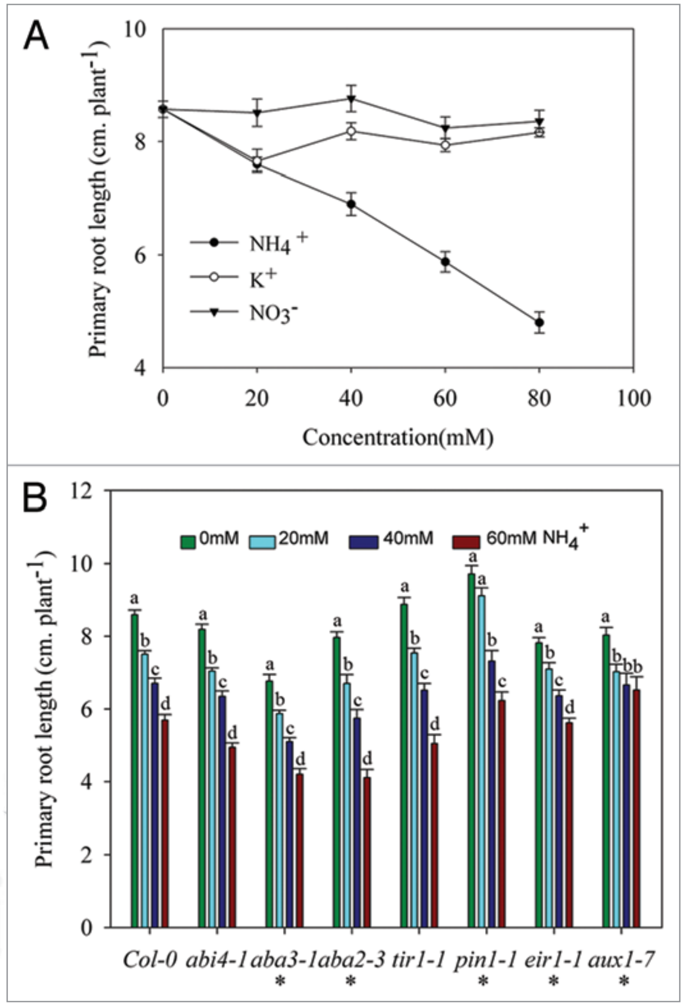

Figure 2. SSA inhibition of the primary root growth in both ABA and auxin-related mutants. (A) Effects of NH_4^+ [as $(NH_4^+)_2SO_4$], K^+ (as K_2SO_4), NO_3^- (as KNO_3), on the length of primary roots in Col-0. (B) Effect of SSA on the length of primary roots of different genotypes (Col-0, *abi4-1*, *aba3-1*, *aba2-3*, *tir1*, *pin1-1*, *eir1-1 and aux1-7*). 5-DAG seedlings of different genotypes were treated with SSA, shoot-supplied K_2SO_4 or KNO_3 in medium for an additional six days. Values are the means of more than 12 seedlings ± SE. Experiments were repeated at least twice independently. Letters indicate significantly different means between ammonium treatments within a given genotype (one-way analysis of variance with Tukey-B multiple comparison test, $p < 0.05$). The asterisk indicates significant difference between the given mutant and Col-0 on the normal growth media (Student's t-test, $p < 0.05$).

elongation. Here, we show the length of the primary root was reduced with increased shoot-supplied $(NH_4)_2SO_4$, but remained nearly unchanged with increased K_2SO_4 or KNO_3 (**Fig. 2A**). These results demonstrate that the SSA inhibition of primary root growth is a result of exposure to the ammonium ion itself, rather than ion stress in general or elevated nitrogen content. However, under conditions of root-supplied ammonium, root tip contact with ammonium is necessary for the inhibition of root elongation.[3] Hence, we propose SSA inhibition of primary root elongation must be a secondary effect of ammonium exposure, whereas the effect of root-supplied ammonium is more primary.

We were particularly interested in the question: What is the role of ABA and auxin signaling in SSA inhibition of primary root growth? We therefore examined primary root growth in ABA-synthesis-defective mutants (*aba2* and *aba3*) and ABA-response-insensitive mutant *abi4* in response to SSA, and found that they displayed similar reductions as wild type under SSA treatment (**Fig. 2B**). However, the primary root length of ABA-synthesis-defective mutants, particularly *aba3*, was less than wild type under the same conditions (**Fig. 2B**). This indicates that proper ABA content was required for normal primary root growth, in agreement with previous observations.[24] Compared with wild type, the primary root elongation of auxin response mutant *tir1* was similar, that of auxin transport mutant *pin1* was significantly increased, whereas auxin transport mutants *eir1* and *aux1* showed slight reductions on normal growth medium (**Fig. 2B**). This data indicate auxin transport is also important for normal primary root growth, and different auxin transporters may play opposite roles in primary root growth. Under SSA treatment, the primary roots of *tir1*, *pin1* and *eir1* displayed similar reductions as wild type, but the primary root of *aux1* was reduced significantly less than others (**Fig. 2B**). This data suggest AUX1 is required for SSA inhibition of primary root elongation. These results are reminiscent of the response of LR development to SSA treatment, suggesting the effect on LR development and primary root growth of SSA may be mediated by the same pathway.

The current study suggests AUX1-mediated auxin transport may play a key signaling transmission role between root system development and environmental stresses experienced in shoots. Further unraveling the molecular mechanisms underlying these processes will be important to understanding the relationships of shoot-derived signals and underground organ development, and the coordination of these elements in the context of plant acclimation to environmental stress.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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