Allantoin accumulation mediated by allantoinase downregulation and transport by Ureide Permease 5 (AtUPS5) confers salt stress tolerance to Arabidopsis plants

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In several reports, variations in the content of purine degradation metabolites has been associated with plant responses to different stress conditions and leaf senescence, but the subjacent molecular mechanisms remained poorly understood. A recent study demonstrated that the ureide allantoin (ALN), a product of the purine degradation pathway, induces *de novo* biosynthesis and deconjugation of the stress hormone abscisic acid (ABA). This was related to a better tolerance to osmotic and water stress. Other studies suggest a protective effect of ALN and allantoate against ROS, but the mechanisms implied remain unknown.

The ureides ALN and allantoate are products of the purine degradation pathway (Fig. 1). ALN is hydrolyzed to allantoate through allantoinase (AtALN).

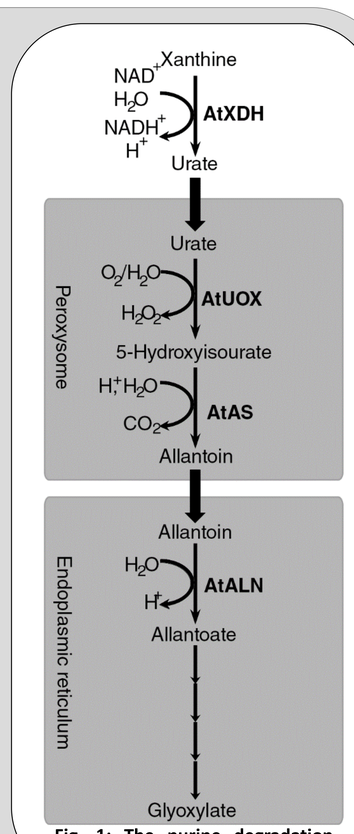


Fig. 1: The purine degradation pathway

A primary objective of this work was to know if ureides concentration increase in Arabidopsis plants subjected to salt stress. (Fig. 2)

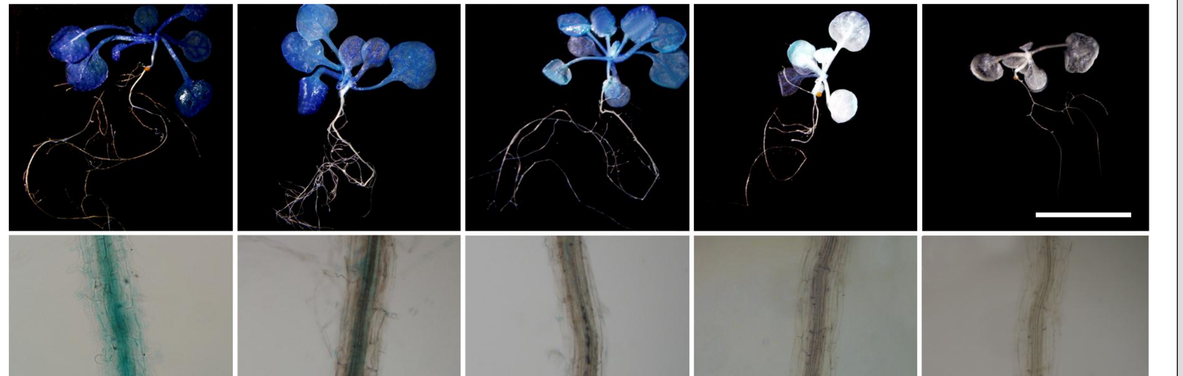


Fig. 2: Response of wt seedlings to different concentrations of NaCl. 14 days-old seedlings were treated in solid MS medium supplemented with 0-250 mM NaCl for 7 days. The same procedure was used for further experiments. **(a)** Representative seedlings grown on vertical plates (bar=1cm) and **(b)** Ureide concentration are shown. DW, dry weight.

We study the expression of genes encoding the two enzymes involved in the sequential ALN synthesis (AtURI and AtAS) and the ALN degrading enzyme (AtALN).
(Fig. 3 and 4)

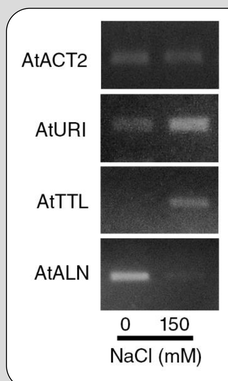
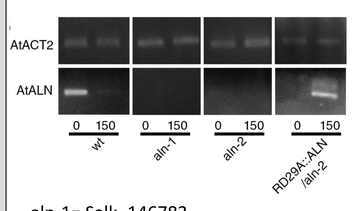


Fig. 3: Gene expression of the *Arabidopsis* purine degradation pathway enzymes under salt stress conditions. RT-PCR of transcripts present in seedlings with or without salt stress. Material was harvested after 24 hours of salt treatment.
AtUOX= urate oxidase/uricase; AtAS= allantoin synthase; AtALN= allantoinase. AtACT2 fragment was used as control.

Fig. 4: Response of the uida gene under the control the AtALN promoter in seedlings subjected to salt stress. 14 days-old seedlings were transferred to solid MS medium supplemented with 0-200 mM NaCl for 7 days. Scale bar=0.5 cm (top) or =100 µm (bottom).

Salt stress increases ALN concentration by AtALN downregulation in Arabidopsis seedlings. Both shoot- and root- AtALN transcription are downregulated by salt, but gene repression occurs in root cells even at low salt concentrations suggesting a role in early plant response to moderate stress conditions.

To know if ALN accumulation results in plant stress protection, we comparatively analyzed genotypes with different capacities of ALN accumulation. (Fig. 5 and 6)



aln-1= Salk_146783.
aln2 =Salk_142607.

Fig. 5: Expression of AtALN mRNA in wt, aln T-DNA insertion lines and RD29A::ALN/aln-2 under salt stress. Material harvested after 24 hours salt treatment was used for RT-PCR. AtACT2 fragment was used as control.

Transcriptional regulation of AtALN is a key factor for salt stress tolerance mediated by ALN accumulation.

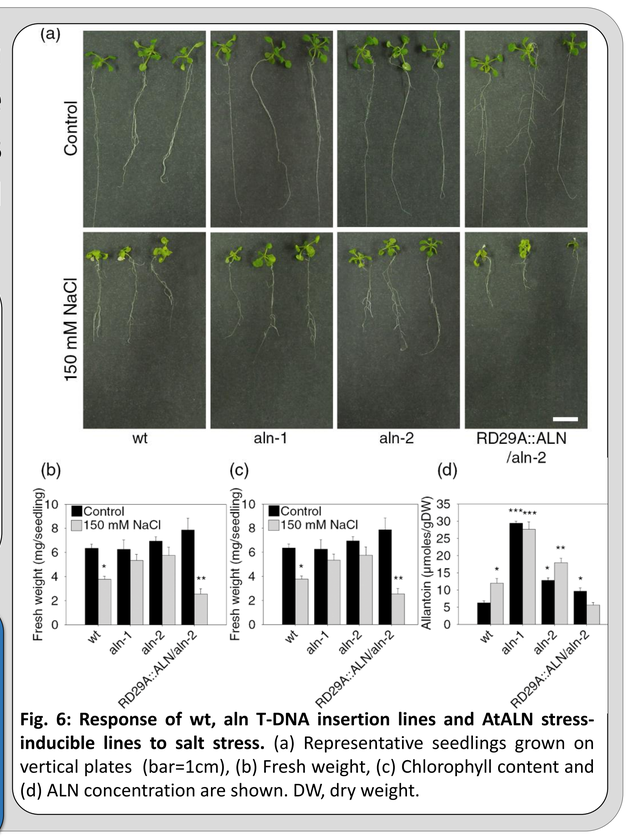


Fig. 6: Response of wt, aln T-DNA insertion lines and AtALN stress-inducible lines to salt stress. (a) Representative seedlings grown on vertical plates (bar=1cm), (b) Fresh weight, (c) Chlorophyll content and (d) ALN concentration are shown. DW, dry weight.

In further experiments, we focused our attention on a potential transport of ALN mediated by AtUPS5.
(Fig. 7 and 8)

In Arabidopsis the ureide permease AtUPS5 is a membrane protein with a high affinity for ALN and is expressed in root cortex and endodermis.

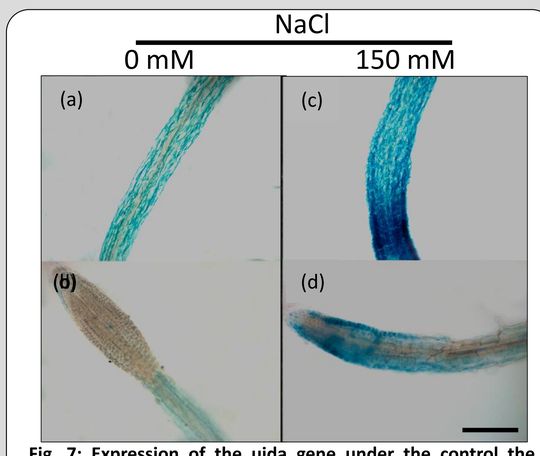


Fig. 7: Expression of the uida gene under the control the AtUPS5 promoter subjected to salt stress. (a, c) Primary root final segment and (b, d) tip are shown. Scale bar=100 µm.

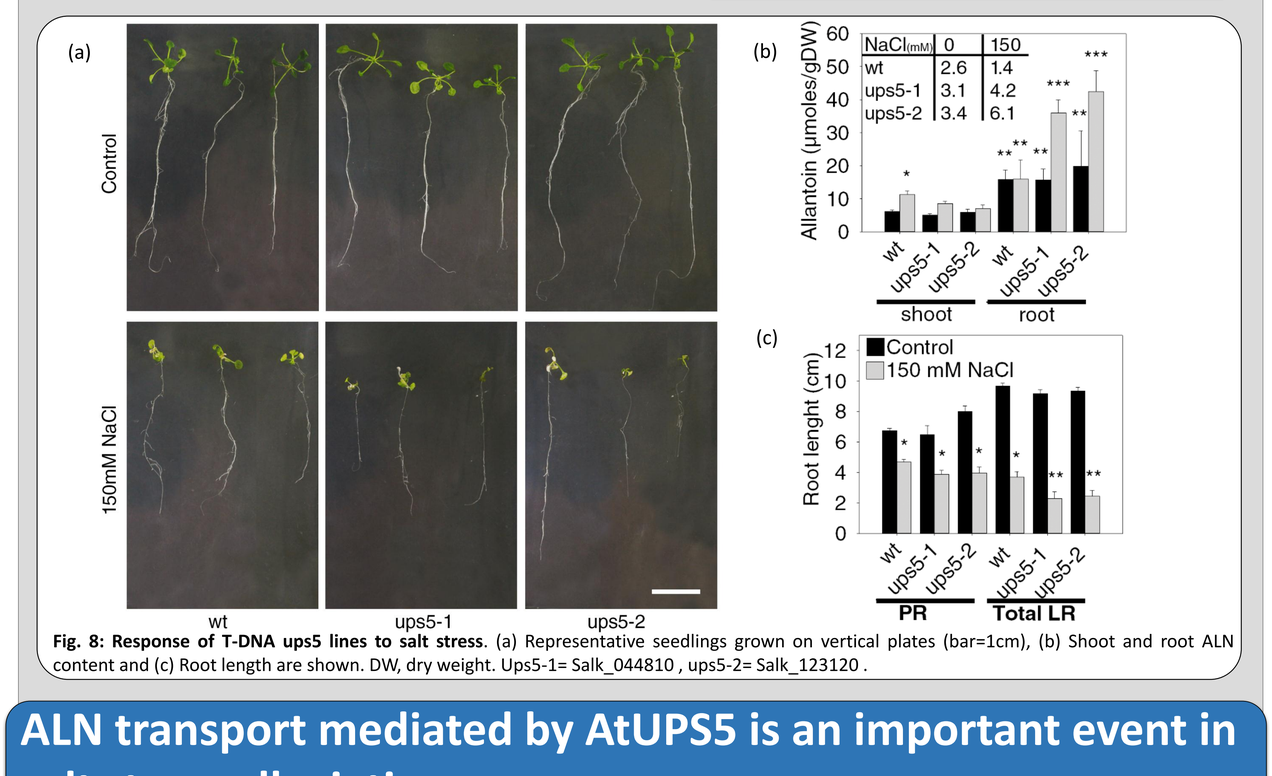


Fig. 8: Response of T-DNA ups5 lines to salt stress. (a) Representative seedlings grown on vertical plates (bar=1cm), (b) Shoot and root ALN content and (c) Root length are shown. DW, dry weight. Ups5-1= Salk_044810 , ups5-2= Salk_123120 .

ALN transport mediated by AtUPS5 is an important event in salt stress alleviation.

¿ALN Long distance Transport or ABA Long distance Signal?

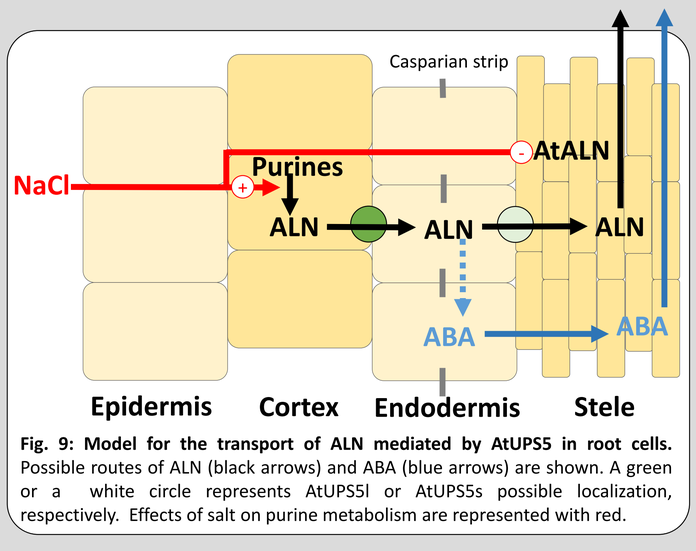


Fig. 9: Model for the transport of ALN mediated by AtUPS5 in root cells. Possible routes of ALN (black arrows) and ABA (blue arrows) are shown. A green or a white circle represents AtUPS5l or AtUPS5s possible localization, respectively. Effects of salt on purine metabolism are represented with red.

AtUPS5 could be necessary for endodermis crossing: ALN could be imported to endodermis through AtUPS5l (long isoform) and exported to the stele by AtUPS5s (short isoform) for long distance transport (Fig. 9) .

AtUPS5 could be involved in the generation long distance signal for plant protection such as ABA.