14. Transformation of Rice(*Oryza sativa*) by Dehydroascorbate Reductase Gene using *Agrobacterium tumefaciens*

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*Agrobacterium*을 이용한 DHAR 유전자의 쌀 형질전환

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Key words : Dehydroascorbate reductase, *So-DR8* gene, Transgenic plant.

<Introduction>

Dehydroascorbate reducyase(DHAR) is regarded as one of the chloroplast enzymes involved in the protection aganist oxidative stress. In plants, peroxide-scavenging was accomplished through the AsA-glthathione pathway, coupled series of redox reactions involving four enzymes: Ascorbate peroxidase(APx), monodeascorbate reductase, DHAR and gluthathione reductase. This pathway has been studied mainly in chloroplasts. In chloroplasts molecular oxygen is reduced to superoxide at the reducing sides of the photosystems. The superoxide radical anion is efficiently removed by superoxide dismutase, and the generated hydrogen peroxide is detoxified to water by APx. To develop rice resistant to reactive oxygen species produced from environmental stresses, a cDNA encoding vector with the DHAR cDNA(*SoDhar*) from *Spinacia oleracea* was constructed under the control of the cauliflower mosaic virus 35S promoter and was introduced into rice calli using *Agrobacterium tumefaciens* EHA101. The expression vector, pIG-dr8, harboring *SoDhar* gene was used for production of transgenic rice plants. Integration of pIG-dr8 gene was confirmed by PCR and Southern blot analyses with genomic DNA.

<Materials and Methods>

• Construction of the DHAR-overexpressin plasmid

A cDNA clone encoding DHAR was isolated by RT-PCR of *Spinacia oleracea* using the following primers; a forward primer, 5'-GCG AAA TGT CGA CCG TTA AA-3', and a reverse primer, 5'-TGT GAC CAT GGA TTA CCC GA-3'. The PCR product (1.0kb fragment) was cloned in the pGEM-T easy vector (Promega, USA). The full-length cDNA was subcloned into the *Xba*I and *Sac*I site of the binary vector pIG121-Hm. and was introduced into the *Agrobacterium tumefaciens* EHA101.

• Rice transformation : Calli Induction, Co-cultivation, Selection and Plant Regeneration

Rice seeds were sterilized in 2.5% NaOCl solution, inoculated in the callus induction medium, incubated at 24 ℃. After 3 weeks, calli derived from the scutella were used for trnasformation. Agrobacterium containing the pIG-dr8 plasmid was grown for for 2 day in YEP medium. The calli were soaked in this bacterial suspension for 2min. Then the calli were transferred on a co-culture medium. After co-cultivation for 3 day, the infected calli were transferred to selection medium containing 125 mg/L cefotaxim and 30 mg or 50 mg/L hygromycin. After selection for 3~4 week, resistant calli were transferred to regeneration medium.

<Results and Discussion>

The DNA sequence of the inserts revealed a 801-base pair (bp) open reading frame (ORF) which can encode 267 amino acids with an expected molecular weight of 29 kilodalton. The nucleotide sequence and its deduced amino acid sequence are available at GeneBank (accession no. AF195783). The soDhar clones were fused under the cauliflower mosaic virus 35S promoter and introduced into rice calli. The expression vector, *pIG-dr8*, harboring *soDhar* gene was used for production of transgenic rice plants(Fig. 1). A cDNA encoding the *soDhar* was introduced into rice calli via Agrobacterium-mediated gene transfer system. We were conducted to obtain the transformed rice calli with *soDhar* gene, using *Agrobacterium tumeaciens* EHA101. Rice calli were transformed and transgenic calli were regenerated on MS medium supplemented with 0.1 mg/L NAA, 2.0 mg/L Kinetin, 50 mg/L hyromycine, and 250 mg/L cefotaxime and were rooted on MS medium with hygromycine and without plant growth regulators. Integration of *pIG-dr8* gene was confirmed by PCR(Fig. 2A) and Southern blot analyses with genomic DNA(Fig. 2B). Transgeic plants from hygromycin-selected calli of rice did not show any morphological difference from wild-type plants. We will develop transgenic rice plants with overexpressing of the *SoDhar* and study their response to photo-oxidative stress in the presence of chemicals and environmental factors. The *SoDhar* gene still requires, however, more experimental evidence for the further study which may help understanding the physiological and biochemical function as well as the genetic regulation of this enzyme.

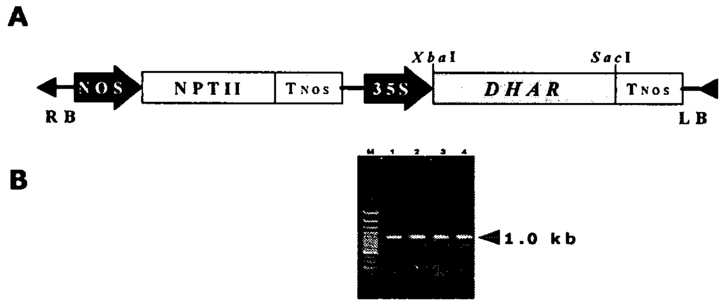

Fig. 1. Construction of the expression vector. For the construction of expression vector, DHAR cDNA has replaced *GUS* in *pIG*121 vector. (A) Structure of *pIG*-dr8. (B) Identification of recombinant expression vector. PCR analysis of the construct, *pIG*-dr8.

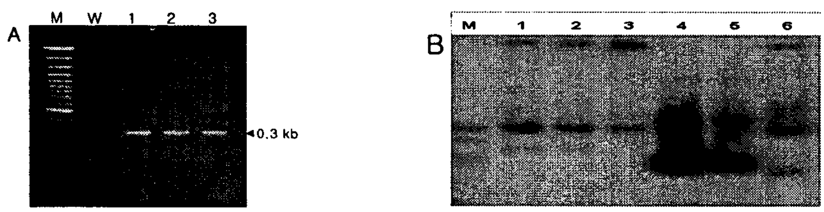

Fig. 2. (A) PCR analysis of transgenic rice plants. PCR amplification with 35S sense1 and 35S antisense1 primers. (B) Southern blot analysis of transgenic rice plants. Numbers indicate independent transgenic lines.