Metabolic engineering of *Arabidopsis thaliana* to increase carotenoid content by using the *Erwinia herbicola crtI* and *Escherichia coli dxs* genes

Jung-Hun Kim, *Sang-Hwal Yoon, *Sook-Hee Lee, *Ju-Eun Kim, *Hye-Min Park, *Eun-Kyung Lee, +Myung-Suk Choi, and *Seon-Won Kim
Division of Applied Life Science(BK21), +Division of Forest science,
*Dept. of Food Science & Nutrition, Gyeongsang National University
Tel : 82-55-751-5974, Fax : 82-55-751-5971

In carotenoid biosynthesis, phytoene desaturase gene *crtI* and DXP synthase gene *dxs* are both rate-limiting step enzymes. These two genes were cloned from *Erwinia herbicola* and *Eschericha coli* chromosomal DNA, respectively. The genes were functionally expressed in lycopene producing *E. coli* and increased the carotenoid production. *Arabidopsis thaliana* was transformed with the genes which were expressed under the control of CaMV 35S promoter. Transit peptide sequence of *Brassica rapa* Rubisco small subunit was located in front of the genes to target the expressed enzymes to plastid in which carotenoids were synthesized. Recognition of these genes and expression level were demonstrated by PCR and southern blotting, and Northern blotting, respectively. Transgenic plants, which were transformed with *E. herbicola crtI*, showed resistance against bleaching herbicide norflurazon, which specifically inhibited phytoene desaturase encoded by native genes of *Arabidopsis thaliana*. Also, *crtI* and *dxs* transgenic plants showed higher amount of carotenoid contents than control transgenic plants.

Acknowledgement

This work was supported by the BioGreen21 Program of the Korea Rural Development Administration, Republic of Korea. The Scholarship of authors was supported by the BK21 Program of Korea.

References

1. Armstrong GA, Alberti M, Hearst JE. Conserved enzymes mediate the early reactions of carotenoid biosynthesis in nonphotosynthetic and photosynthetic prokaryotes. *Proc Natl Acad Sci* USA. (1990) Dec; 87 (24): 9975-9.
2. Sprenger GA, Schorken U, Wiegert T. Identification of a thiamin-dependent synthase in *Escherichia coli* required for the formation of the 1-deoxy-D-xylulose 5-phosphate precursor to isoprenoids, thiamin, and pyridoxol. *Proc Natl Acad Sci* USA. (1997) Nov 25; 94 (24): 12857-62.
3. Steven J. Clough, Andrew F. Bent. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *The Plant J.* (1998) 16(6) 735-743.
4. Norihiko Misawa, Shigeyuki Yamano, Hartmut Linden. Functional expression of the *Erwinia uredovora* carotenoid biosynthesis gene *crtI* in transgenic plants showing an increase of -carotene biosynthesis activity and resistance to the bleaching herbicide norflurazon. *The Plant J.* (1993) 4(5) 883-840.