Introduction

Although the major N forms available to crop plants in most agricultural soils are ammonium and nitrate, urea is found ubiquitously in the soil and additionally represents the most widespread form of nitrogen fertilizer used in agricultural plant production. Due to its fast hydrolysis by urease the concentration of urea in agricultural soils is in general below 70 µM (Gaudin et al., 1987) and most of the urea derived nitrogen is thought to be taken up from the soil solution in the form of ammonium (Marschner, 1995). However, biphasic uptake kinetics of labelled urea in *Chara* cells indicated that membrane transport systems might facilitate the uptake of urea into plant cells (Wilson and Walker, 1988). By heterologous expression of plant genes in yeast and frog oocytes, two different transport systems were identified, that allow the membrane permeation of urea. Several aquaporins of the TIP, NIP and PIP families have been identified as low affinity transport systems (Liu et al, 2003a, Biela et al., 1999), while AtDUR3 encodes a H^+/urea cotransporter (Liu et al, 2003b), that facilitates high affinity urea transport in heterologous systems.

In addition to externally supplied urea, internal production of urea represents an important intermediate of the N metabolism in higher plants. Urea is amongst others increasingly generated during protein degradation, a process of particular importance for the retranslocation of nitrogen during plant senescence. In senescence the earliest and most drastic change in plant cellular structures is the breakdown of chloroplasts, which hold the majority of the leaf protein (Matile, 1992). The degradation of these photosynthetic proteins results in the generation of mostly free amino acids, in particular arginine. This is assimilated in the mitochondria within the ornithine cycle (urea cycle) by degradation into ornithine and urea (Polacco and Holland, 1993; Marschner, 1995). Urease is thereby the only enzyme known in plants that is able to recapture nitrogen from urea, which otherwise would remain an unavailable N source. It has been localized to the cytoplasm (Polacco and Holland, 1993) and was detected in particular in generative tissues of plants (Hogan et al., 1983), although it seems to be synthesized in almost all organs (Holland et al., 1987). The product of the urease reaction, ammonia, is then assimilated via the GS-GOGAT (glutamine synthetase-glutamate synthase) pathway into glutamine (Marschner, 1995). To date it is unclear if urea also has a meaning for short-term storage and long-distance transport of N.

Methods

Arabidposis thaliana plants were grown hydroponically under controlled conditions to allow changing nitrogen regimes and applying different N forms and to maintain intact roots for uptake studies. By applying coloured threads to leaves their age could be defined at any given time point. Additionally, axcenic conditions were applied to exclude urea degradation by microorganisms. Two independent T-DNA insertion lines lacking *AtDUR3* gene expression were used to quantify the contribution of AtDUR3 to urea transport. In order to visualize the promoter activity in roots and shoots transgenic lines expressing *AtDUR3*^{pro}*::GFP* and *AtDUR3*^{pro}*::GUS* were generated. Urea concentrations were measured by a colorimetric assay allowing high sensitivity down to micromolar ranges. Northern and western blot analyses were carried out to investigate regulatory mechanisms.

Results and Discussion

The physiological characterisation of the urea transporter AtDUR3 in *Arabidopsis thaliana* revealed that it is localized at the plasma membrane of the rhizodermis, including root hairs, as well as the cortex in more basal root zones under nitrogen deficiency. The two independent *atdur3* T-DNA insertion lines showed impaired growth on urea as a sole nitrogen source. Uptake studies using ^{15}N-labeled urea confirmed that AtDUR3 represents the major transporter for high-affinity uptake of urea with a Km of 4µM, suggesting that the high

substrate affinity of AtDUR3 reflects an adaptation to the low urea levels usually found in soils.

In leaf samples urea concentrations of different plant and leaf age showed marked differences in urea concentrations after plants turned into generative growth, with lower urea concentrations in younger or sink leaves and higher concentrations in leaves of more advanced leaf or plant age. In parallel, the mRNA abundance of *AtDUR3* increased with leaf age. Additionally, transgenic *AtDUR3*-promoter-GUS lines indicated a localization of the *AtDUR3* promoter activity in the vascular bundle of old leaves, suggesting a function of AtDUR3 in nitrogen retrieval during senescence as well as an involvement of AtDUR3 in maintaining elevated urea concentrations. Indeed, a contribution of AtDUR3 to increased urea concentrations was evident by the analysis of *atdur3* T-DNA insertion lines. As an easily soluble molecule containing 46% nitrogen, urea might represent a suitable candidate for intra- or intercellular nitrogen transport.

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