Light regulation of 4-hydroxybenzoic acid biosynthesis in hairy roots of *Daucus carota* L.

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Introduction

Daucus carota, **a food crop is known to produce 4-hydroxybenzoic acid (4-HBA). Biosynthesis of this phenolic acid has recently been elucidated in hairy root cultures of *D. carota* from this laboratory [1, 2, 3, 4]. However regulation of this biosynthetic pathway has not been worked out. Under continuous light white, hairy roots turned into green hairy roots. Anatomical study confirmed the presence of chloroplasts in green hairy roots. In plants, light perform remarkable regulatory functions in metabolic reactions. Therefore, these green hairy roots has provided us a new opportunity to study the light regulation of 4-HBA biosynthesis in *D. carota*.**

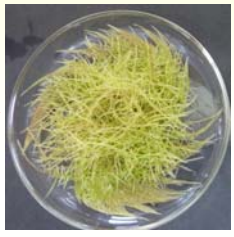
Greening of hairy roots

Hairy root cultures are maintained in liquid B_5 media in the dark. Under the continuous illumination, these hairy root become green.

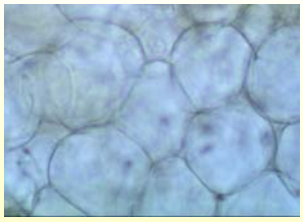
Anatomical examination confirmed the presence of chloroplast.

Normal white hairy root

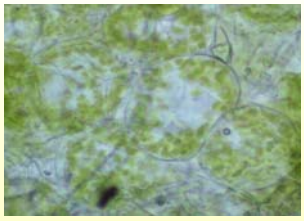
under continuous illumination



Green hairy root



Portion of T.S of white hairy root (magnified)



Portion of T.S of green hairy root (magnified)

References

[1] Sircar D, Roychowdhury A and Mitra A. *J Plant Physiol* (2007) 164: 1358 - 1366

[2] Sircar D and Mitra A. *J Plant Physiol* (2008) 165: 407- 414

[3] Sircar D and Mitra A. *J Plant Physiol* (2009) 166: 1370 - 1380

[4] Sircar D et al. *Acta Physiol Plant* (2011) <in press>

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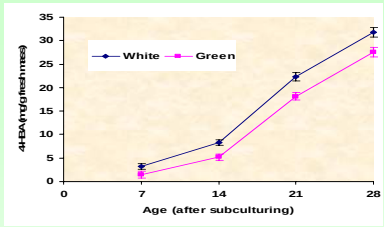
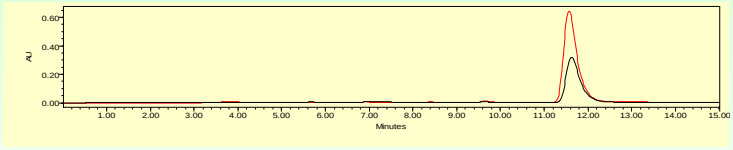
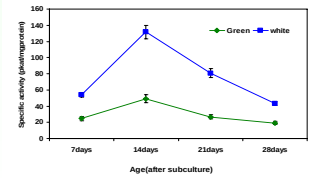
Result Highlights



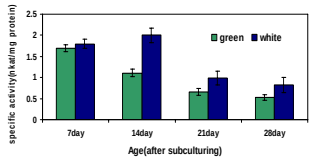
Time course accumulation of 4-HBA in white and green hairy roots.
For each study, 0.5 g hairy roots were harvested periodically at regular interval of seven days and wall-bound 4-HBA content were analyzed by HPLC



HPLC chromatograms showing reduced level of 4-HBA content in green hairy root as compared to the white roots. Red- white**, Black-** green



Time course PAL activity in green and white hairy roots

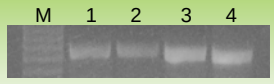


Time course SKDH activity in green and white hairy roots

Expression study

Total RNA was extracted from both 14 days old white and green hairy root cultures.

cDNAs were prepared and amplified by gene specific primers of PAL; amplified product was analyzed by agarose gel electrophoresis.



Amplified product of PAL gene using gene specific primers. M-marker; 1, 2- green; 3, 4- white

Major Observations

Reduced level of 4-hydroxybenzoic acid accumulation was observed in green hairy roots as compared to white ones.

Phenylalanine ammonia-lyase (PAL) and Shikimate dehydrogenase (SKDH) activities were also found to be reduced in green hairy roots. This was consistent with the gene expression profile of PAL in green hairy roots, where a clear low level of expression was observed.

Conclusion

These observations raised a fundamental question as if chlorophyll biosynthesis reduces the carbon flow towards 4-hydroxybenzoic acid formation by some unknown route of metabolic diversion. Work is being continued to answer this question.