【別紙２】

論 文 名 : Studies on physiological function of *Oryza sativa* nucleotide pyrophosphatase/phosphodiesterase 1
(イネ nucleotide pyrophosphatase/phosphodiesterase 1 の生理機能に関する研究)

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（以下要約を記入する）

Nucleotide pyrophosphatase / phosphodiesterasee (NPP) is a widely existing enzyme in both mammals and plants, which catalyzes the reaction of pyrophosphate and phosphodiester bonds of nucleotides and sugar nucleotides. Mammalian NPP is known to be involved in nucleotide signaling, cell differentiation, nucleotide recycling and concentration adjustment. The details of the physiological functions of NPP in plants are still obscure. In this study, I clarified the enzymatic properties of rice NPPs, and created and analyzed *npp1* mutants in order to elucidate the physiological functions of NPP 1 in plants.

In previous studies, *NPP* genes (*NPP1* - *NPP6*) corresponding to six cDNAs have been identified from rice shoot cDNA libraries using rice *NPP1* cDNA as a probe. For elucidation of the function of NPP, NPP1, NPP2, and NPP6 enzymes were purified from the culture cells of transgenic rice, and their enzyme characteristics were analyzed. Whereas NPP1 and NPP6 recognized nucleotide sugars, NPP2 did not recognize these compounds as substrates but preferentially hydrolyzed UDP, ADP, and adenosine 5′-phosphosulfate (APS). NPP1 best hydrolyzed ADP-glucose, ADP-ribose, and ATP, while ADP and ADP-glucose were the best substrates for NPP6.

In order to clarify the physiological functions, *Tos17* inserted *npp1* mutant was generated and analyzed. In the seedling shoots of npp1 mutant, the hydrolysis activity to ADP-glucose was reduced to 8% of the wild type (WT), indicating that NPP1 functions as a major ADP-glucose hydrolyzing enzyme in rice plant. Changes in ADP-glucose levels are known to affect starch biosynthesis. When the starch contents in leaves of *npp1* mutants and transgenic plants with overexpression of *NPP1* were measured, it was found that the starch contents varied depending on the expression level of *NPP1*. Thus, it was strongly suggested that *NPP1* is involved in biosynthesis of starch as a physiological function.

Global warming is one of the most serious environmental issues in recent years. In order to investigate the influence on the growth physiology of rice by the disruption of *NPP1*, we performed analyses using thermography and photosynthesis measuring equipment. The plant body temperature in *npp1* mutant was lower than WT and the stomatal conductance of leaves increased. The photosynthetic rate also increased in the *npp1* mutants, and it was significantly increased in the mutant even in the same intercellular CO_2 concentration (C_i), indicating that the *npp1* mutant has a

mechanism capable of performing efficient photosynthesis. When this npp1 mutant was cultivated under high CO_2 condition of 28℃ / 23℃ and 160 Pa, the plant growth and starch accumulation were promoted in comparison with those of WT. A similar phenomenon was observed even under high temperature conditions (33℃ / 28℃, 160 Pa). From these results, it was strongly suggested that the functional deficiency of the *NPP1* gene lowers the plant temperature by opening the stomata, promotes photosynthesis, and causes the accumulation of starch and sucrose.

To characterize changes in the proteome of npp1 leaves under high temperature + high CO2 (HT + ECO2), I carried out a quantitative proteomic analysis. Proteins extracted from leaves of WT and npp1 plants grown under normal (28/23 ℃ and 40 Pa CO2) and HT + ECO2 (33/28 ℃ and 160 Pa CO2) conditions were labeled by iTRAQ (isobaric tag for relative and absolute quantitation), followed by tandem mass spectrometry analysis. Using this approach, 103 differentially expressed proteins were successfully identified among 1701 detected proteins in total. The general trend indicates that the response of the npp1 mutant to the HT + ECO2 treatment is due, at least partly, to changes in the expression of proteins from the following groups: photosynthesis, carbohydrate metabolism, protein synthesis, and signaling. In contrast, most of the proteins in WT were not induced with HT + ECO_2.

Furthermore, the expression of 14-3-3 protein interacted to various signaling proteins such as kinase and phosphatase was also increased in the *npp1* mutant. 14-3-3 proteins have been localized to the chloroplast stroma and the stromal side of thylakoid membranes, thereby implicating a potential role in starch regulation. It has been reported that many proteins are phosphorylated in the chloroplast, and they are related to the light - dark reaction of photosynthesis. Phosphoproteome in isolated chloroplasts were analyzed, the results showing that chlorophyll a / b binding protein was significantly phosphorylated in the *npp1* mutant under HT + ECO_2. I consider that the high phosphorylation status in *npp1* under HT and ECO_2 could be related to the activation of growth and carbohydrate accumulation of the rice plant.