Transformation of *Artemisia adamsii, Endemic to a Gobi Desert,* with CLP, Dhn5 to Enhance a Tolerance against Environment Stresses.

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Objectives

Freezing and drought tolerance in plants are very important for survival in the desert. In an effort to reduce desertification in Gobi, a molecular breeding of *Artemisia adamsii* using the CLP(chitinase like protein, antifreeze protein) and Dhn5(dehydrin5) genes from barley(*Hordeum vulgare* L.) is being performed by constructing those genes in pGA748 under 35S promoter and introducing them into *Artemisia adamsii* via *Agrobacteria.*

Materials and Methods

Materials - plant : *Artemisia adamsii*
medium : MSA(modified MS medium with NAA, BA added),
MSAR(regeneration medium)

Methods - *Agrobacterium*-mediated Transformation, PCR, Callus culture, SDS-PAGE, Western analysis, PEG test, Ion leakage test

Results and Discussion

We had found the optimal hormone combination of NAA 0.05mg/L and BA 0.5mg/L for the best growth of callus in *Artemisia adamsii.* In addition, the highest rate of callus induction was observed with hypocotyl. as an initial explant to start with. The CLP and Dhn5 gene were constructed in pGA748 and introduced into *Agrobacterium*(LBA4404). The explants of leaf, stem, and root from 14 weeks-old seedling of *Artemisia adamsii* were cocultivated with *Agrobacterium tumefaciens* and several transgenic cell lines were stably established.

An introduction of the CLP gene was confirmed by PCR with CLP primer as well as NPRII primer showing the expected bands of 430 bp and 700 bp, respectively. Higher levels of CLP, Dhn5 expression were detected in western analysis with the transgenic callus lines than nontransgenic callus. There were variations in the level of the proteins expressed among the transgenic line and the lines of CLP(cs1-5, 1-7, 4-4) and Dhn5(ds1-5, 2-2, 2-3) lines were determined to produce to higher levels. In addition, those Dhn5 transgenic lines exhibited better growth than nontransgenic callus in presence of 10 and 20% PEG. Among the CLP tansgenic lines, both cs1-5 and cs1-7 showed a tolerance to some extents against leakage from freezing.

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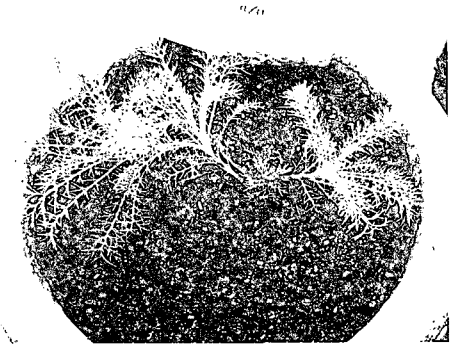

Figure 1. Plants of *Artemisia adamsii* grown in the pot.

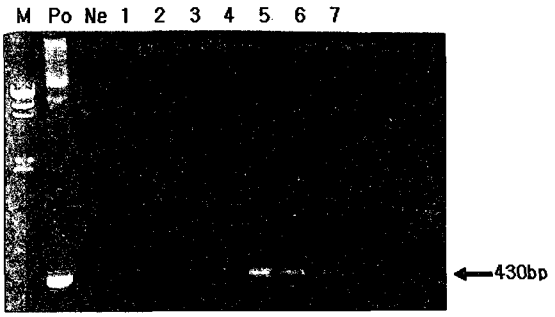

Figure 2. Confirmation of the presence of CLP gene introduced in transgenic callus cell by PCR with CLP primers.

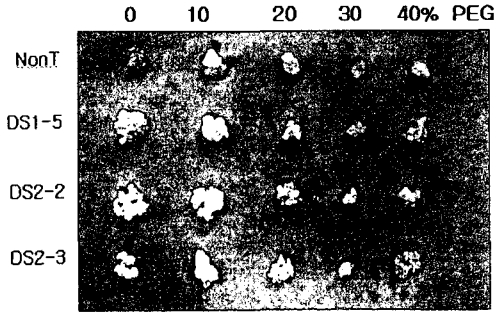

Figure 3. Growth of transgenic callus of *Artemisia adamsii* with a barely Dhn5 gene in PEG(0 to 40%).

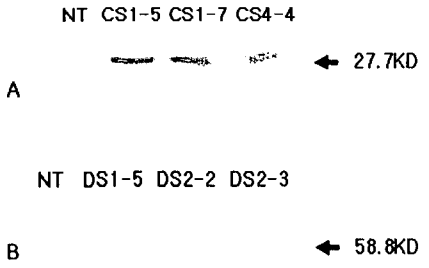

Figure 4. Western analysis of total proteins in CLP(A), Dhn5(B) transgenic calli with anti-CLP, anti-Dhn5 serum.