

ABSTRACTS (MASTER THESIS)

Purine permeases of *Coffea canephora*, CcPUP1 and CcPUP5, are involved in the uptake of adenine

**(Graduate School of Agriculture,
Laboratory of Plant Gene Expression, RISH, Kyoto University)**

Hirobumi Kakegawa

Purine permeases (PUPs) are a large plasma membrane-localized transporter family in plants. These transporters have been shown to function in the proton-coupled uptake of various nucleotide bases and their derivatives, including adenine, cytokinins, and nicotine. Arabidopsis PUP1 was the first member of PUP family identified as the gene which complements the growth of a yeast mutant of adenine uptake. It was shown that the adenine uptake activity of AtPUP1 is inhibited by carbonyl cyanide m-chlorophenyl hydrazine (CCCP). These results suggest the function of AtPUP1 as proton symporter. The adenine uptake activity was shown to be competitively inhibited by the addition of purine derivatives such as cytokinins and caffeine. These results implicate that AtPUP1 mediates the uptake of a broad range of substrates. Arabidopsis contains 21 PUP members in the genome. To date, only four PUP genes have been analyzed.

Rice (*Oryza sativa*) contains 12 PUP family proteins. However, only one member, OsPUP7, was characterized. OsPUP7 was shown to be involved in growth and development. Direct transport assay was not shown, but this transporter possibly mediates the cytokinin transport. Although the direct transport activity is not measured as well, OsPUP7 conferred yeast the sensitivity to caffeine. These results suggest the caffeine uptake activity of OsPUP7 [1].

Nicotine and caffeine are nucleotide base derivatives found in plants. The nicotine uptake activity was reported in a tobacco PUP, NtNUP1 of *Nicotiana tabacum*. NtNUP1 was reported to be involved in the uptake nicotine from the apoplast. Direct uptake activity for nicotine was measured in yeast. NtPUP1 is also shown to take up pyridoxamine, pyridoxine, anatabine, in addition to nicotine.

In this study, we hypothesized that purine permeases of *Coffea canephora* have the ability to take up caffeine in addition to adenine. We have identified 15 members of PUP protein in the genome of *C. canephora*. Based on the yeast sensitivity assay, we selected two candidates, i.e. CcPUP1 and CcPUP5. Direct transport assay was conducted with radioactive compounds. It was shown that both CcPUP1 and CcPUP5 are adenine uptake transporters, but not caffeine uptake transporters. Inhibition assay indicated that this transporter was not inhibited by the excess amount of caffeine. We thought insensitivity to caffeine is important for *C. canephora* PUPs, because adenine uptake transporters of *C. canephora* need to distinguish adenine from caffeine to effectively uptake adenine in cells containing high concentration of caffeine. It can be suggested that purine permeases in origin had the uptake ability for both adenine and caffeine, and that these proteins have been evolved to distinguish adenine from caffeine in plants synthesizing caffeine such as *C. canephora*.

Reference

[1] Kakegawa, H., Shitan, N., Kusano, H., Ogita, S., Yazaki, K., Sugiyama, A. Uptake of adenine by purine permeases of *Coffea canephora* Bioscience, Biotechnology, and Biochemistry (in press).