Studies on isoprene emission from tropical tree: hormonal regulation under stressed condition and characterization of emission behavior

Abstract

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Over the past 30 years, much attention has been focused on understanding the processes that control emission rates of the C<sub>5</sub>H<sub>8</sub> molecule isoprene, from vegetation. Isoprene significantly affects air quality and tropospheric chemistry. Meanwhile, isoprene has been considered to protect leaves from many environmental stresses. It is therefore highly desired to uncover the underlying regulatory mechanisms of isoprene formation in response to environment stresses to develop entirely mechanistic and process-based regulatory models. In this thesis, I addressed the following points using tropical trees: (1) regulatory relationship between isoprene emission and antioxidation system under drought stress; (2) hormonal control of isoprene biosynthesis; (3) optimization of G-93 parameters and characterization of emission behavior of tropical trees.

In the first part, I explored the molecular regulatory mechanism of isoprene emission in relation to the antioxidation system of tropical tree *Ficus septica*. It was observed that the isoprene emission was increased during drought stress and resulted in significantly increased leaf isoprene concentration due to reduced stomatal conductance. The isoprene synthase (IspS) protein level showed a positive correlation with the isoprene emission rate in stressed plants. The antioxidant genes transcript like peroxidase 2 (*POD2*), *POD4*, copper-zinc superoxide dismutase 2 (*Cu-ZnSOD2*), and manganese superoxide dismutase 1 (*Mn-SOD1*) also increased during the drying period, while those of ascorbate peroxidase 1 (*APX1*) decreased. However, there was only a weak correlation between isoprene emission and antioxidant enzyme gene expression, indicating that the regulation of isoprene biosynthesis is not directly linked to the antioxidant defense network in drought-stressed *F. septica*.

Since naturally occurring plant hormone jasmonic acid (JA) has been implicated in the

regulation of isoprene emission in our previous study, we investigated the impact of exogenous JA treatment on the isoprene emission. Foliar spraying of 50  $\mu$ M jasmonic acid (JA) on *Ficus septica* decreased isoprene emission, and the emissions increased after relief from JA application. We explored the molecular regulatory mechanism of isoprene emission by analysis of photosynthetic rate, gene expression of 2-C-methyl-D-erythrytol 4-phosphate (MEP) pathway, hormone signaling and circadian rhythm processes, and metabolite pool sizes of MEP pathway. Isoprene emission strongly correlated with gene expression and protein levels of IspS, indicating transcriptional and possible translational modulation of *IspS* by JA. Among the transcriptional factors involved in hormone or circadian rhythm signaling, *MYC2* (JA) and *LHY* (circadian clock) showed negative correlation with isoprene emission. Putative *cis*-elements predicted on the promoter of *F. septica IspS* (G-box for *MYC2* and circadian for *LHY*) supports our proposal that isoprene emission is regulated by coordinated transcriptional modulation of *IspS* gene by phytohormone and circadian rhythm signaling.

In the last part, I improved the performance of the G-93 model in predicting isoprene emissions from six tropical tree species. I conducted diurnal leaf-scale observations under laboratory conditions. A new iterative method was developed, named "Ping-Pong" method that uses mutual and repetitive step-by-step optimization of the temperature ( $C_T$ ) and light ( $C_L$ ) dependency variables of G-93 model. Results showed that the optimized G-93 well captured the light and temperature dependent increase and the decrease of isoprene emission rate in all tropical trees assessed. This result also revealed that estimated Q<sub>10</sub> values for isoprene emission from tropical trees were much higher than that of the temperate plant *Populus nigra* under same experimental conditions.