University of Victoria | Graduate Studies

Notice of the Final Oral Examination for the Degree of Master of Science

of

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BSc Hons (Nanjing Agricultural University, 2012)

"Characterization of a Putative Flavonoid 3', 5'-Hydroxylase (PtF3'5'H1) in *Populus***"**

Department of Biology

Friday April 17, 2015
2:00 P.M.
David Strong Building
Room C108

Supervisory Committee:
Dr. Peter Constabel, Department of Biology, University of Victoria (Supervisor)
Dr. Juergen Ehlting, Department of Biology, UVic (Member)
Dr. Alistari Boraston, Department of Biology and Microbiology, UVic (Outside Member)

External Examiner:
Dr. Reinhard Jetter, Department of Botany, University of British Columbia

Chair of Oral Examination:
Dr. Gina Harrison, Department of Education, Psychology and Leadership Studies, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

Proanthocyanidins (PAs), also known as condensed tannins (CTs), are oligomers or polymers of flavan-3-ols. They have a very important role in plant-environment interactions, such as plant-herbivore, plant-microbe, and potential in light stress tolerance. In poplar, PAs can make up as much as 30% of the leaf dry weight. The synthesis of PAs in poplar was demonstrated to be inducible by both abiotic and biotic stresses. The B-ring hydroxylation pattern of flavan-3-ols directly affects the structure of PAs, and many studies have shown that B-ring hydroxylation of PAs is associated with their biological functions such as leaf litter decomposition rate and anti-herbivore activity. Anthocyanins are very important colour pigments in plants, and share the intermediate leucoanthocyanidin with PAs as precursors. The role of anthocyanins in plant pollination, light stress tolerance, and seed dispersal has been well studied. A change in B-ring hydroxylation pattern can modify the colour of anthocyanins dramatically, and also change their biological function. Flavonoid 3'-hydroxylase and flavonoid 3', 5'-hydroxylase (F3'H and F3'5'H) are two key enzymes involved in the modification of B-ring hydroxylation pattern of both PAs and anthocyanins. The objective of this study is to characterize the possible role of flavonoid 3', 5'-hydroxylase in PA and anthocyanin biosynthesis in poplar. A candidate F3'5'H was identified in the *Populus trichocarpa* genome database based on previous expression profile experiments, called PtF3'5'H1. PtF3'5'H1 shares high sequence similarity with previous characterized F3'5'H from other plants. To test the function of PtF3'5'H1 directly, transgenic hybrid poplar plants overexpressing PtF3'5'H1 were generated. Contrary to expectation, the hydroxylation pattern of the PA in the transgenic poplars was not significantly modified. Likewise, overexpression of PtF3'5'H1 in poplar did not change the overall amount of PAs. These results suggest that PtF3'5'H1 may not be directly involved in the production of PAs in poplar, or that the overexpression of PtF3'5'H1 in poplar PA synthesis is limited by a requirement from other factors. The transgenic PtF3'5'H1 overexpressing poplar did show an alteration in anthocyanin. In leaves of transgenic poplars, several putative delphinidin derivatives were observed at greater levels than the wild type, indicating that PtF3'5'H1 participates in the anthocyanin production in poplar. By contrast, transiently introducing PtF3'5'H1 into *Nicotiana benthamiana* had no effect on the anthocyanin profile. I conclude that PtF3'5'H1 is very likely to be involved in the anthocyanin synthesis in poplar, while the function of PtF3'5'H1 in poplar PA synthesis has yet to be demonstrated.