Ureide synthesis, accumulation and transport in Arabidopsis plants under salt and osmotic stress

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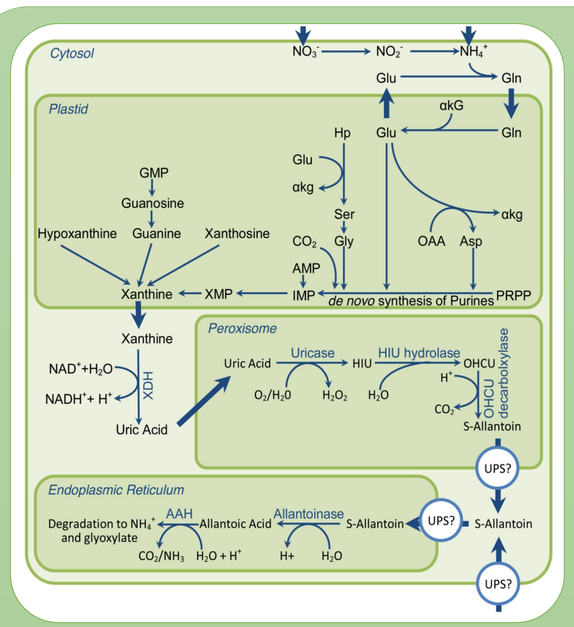

Fig. 1: Pathway of ureides allantoin and allantoic acid synthesis. In plastids, purines are synthesized *de novo* and can be degraded to xanthine in an irreversible way. In cytosol, xanthine dehydrogenase (XDH) oxides xanthine to uric acid, which enters the peroxisomes and is subsequently degraded by uricase (URI), HIU Hydrolase and OHCU decarboxylase. The two last steps are carried out by one enzyme with dual activity (allantoin synthase; TTL). Allantoin is converted in allantoic acid by allantoinase (ALN) and allantoic acid is degraded by an hydrolase (AAH). These two enzymes resides in the ER, suggesting that export of ureides occurs via the secretory pathway. However, there is no information about the allantoin transport within the cells being ureide permeases (UPS) candidates for this function.

In Arabidopsis, microarrays studies showed an up regulation of ureides synthesis genes (AtXDH, AtURI) during osmotic (Fig. 2A) and salt (Fig.2B) stresses. On the contrary, allantoinase gene expression is strongly reduced after stress suggesting that allantoin may accumulate in the cells. Recently, allantoin accumulation was associated with the ABA pathway stimulation, and a following enhancement of stress response. However, some aspects are still controversial and allantoin also could potentially have other functions in stress response e.g. compatible osmolyte, active oxygen scavenger)

The ureides allantoin and allantoic acid play a central role in transport of organic nitrogen in nodulating tropical legumes. They are synthetized from purines in a pathway comprising several enzymes localized in different compartments (Fig. 1). However, the complete enzyme set for ureide synthesis and a family of ureide permeases are widely distributed in the plant kingdom suggesting their participation in physiological processes not properly characterized yet.

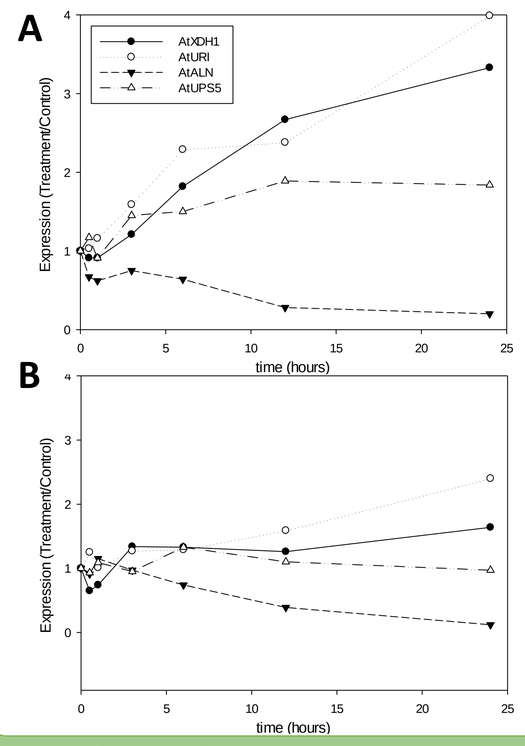

Fig. 2: Ureides metabolism genes expression under osmotic (A) and salt (B) stresses. Arabidopsis Col-0 seeds were sown on rafts in Magenta boxes containing MS-Agar-media. After 2 days at 4ºC, boxes were transferred to a long day chamber (16/8 hrs light/dark) . At day 11, the drafts were transferred to MS-liquid-media. At day 16, osmotic (A) or salt (B) stress treatment started. Mannitol and NaCl were added to a concentration of 300 and 150 mM in the Media, respectively. RNA was isolated and hybridized to the ATH1 Chip (Data from Killian *et al.* 2007)

The aim of this work was to determine if allantoin accumulation promotes abiotic stress resistance in Arabidopsis. Phenotype and water parameters of wt and ALN knockout mutants were studied under normal and stress conditions. In addition, response of an ALN stress-induced transgenic line was analyzed.

1. Nitrogen source and C/N relation are determinant for allantoin concentration.

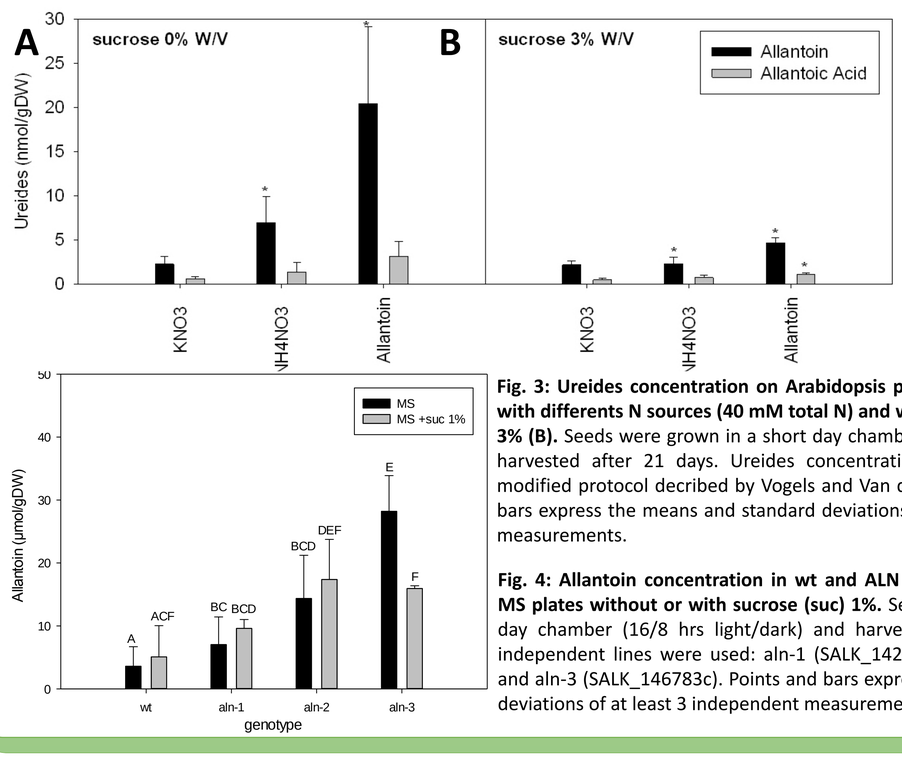

Fig. 3: Ureides concentration on Arabidopsis plants grown on MS plates with differents N sources (40 mM total N) and without (A) or with sucrose 3% (B). Seeds were grown in a short day chamber (8/16 hrs ligh/dark) and harvested after 21 days. Ureides concentration was determined by a modified protocol decribed by Vogels and Van der Drift (1966). Points and bars express the means and standard deviations of at least 3 independent measurements.

Fig. 4: Allantoin concentration in wt and ALN knockout plants grown on MS plates without or with sucrose (suc) 1%. Seedlings were grown in long day chamber (16/8 hrs light/dark) and harvested after 21 days. 3 ALN independent lines were used: aln-1 (SALK_142607), aln-2 (SALK_013427c) and aln-3 (SALK_146783c). Points and bars express the means and standard deviations of at least 3 independent measurements.

2. Allantoin accumulation enhances salt stress tolerance.

To find out whether allantoin improves stress tolerance or not, ALN knockout lines that showed allantoin accumulation were used (aln-2, aln3). They showed better hydric status than wt under salt stress as measured by Water Content (Fig. 5C) and Water Saturation Deficit (Fig. 5D).

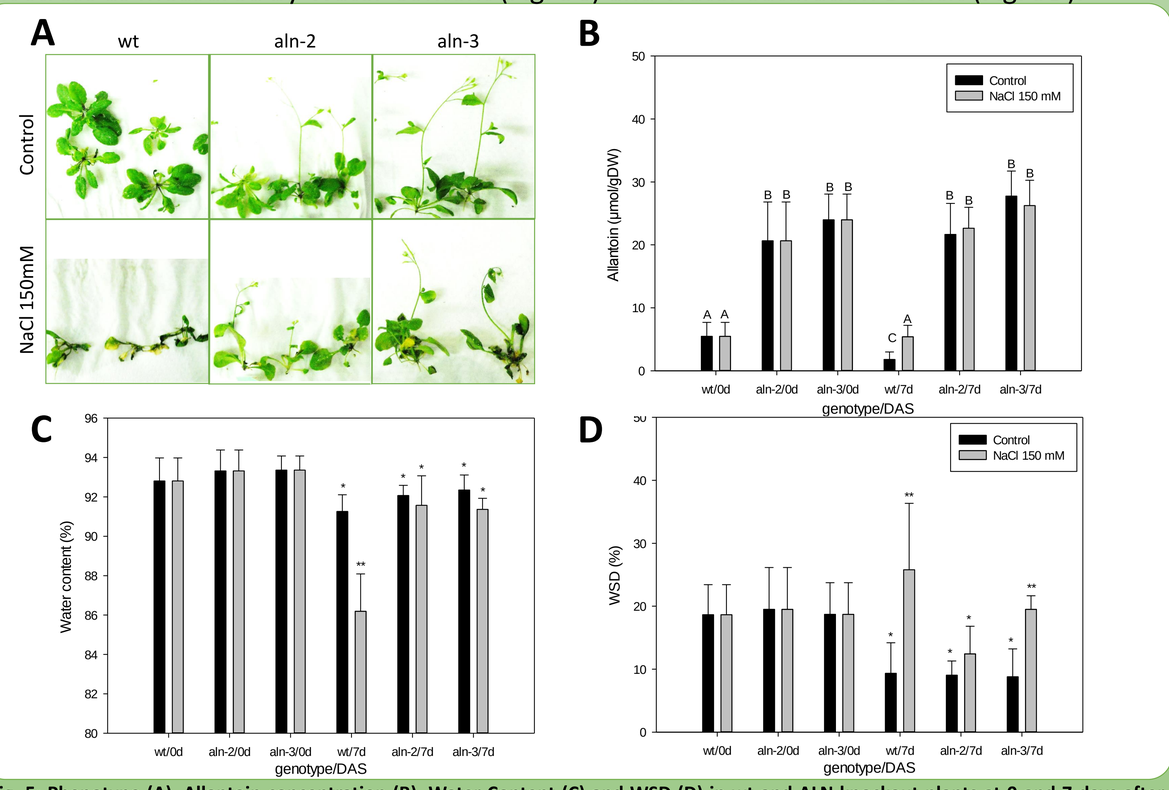

Fig. 5: Phenotype (A), Allantoin concentration (B), Water Content (C) and WSD (D) in wt and ALN knockout plants at 0 and 7 days after Salt Stress (DAS). Seeds were germinated and grown in a long day chamber (16/8 hrs ligh/dark) in plates with MS 0.5X for 21 days. Plants were transferred to an hydroponic system with MS 0.5X for 14 days. Plants were put under Control (MS 0.5X) or Salt Stress (MS 0.5 plus NaCl 150mM) conditions. Material was harvested at 0 and 7 days after stress treatment. Phenotype pictures (A) corresponds to plants 7 days after stress treatment. Allantoin concentration (B), Water content (C) and WSD (water saturation deficit) was also determined (D). Points and bars express the means and standard deviations of at least 5 independent measurements.

3. Down-regulation of ALN gene expression is necessary for plant response to salt stress conditions.

To determine the physiological relevance of allantoinase gene repression on resistance to stress, transgenic lines were generated on the genotype aln-1, in which ALN coding sequence was introduced under the control of the stress inducible promoter RD29A (RD29A::ALN/aln-1). This line showed less growth (Fig. A and C) and reduced germination (Fig. B) than wt under salt stress conditions. However, aln-3 germination was also affected, suggesting another implications of ALN regulation during germination at stress conditions.

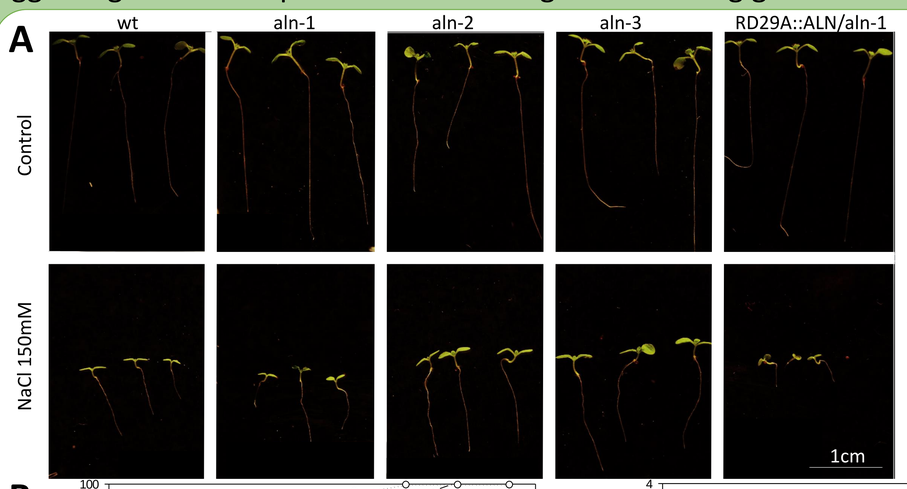

Fig. 6: Phenotype (A), Germination (B) and Root length (C) in wt, ALN knockout plants and a ALN stress induced transgenic line grown at Control and stress conditions. Plants were grown in a long day chamber (16/8 hrs ligh/dark) in plates with MS 0.5X for 8 days. NaCl was added to a final concentration of 150 mM in stress treatment. Points and bars express the means and standard deviations of at least 14 independent measurements.

<u>Conclusions:</u> The presence of NH_4^+ as N source and salt stress conditions both promote allantoin accumulation in Arabidopsis plants. Allantoin accumulation improves water status of plants suggesting a possible function of this compound as a compatible osmolyte. In addition, a putative higher allantoin concentration in transgenic lines promotes increased germination rates and seedling growth.