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# Development of Efficient Plant Transformation System for Orchardgrass

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## Objectives

Grassland agriculture is highly dependent upon a reliable source of forage as the primary feed base for ruminant livestock. Forage plant breeding has been largely based on phenotypic selection following sexual recombination of natural genetic variation found within ecotypes. Biotechnology offers opportunities to enhance the sources of useful genes accessible for development of new cultivars. Orchardgrass (*Dactylis glomerata* L.) is one of the most widely cultivated forage grass in Korea. We are interested in developing molecular breeding methods to improve its abiotic stress resistance and nutritional quality. To examine whether the introduction of *AtNDPK2* cDNA into the genome of orchardgrass could enhance tolerance to multiple environmental stresses, transgenic orchardgrass was generated.

## Materials and Methods

1. Plant material and transformation: Embryogenic calli were induced from mature seeds of orchardgrass. Embryogenic calli were infected with *Agrobacterium* for 10-30 min, and vacuum was applied for 1 to 5 min. *Agrobacterium tumefaciens* strain, EHA105 containing plasmid carrying *AtNDPK2*, a multiple stress resistance gene, under the control of CaMV35S promoter in the T-DNA region was used.
2. Selection and regeneration: After 3 days of co-cultivation in the dark, calli were subjected to 2 cycles of selection on the MS-based regeneration medium containing 1 mg/l 2,4-D, 3 mg/l BAP, 50 mg/l kanamycin, 300 mg/l cefotaxime and 30 g/l maltose. Regenerated plantlets were isolated and transferred to rooting medium.

## Results and Discussion

1. Highly embryogenic calli were obtained in the callus induction medium supplemented with 3 mg/l dicamba. Addition of maltose to the regeneration medium resulted in higher regeneration efficiency.
2. Vacuum treatment during *Agrobacterium* infection enhanced transformation efficiency of embeyogenic calli.
3. PCR analyses with genomic DNAs of transgenic plants revealed the integration of *AtNDPK2* gene into the genome of transgenic plant. Study on the effects of constitutive expression of the *AtNDPK2* gene in orchardgrass is currently in progress.

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