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# Osmotic Stress Tolerance of Transgenic *Panax ginseng* Through the Introduction of the Glyceraldehyde-3-Phosphate Dehydrogenase Gene

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## Objectives

*Panax ginseng* is one of important medicinal plants in the world. The cultivation of ginseng is troublesome and a period of 4-6 years is required to harvest the roots. Red skin disease and symptom of repeated cultivation were important problem to produce the good quality of ginseng roots, which is caused from the accumulated salts in soil by over accumulation of fertilizer in the ginseng cultivation field. In this study, Transgenic *Panax ginseng* plants were produced by introducing the glyceraldehydes-3-phosphate dehydrogenase (GPD) gene. We confirmed that the over-expressed GPD gene creates osmotic stress tolerance in transgenic ginseng plants.

## Materials and Methods

1. Plant materials: *Panax ginseng*, embrygenic callus
2. Embrygenic callus induction: MS+1.0 mg/l 2,4 D+3% sucrose + 1% agar
3. Transformation and regeneration of transgenic plants: We obtained transformed callus in the selection medium: MS basal salt, 25 mg/l kanamycin, 300 mg/l cefataxime, 1.0 mg/l 2,4-D. and regenerated plants in the HF medium.
4. Analysis of transgenic plantlets: PCR analysis and Southern blot.
5. Bioassay for osmotic stress tolerance: Leaf and petiol explants were treated with 0.5 M of sucrose or sorbitol.

## Results and Discussion

1. Embrygenic callus induction: Koera ginseng seeds were sterilized with 1% sodium hypochlorite solution. After careful dissection of the zygotic embryos from the seeds, *P. ginseng* cotyledons were cultured on MS medium with 1% agar, 3% sucrose and 1.0 mg/l 2,4-D. The embyogenic callus was induced from the ginseng cotyledons after 1 month.
2. Genetic transformation: Embryogenic callus was co-cultivated with *A. tumefaciens* on MS agar medium containing 1.0 mg/l 2,4-D and 3% sucrose for 3 days. To select transformed embrygenic callus, each callus was transferred onto the medium with 300 mg/l cefotaxime and 25 mg/l kanamycin for three weeks and transferred to the hormone-free selection medium (300 mg/l cefotaxime and 25 mg/l kanamycin) and sub-cultured three times to the same medium.
3. Analysis of transgenic plantlets: A total of 25 putative plants was obtained and examined by PCR analysis. NPT II and GPD genes were detected in 15 transgenic plantlets. Southern hybridization analysis was carried out on the PCR positive transgenic plantlets. All of these plantlets showed a hybridization band with the NPT II probe.
4. Bioassay for osmotic stress tolerance: To test whether or not transgenic ginseng with GPD gene gained enhanced tolerance to osmotic stress, healthy leaf and petiole of wild-type or transgenic in vitro grown plants cut and then treated with 0.5 M of sucrose or sorbitol for 12 h. The treated explants were re-hydrated by half-dilution in water at 5-min intervals. The leaf of wild-type plants exhibited severe necrosis all over the surface, and died after 3 d, whereas the transgenic plants were tolerant against the osmotic stress.

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