Increased Stress Tolerance in Transgenic Alfalfa Expressing *At.* DREB1C Gene by *Agrobacterium*-Mediated Genetic Transformation

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The XXI International Grassland Congress / VIII International Rangeland Congress took place in Hohhot, China from June 29 through July 5, 2008.

Proceedings edited by Organizing Committee of 2008 IGC/IRC Conference

Published by Guangdong People's Publishing House

Increased stress tolerance in transgenic alfalfa expressing *At*. DREB1C gene by *Agrobacterium*-mediated genetic transformation

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Key words : transcription factor DREB1C ,alfalfa ,*agrobacterium*-mediated ,transgenic plant ,transformation

Introduction Abiotic stresses are multigenic as a quantitative trait in multiple signaling pathways (Knight and Knight , 2001) . The transcription factor DREB can induce many genes related to stresses to express in order to regulate the stress responses of plants . The objectives of this study were that transferring *dreb1c* gene into alfalfa to improve its stress tolerance by *Agrobacterium*-mediated genetic engineering and tissue cultural technology .

Materials and Methods

Transformation and regeneration of the plant

Medicago sativa L . Baoding as plant materials and *Agrobacterium tumefaciens* strain GV3101 harboring a binary vector pCAMBIA1301 were used in this study . There are two genes on T-DNA region of this plasmid . A Cauliflower mosaic virus 35S promoter is used to drive the selective gene resistant to hygromycin . The dehydration responsive element binding (DREB) protein gene is also driven by CaMV 35S promoter . The explants were transformed by *Agrobacterium* . After 3 days of co-culture in the dark , the explants were transferred onto the callus induction medium (improved SH medium supplemented with 2 ,4-D 2 mg/L , KT 0 .2 mg/L , 30 mg/L sucrose , 30 mg/L hygromycin and 300 mg/L cefotaxime , pH5 .8) . After three weeks , the calli were transferred onto agar-solidified (0 .8% w/v) UM medium (supplemented with 0 .6 mg/L KT , 30 mg/L hygromycin , 300 mg/L cefotaxime , 2 g/L casein hydrolysate , 30 mg/L sucrose and pH5 .8) to induce the shoot . After about four weeks , hygromycin-resistant shoots were transferred to 1/2 MS basal agar-solidified (0 .8%) medium containing 15 mg/L sucrose without any regulator for rooting , pH5 .8 . All plant materials were cultivated in a growth chamber at 24±1℃ under a 12-h light period with a 25 μmol · m^{-2} · s^{-1} white fluorescent light . In order to optimize the antibiotic concentrations for the selection of transgenetic plants , the sensitivity of the explants to cefotaxime and hygromycin was separately evaluated .

Molecular confirmation of DREB gene in alfalfa

These transgenic plants were confirmed by PCR and Southern blotting .

Physiological detection

These transgenic plants were placed at 4℃ for 10 h to identify the cold tolerance .

Results and analysis Cefotaxime which was used to suppress the growth of *Agrobacterium* in this study , was found that 100 mg/L of cefotaxime effectively inhibited *Agrobacterium* , and 500 mg/L of cefotaxime did not inhibit the induction of callus . One month later , on the medium containing 30 mg/L hygromycin there was not explants surviving . In our transformation experiments , after co-cultivation of explants with *Agrobacterium* , 30 mg/L of hygromycin was used to select the transgenic plants and 300 mg/L cefotaxime was used to inhibit the growth of *Agrobacterium* . After 90 days , transgenic plants were regenerated . The analysis of PCR and Southern blotting confirmed these plants were transgenic . Physiological detection shown these transgenic plants had better ability of tolerance to low temperature than the CK .

Discussion Transcription factor *At*. DREB1C was confirmed to play an important role in improving the cold tolerance of alfalfa by this study .

Reference

Knight H , Knight MR .2001 . Abiotic stress signaling pathways : specificity and cross-talk . *Trends Plant Sci* . 6 : 262-267 .