A-2

Drought and Cold Tolerance Enhanced by Transformation of ABF3 gene in Lettuce (*Lactuca sativa* L.)

Enkhchimeg Vanjildorj[1], Tae-Woong Bae[1], Key-Zung Riu[1], Soo-Young Kim[2], Chang-Hyu Bae[3] and Hyo-Yeon Lee[1]*

[1]*College of Agricultural and Life Science, Cheju National University, Cheju 690-756, Korea*
[2]*Kumho Life and Environmental Science Laboratory, Kwangju 500-712,*
[3]*Korea School of Plant Production Science, Sunchon National University, Sunchon 540-742, Korea*

Objectives

Our experimental main objective was to transform ABF3 gene to lettuce cotyledon explants via *Agrobacterium tumefaciens,* to determine the transgene transmission to T_1 progeny, and the influence of ABF3 gene regulation on drought and cold tolerance enhancement of lettuce.

Materials and Methods

1. Material

Plant - Lettuce (*Lactuca sativa* L.) cv. Chongchima's (leaf type) cotyledon explants were used.

Agrobacterium strain EHA 105/ pCUM-VB3 carried out *ABF3* as a target gene, *gfpgus* as a reporter gene and *hph* as a selectable marker gene.

2. Methods:

Cotyledon explants were infected by *Agrobacterium,* and selected on shoot induction and selection medium1 containing 20 mg/L hygromycin. Then, the appeared hygromycin-resistant calli were transferred to shoot induction and selection medium2 containing 25 mg/L hygromycin. The putative transgenic plants were confirmed by PCR and GUS analysis. The transgene segregation to T_1 progeny was determined on MS containing 30 mg/L hygromycin medium. Drought (at 15℃ for 30 days) and cold (at -4℃ for 2 days) stress tolerance assays were tested.

Results and discussion

Transgenic plants showing *in vitro* resistance to 20 and 25mg/L hygromycin were obtained by using a two-step selection/regeneration procedure. The gene transformation efficient was 10.9%. Those transgenic plants exhibited normal growth in terms of phenotype and yield of seeds. And the transgene segregation ratio to T_1 progenies was 3:1 in the eight lines and 1:1 in the only one line (semidominant). Each leaves of putative transformants showed GUS activity except for semidominant plants. PCR confirmed the integration of transformed genes in T_0 and T_1 plants genome. Those of *35S-ABF3*(468-bp), *hph*(0.9-kb), and *gus*(1-kb) genes were detected on all lines of putative transformants. The gene escape was not observed. The transgenic lettuce plants displayed more tolerant to drought and cold stresses than that of wild-type plants. These *in vivo* assay results indicated that overexpression of *35S-ABF3* can significantly improve drought and cold tolerance in lettuce plants. The enhancing stress tolerance of lettuce as well as the other crop plants is very important not only academically but also agronomically. In other word, it's a key to the increase agricultural productivity in many parts of the world is being damaged the ecological balance.

*Corresponding author : Hyo-Yeon Lee, Tel : 064-754-3347, E-mail : hyoyeon@cheju.ac.kr