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Nitric Oxide –mediated inhibition of OsDHODH1 gene expression is independent to the Cytochrome c Oxidase activity in rice

Nkulu Kabange Rolly[1], Bong—Gyu Mun[1], Byung-Wook Yun[1*]

[1]Laboratory of Plant Functional Genomics, School of Applied Biosciences, Kyungpook National University, Daegu, South Korea

[Introduction]

Nitric oxide (NO) interacts with the plant mitochondrial electron transport chain (ETC) at the cytochrome c oxidase (COX). The plant DHODH links physically de novo pyrimidine biosynthesis pathway to the mitochondrial respiratory chain (MRC) via the ETC. NO was shown to have a high affinity with the CcOX, which is also the primary site for oxygen binding and consumption. A late in vitro enzymatic assay supported that inhibition of the CcOX responsible for reduction of O2 to H2O by NO resulted in indirect suppression of DHODH activity. However, the mechanism underlying the inhibition of the plant DHODH by binding of NO to the CcOX is poorly understood. In this study, we monitored by qPCR the transcriptional level of OsDHODH1 in relation with the activity of two main subunits of the CcOX containing the binuclear center where NO and O2 competitively bind.

[Materials and Methods]

Expression of the nitrate reductase related genes OsNIA1 and OsNIA2, OsNOE1 encoding the rice catalase, was measured as well in roots of different rice cultivars upon their exposure, for 1hour, to 1mM SNP-induced nitrosative stress at seedling stage. Our finding revealed that short term NO application profoundly resulted in significant downregulation of OsDHODH1 expression in rice. In contrast, two principal CcOX subunits, OsCOX1 and OsCOX5B, were not suppressed by NO; which would indicate a high competition of O2 and NO for binding to the binuclear center of the CcOX or a CcOX-independent inhibitory effect of OsDHODH1 by NO. Furthermore, expression of OsNIA1 and OsNIA2 were both upregulated, with OsNIA1 highly induced as compared to OsNIA2. Due to high affinity of NR genes with NO, high accumulation of SNO would be expected. In silico analysis using GPS-SNO 1.0 algorithm revealed a possible binding of NO to the Cysteine residue -196 of OsDHODH1. Thereby, supporting the hypothesis of protein S-nitrosylation

[Results and Discussions]

Owing to the above results, this study suggests that inhibition of OsDHODH1 by NO would be a result of direct interaction, rather than indirect relationship through the cytochrome c oxidase.

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*Corresponding author: Tel. +82-51-950-5712, E-mail. bwyun@knu.ac.kr