E201

## Characteristics of the Genes of Blue Copper-Binding Protein II & III in *Arabidopsis thaliana*

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We obtained partial clones encoding blue copper-binding protein(BCB) and nitrite reductase(NiR) in PCR reaction of genomic DNA by using degenerated primers for nir of higher plants. By using partial bcb as a probe, genomic DNA blot analysis in low stringent hybridazation condition(55°C) showed that genes of bcb are multigene family consisting of at least 3 copies. Searching for other clones like bcb in the database of expressed sequence tags in National Center for Biotechnology Information, it was confirmed that there are at least 5 kinds of clones in Arabidopsis sequence analysis of two clones obtained from in these, further Arabidopsis Biological Resource Center showed that there are copper-binding site and amino acid sequence having homology on bcb. These clones were named bcb//(U57320) and bcb///(U65650), genes of copper-binding proteins with 200 aa and 221 aa respectively. bcb showed 50% homology with bcbll, 38% with bcblll in terms of amino acid sequences. Especially, bcbll and bcblll had 57% homology. We isolated genomic clones of bcbll and bcbll showing 2 exons and 1 intron. It is conceivable that bcb // and bcb /// with transit peptide move into organelles.

E202

## Activation of Nitrite Reductase by Light & Nitrate in Hot Pepper ( Capsicum annum L.) Seedling

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Two Key enzymes of nitrate(NO<sub>3</sub><sup>-</sup>) assimilation, nitrate reductase (NR) and nitrite (NO<sub>2</sub><sup>-</sup>) reductase (NIR) are regulated by environmental and metabolic conditions. In plant the major factors of activation of these enzymes are light and nitrate. In oder to study nitrate assimilation in hot pepper, we isolated a *nir* partial clone in hot pepper by degenerative polymerase chain reaction. DNA fragment of 581-bp was obtained and sequenced. The deduced amino acid sequence was highly homologous with that for other plants (PCC7942, kidneybean, corn, tobacco, spinach). Genomic DNA blot analysis revealed that NIR is encoded by a small multigene family and this result supported by NIR-electrophoretic assay showed that NIR have more than two isoforms. By sodium dithionite assay, light and nitrate dependent activation of this enzyme was tested. It is under investigation whether light and nitrate affects *nir* transcripts level by through the RNA blotting assay.