Nitrogen metabolism

483(3pI01)

REDUCTION OF NITRATE AND NITRITE IN TRANSGENIC PLANTS THAT HAVE HIGHLY REDUCED NITRITE REDUCTASE ACTIVITY(4)

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Nitrite reductase (NiR) is the second enzyme in the primary assimilation pathway of nitrate. However, we have found that transgenic plants that have much reduced NiR activity still had substantial ability (40 to 60% of that of wild type) to assimilate nitrogen dioxide. Here we report the results obtained by nitrite reductase activity staining and immunoblot analysis.

Transgenic tobacco plants (H. Vaucheret et al. Plant Journal, 2: 559-569,1992) that express the antisense RNA of nitrite reductase gene were grown for nine weeks and used here. The leaves were frozen in liquid nitrogen and homogenized in extraction buffer. The homogenate was centrifuged and the resulting supernatant was used as crude enzyme extract. After native PAGE, the gels were subjected to nitrite reductase activity staining using methylviologen as an electron donor and to immunoblot analysis using an antibody anti spinach NiR. Two bands appeared in the native PAGE gel stained NiR activity, one of which appeared to comigrate with NiR polypeptide.

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ANALYSIS OF COMPOUNDS BEARING UNIDENTIFIED NITROGEN DERIVED FROM NITROGEN DIOXIDE ABSORBED IN PLANT LEAVES

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Plant leaves can absorb and metabolize nitrogen dioxide (NO_2) gas in atmosphere. We have discovered that the metabolic fate of NO_2 nitrogen (NO_2-N) in the leaves somewhat differs from that of nitrate. Twenty to 30% of the total NO_2-N is converted into unidentified nitrogen (UN) compounds which are not digestible and/or recoverable by the Kjeldahl method. In this study, we aimed at identification of UN-bearing compounds from the extract of leaves fumigated with NO_2.

About 5-week-old Arabidopsis thaliana ecotype C24 seedlings were fumigated with 4.0±0.4 ppm $^{15}NO_2$ (51.6 atom% ^{15}N) for 4 hours in the light of 70 $\mu Em^{-2}s^{-1}$ and 340±80 ppm CO_2 concentration at 22.0±0.3°C and relative humidity of 70±4%. Then, the leaves were harvested, freeze-dried and ground into powder. The leaf powder was homogenized, and the homogenate was centrifuged. The supernatant fraction was separated by an FPLC using a Superdex 75 HR 10/30 and a Dowex 50W column and HPLC using a Zorbax BP-CN column. An HPLC fraction (peak 1) was further separated by a TLC on a Funacel SF cellulose plate. We are currently analyzing ^{15}N-enriched fractions.

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ANALYSIS OF GENES INVOLVED IN THE METABOLISM OF NO_2 IN ARABIDOPSIS THALIANA

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Plants can absorb and metabolize nitrogen dioxide (NO_2) gas, but little is known about the genes involved in the metabolism of this nitrogen oxide. We have been investigating such genes using two T-DNA tagged *Arabidopsis thaliana* plant lines; activation-tagged lines and enhancer-trap lines.

To obtain the plant having high ability to absorb NO_2, about 5-week-old T-DNA tagged plants were fumigated with 0.1ppm ^{15}N-labelled NO_2 in the light for 1 hour, after which a leaf strip was harvested, dried and analyzed for the ^{15}N content (atom% excess) using an EA-MS as described previously (1).

To obtain the gene induced with NO_2 gas fumigation, mutant plants (Arabidopsis Biological Resource Center at the Ohio State Univ .) that contain promoter-less β-glucuronidase (GUS) gene, were similarly fumigated with NO_2, after which leaves were harvested and analyzed for GUS activiy. Results will be presented and discussed.

(1) Morikawa et al., *Plant Cell Environ.*, 21: 180-190 (1998)

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ANALYSIS OF TRANSGENIC RICE EXPRESSING ANTISENSE RNA FOR CYTOSOLIC GLUTAMINE SYNTHETASE

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We have suggested from localization studies that cytosolic Glutamine Synthetase (GS1) is important in the export of glutamine from the senescing leaves. To obtain more direct evidences, antisense methods were adopted to reduced the content of GS1 in vascular bundles. A fragment of GS1 cDNA was fused with thioredoxin h promoter in the antisense orientation, and the chimeric construct was introduced into rice calli with Agrobacterium-mediated methods. Resulting 14 lines of transformant at T0 generation were analyzed.

Some of these transformants, confirmed the gene transfer by Southern analysis, showed retardation of plant growth and leaf senescence.Transformants contained 30-80% less GS1 content in senescing leaf blade than the wild-type, on a basis of soluble protein. these observed a negative correlation between the content of GS1 protein and the content of either Rubisco or total N, suggesting that the presence of less GS1 protein causes delay of nitrogen export and hence delay of leaf senescence. Analysis of amino acid composition in phloem sap is now in progress.