Combating photooxidative stress and redirection of secondary metabolism in green hairy roots of *Daucus carota* L.

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by

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ABSTRACT

Hairy root culture of *Daucus carota* when cultivated under continuous illumination (250 $\mu mol.m^{-2}.s^{-1}$) turned green. When compared with normal hairy roots, accumulation of both soluble and wall-bound phenolic contents, mostly 4-hydroxybenzoates were to be reduced to half in green hairy roots cultivated under continuous light. Besides, suppression of *p*-hydroxybenzaldehyde dehydrogenase (HBD) activity was also evident in the green hairy roots.

Anticipating photo-oxidative stress response in green hairy roots as one of the reasons behind the reduced accumulation of phenolic compounds, activities of several antioxidant enzymes were measured. When compared with normal hairy roots, green hairy roots showed an enhanced superoxide dismutase (SOD) activity. Interestingly, SOD-suppressed root showed three-fold enhanced caffeic acid glucoside accumulation in the soluble fraction as compared to untreated ones. While ascorbate peroxidase activity showed marginal increase in green hairy roots, a decrease in the activities of guaiacol peroxidase and catalase were observed. SDS-PAGE of crude protein profile from green hairy roots showed a distinct band of RuBisCO, which was absent in normal hairy roots. RT-PCR based expression analysis of betaine aldehyde dehydrogenase showed enhanced transcript levels in green hairy roots as compared to normal hairy roots, whereas reverse trends were observed with the transcripts accumulation for phenylalanine ammonia-lyase and chalcone synthase. These findings corroborated with the *in vitro* BADH activities in hairy roots, and thus indicated an important role of this stress enzyme in combating photo-oxidative stress in green hairy roots upon continuous light exposure. However, comparative analysis of emitted volatiles from green hairy roots revealed higher monoterpene and sesquiterpene contents than normal hairy roots, though methyl salicylate content was more in normal hairy roots than in green ones. Application of clomazone, an inhibitor of 1-deoxy-D-xylulose 5-phosphate synthase (DXS), reduced the amount of total monoterpenes and sesquiterpenes in green hairy roots compared to normal hairy roots, whereas methyl salicylate content was higher in inhibitor-treated green and normal hairy roots than normal green and normal hairy roots. Because methyl-erythritol 4-phosphate (MEP) and phenylpropanoid pathways respectively contribute to the major routes of monoterpenes and phenolic acids biosynthesis, the levels of activities of several key enzymes regulating these pathways were measured in both green and normal hairy root lines. Of these, 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) is a key enzyme of the MEP pathway, pyruvate kinase (PK) is an enzyme of primary metabolism related to the MEP pathway, shikimate dehydrogenase (SKDH) is involved in biosynthesis of aromatic amino acids, and phenylalanine ammonia-lyase (PAL) catalyzed the first step of phenylpropanoid biosynthesis. Activities of DXR and PK were higher in green hairy roots as compared to normal ones, whereas the opposite trend was observed for SKDH and PAL activities. Gene expression analysis of DXR and PAL showed trends similar to those for respective enzyme activities. Based on the above

observations it has been suggested that a possible diversion of metabolites towards isoprenoid biosynthesis from primary metabolism, limiting phenolic biosynthetic pathway in green hairy roots grown under continuous light.

Keywords: *Daucus carota* L.; Betaine aldehyde dehydrogenase (BADH); Green hairy roots; 4-Hydroxybenzoic acid; 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR); Isoprenoid; Methyl-erythritol-4-phosphate (MEP); Phenylpropanoid; Volatile terpenoid