726(S814)

EXPRESSION OF 11 NITRATE TRANSPORTER (*AtNRT*) GENES IN *ARABIDOPSIS THALIANA* <u>Mamoru OKAMOTO</u>¹, J. J. VIDMAR², A.D.M.GLASS¹ ¹Dept of Botany, University of British Columbia, Vancouver, B.C. CANADA V6T 1N4 ²Agrigenomics, Edmonton, AB, CANADA, T6G 2E1

Higher plants possess both high- and low-affinity nitrate transporters. Since the first Arabidopsis thaliana low-affinity nitrate transporter gene (AtNRT1.1) was isolated in 1993, three low-affinity, and seven high-affinity nitrate transporter gene homologues (AtNRT2) have been identified in this species. We investigated the transcript abundance of all eleven genes in response to NO₃ provision, by relative RT-PCR. Each gene showed different levels and/or expression patterns. AtNRT2.1, 2.2, and AtNRT1.1 were highly induced by 1 mM NO3, peaking at 3 to 12 hours, and declining during 3 subsequent days. AtNRT2.5 transcript was the least abundant among the NRT2 family in roots, but the most abundant in shoot tissues. Shoot and root AtNRT2.7 mRNA levels, which were moderate under non-inducing conditions, were strongly suppressed by nitrate provision. AtNRT2.3, 2.4, and 2.6 showed a constitutive pattern of transcript abundance in roots and shoots. The localization of members of the AtNRT2 family, by promoter::uidA fusions, will be demonstrated.

727(S815)

FUNCTIONAL ANALYSIS OF LOW-AFFINITY SULFATE TRANSPORTER Sultr2;2 IN ARABIDOPSIS

Hideki TAKAHASHI¹, Naoko YOSHIMOTO², Tomoyuki YAMAYA^{1.3}, Kazuki SAITO² ¹RIKEN Plant Science Center, ² Chiba Univ. Fac. Pharm. Sci., ³ Tohoku Univ. Grad. School Agr. Sci.

Sulfate transporter gene family of Arabidopsis is consisted of 11 isoforms classified into 4 distinct groups by their functions. Sultr2;2 belongs in the group of the low-affinity, vascular tissue-specific isoforms. Sultr2;2 mRNA was accumulated both in leaves and roots under sulfur-limited growing conditions. Transgenic Arabidopsis plants expressing the fusion gene construct of the 5'-region of Sultr2;2 and green fluorescent protein (GFP) showed accumulation of GFP in the phloem of roots and bundle sheath cells of leaf vascular tissues. Antisense suppression of Sultr2;2 caused overaccumulation of Sultr3;3 mRNA in leaves suggesting a possible compensatory role of Sultr3;3 for the loss of sulfate transport activities driven by Sultr2;2. Cell typespecific expression of Sultr3;3 is studied to identify its function.

728(S816)

CHARACTRIZATION OF CHROLOPLASTIC SULFATE TRANSPORTERS IN *ARABIDOPSIS* <u>Akiko TAKAHASHI</u>¹, Kazuki SAITO², Tomoyuki YAMAYA^{1,3}, Hideki TAKAHASHI¹ (RIKEN Plant Science Center¹; Chiba Univ. Fac. Pharm. Sci.²; Tohoku Univ. Grad. School Agr. Sci.³)

Multiple isoforms of sulfate transporters are responsible for distribution of sulfate between different cell compartments in plants. Among the 11 isoforms of the sulfate transporter gene family, Sultr4; 1 encoded an unique transporter localizing in the chloroplast. Recently, we have isolated Sultr4;2 as a homologue of Sultr4;1 in Arabidopsis genome. Sultr4;2 cDNA encoded a 677 amino acids polypeptide showing similarities with predicted ORFs for higher plant-type sulfate transporters in cyanobacteria and algae. The N-terminus region of the protein was predicted to have signal peptides for chloroplast localization. Sultr4;2 mRNA was abundantly accumulated under low sulfate conditions. The increase of mRNA levels of Sultr4;2 by sulfur limitation was stronger than in the case of Sultr4; 1. Organelle and tissue specificity of Sultr4;2 is studied using green fluorescent protein (GFP) as a reporter.

729(S817)

CHARACTERIZATION OF SODIUM DEPENDENT SULFATE TRANSPORTER HOMOLOGUE FROM *Arabidopsis*

Motoko AWAZUHARA¹, Hideki TAKAHASHI², Asuko KIMURA¹, Kazuki SAITO¹ (¹ Chiba Univ. Fac. Pharm. Sci., ² RIKEN Plant Science Center)

In higher plants, multiple isoforms of sulfate transporters are responsible for the uptake and distribution of the sulfate anion. We have isolated an Arabidopsis cDNA (ASST11) encoding a homologue of rat sodium dependent sulfate transporter. ASST11 encoded a 540 amino acid polypeptide that was predicted to be a membrane-bound transporter with 12 membrane spanning domains. However, ASST11 was unable to complement the yeast mutant CP154-7A lacking two sulfate transporter genes. ASST11 mRNA was accumulated both in roots and leaves of Arabidopsis, and the expression was slightly induced under sulfur deficient condition. A fusion gene construct carrying the coding sequence of ASST11 in the antisense direction under CaMV 35S promoter was introduced in Arabidopsis plants. Growth was not affected by suppression of ASST11 both under normal and sulfur deficient conditions. Fluctuation of metabolite levels and mRNA levels of sulfur assimilation genes are characterized in these antisense transgenic plants.