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A Simple Method for Protoplast Transformation of Trp1 Mutant in *Arabidopsis thaliana*

Seonhee Lim*, Yeongsoon Kim, and Hyeonsook Cheong
Department of Genetic Engineering, Chosun University

The tryptophan requiring trp1 mutant of *Arabidopsis* are blue fluorescent under UV light because they accumulate anthranilate compounds. Trp1 plants are defective phosphoribosyl anthranilate transferase activity. Protoplast is the most powerful tool for homologous recombination. We constructed two vectors for gene targeting of PAT gene(pHS113, pHS117). In order to establish system for genetic manipulation of *Arabidopsis* at the protoplast level, we performed a experiment which allows the efficient transformation and regeneration of *Arabidopsis* tryptophan mutant. Root explants excised from plantlets grown in liquid medium were cultured in callus inducing medium containing 2.0mg IAA, 0.5mg 2,4-D, and 0.5mg IPAR. After 10 days, root culture showing cell proliferation was used for protoplast isolation. The protoplast suspension was incubated in liquid medium and obtained high plating efficiency. Root protoplast manipulation in trp1 mutant induced faster and higher cell division rather than one from the mesophyll cell.

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Identification of Endonuclease Activity Induced by Oxidative Stress in Tobacco Chloroplasts

Kim, Jin Hyun*[1], Shin, Sung Ho[2] and Lee, Woo Sung[1]
[1]Department of Biology, Sung Kyun Kwan University
[2]Agency for Defence Development

We were interested in DNA degradation in chloroplasts under superoxide, one of the active oxygens, stress. When herbicide methyl viologen (MV), which artificially generates superoxide in chloroplast, was applied to young tobacco (*Ncotiana xanthi*) leaf, DNA degradation, observed by agarose gel electrophoresis, occurred in a MV dose-dependant manner. The DNA degradation was mediated by DNA endonuclease, evidenced by the following observations ;
(1) Heat-treated chloroplast extracts prevented the DNA degradation.
(2) Endonuclease inhibitors (aurintricarboxylic acid and Zn^{2+}) also prevented the degradation.
(3) Chloroplast extracts treated with MV were able to cleave supercoiled plasmid, generating nicked and linear DNA.
Transgenic tobacco (pAL100), carrying over expressed Mn-superoxide dismutase in chloroplast, was used to substantiate the superoxide-mediated induction of the endonuclease. Compared to the untransformed control plant, pAL100 exhibited significantly enhanced protection of DNA degradation under MV treatment. Collectively, this study demonstrated that plant chloroplasts have a DNA endonuclease which is induced (or activated) by superoxide, or possibly other active oxygens.