P 24 Transformation of Barley (*Hordeum vulgare*) Using Mature Embryos

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Objectives

We have introduced a freezing tolerance-related gene (CLP: chitinase like protein) into the callus derived from mature embryos of Saechalsalbori. The transgenic plants were regenerated stably and analyzed further for any increase in freeze tolerance. The results will be reported in this.

Materials and Methods

1. Materials:
   - plant: Saechalsalbori
   - vector: pMJ21 (from MyungJi University), pMJU, pMJRD
   - medium: DBC2 (MS medium supplemented with 30g/l of maltose, 2.5mg/l of 2,4-D, 0.1mg/l of BAP, and 5.0 μM of CuSO4)
   - Freezing tolerance-related genes: CLP, Blt101, GLP, TLP, BCBF1 (from Dr. D. W. Choi)
2. Methods: Regeneration was done by following the protocol of Plant Science 138, Myeong-Je, Cho (1998) with minor modificaton and transformation of embryogenic calli was performed using the biolistic PDS-1000/He (Bio-Rad).

Results and Discussion

We introduced pMJUCLP containing a CLP gene, a tentative antifreeze gene, under the control of the maize ubiqutin promoter and terminated by nos into barley embryogenic calli. The embryogenic calli were derived from 200 mature embryos and cultured under dark condition for 1 month before particle bombardment. On 2 days after transformation, calli were transferred on the selection medium (DBC2 with 5mg/l PPT) and grown for 20 days. The embryogenic callus showing the features of shiny, compact and nodular structures was selected individually from the scutellum and cut into small pieces (about 3~4mm) and transferred onto fresh medium. They were allowed to grow under light condition. After additional 3 weeks, green-callusing sectors were appeared and maintained on DBC2 with 5mg/l PPT. The 15 cell lines survived under the selection were picked and transferred onto FHG regeneration medium and grown for 1 month. It turned out that only 8 transformants lines out of 15 were successfully regenerated. 6 plants were regenerated fully after transferring of the 8 transformants onto rooting medium (DBC2 without phytohormone). Overall 3% of transformation efficiency was achieved in this trial. The tentative transgenic plants are being confirmed by showing the presence of a bar gene and tested for any increase in freezing tolerance.

Besides, transgenic plant having an empty vector control (pMJU) was also generated as control. At this point we are conducting other sets of transformation with genes for Blt101, BCBF1, GLP, TLP under the control of the promoter from either maize ubiqutin or *Arabidopsis* rd29A.

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