E207 Ca^{2+}/calmodulin-dependent Activation of Glutamate Decarboxylase and Nicotinamide Adenine Dinucleotide Kinase Isolated from Tobacco Plants

Suk-Heung Oh* and Song Joong Yun[1]
Department of Biotechnology, Woosuk University,
[1]Faculty of Biological Resources Science, College of Agriculture,
Chonbuk National University

To test how calmodulin and calmodulin methylation affect the activation of glutamate decarboxylase (GAD) and nicotinamide adenine dinucleotide (NAD) kinase, GAD and NAD kinase were partially purified from tobacco plants. GAD was also partially purified from *E. coli* transformed with a plasmid carrying a cloned tobacco GAD gene. We find that GAD from the transformed *E. coli* showed 60-fold Ca^{2+}/calmodulin-dependent activation. However, GAD from tobacco plants was stimulated only about 3.8-fold by the addition of calmodulin in the presence of calcium, suggesting high background activity of the enzyme was possibly due to bound endogenous tobacco calmodulin. A monoclonal antibody against petunia GAD interacted strongly with both GAD from tobacco plants and GAD from cloned gene. NAD kinase from tobacco plants showed a complete Ca^{2+}/calmodulin dependency for activity. Unmethylated calmodulins activated GAD in a manner similar to methylated calmodulin. However, the maximum level of NAD kinase activation obtained with unmethylated calmodulins is approximately 4-fold higher than methylated calmodulins. These data suggested that endogenous tobacco calmodulin may interact more tightly with GAD than NAD kinase and that calmodulin methylation affects the activator properties of calmodulins for tobacco NAD kinase but not for GAD.

E208 Induction of Multiple Shoots and Transformation by *Agrobacterium tumefaciens* from the Leaf of *Taraxacum mongolicum* Hand.

Sang Eun Yeo* and Kwang Soo Roh
Department of Biology, Keimyung University

A high frequency regeneration system *in vitro* and genetic transformation in dandelion (*Taraxacum mongolicum* Hand.) were studied. Leaf explants of dandelion were inoculated to induce the multiple shoot and organ formation using various concentrations of IAA and BA on MS medium. The multiple shoots of dandelion were obtained from leaf explants which were cultured on MS medium containing 1 μM IAA and 1 μM BA after 2 weeks, and plantlets could be transferred to soil for further growth. Leaf explants of dandelion were cocultured with *Agrobacterium tumefaciens* LBA4404 harboring a binary vector pBI121 carrying the CaMV 35S promoter-GUS gene fusion as a reporter gene and NOS promoter-NPT II gene as a positive selection marker for 10 mins, then transferred to MS medium containing 1 μM IAA, 1 μM BA, 100 μg/mℓ carbenicillin and 50 μg/mℓ kanamycin sulfate. After two weeks of subculture of the explants, kanamycin-resistant shoots were formed on explants survived. The transformants showed high levels of the GUS-positive activity by using the GUS histochemical. assay.