Summary

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Title	Studies on Isoprene Emission from Tropical Trees: Molecular Characterization of Regulatory Mechanism and Optimization of Emission Model

Key words（**Isoprene emission**） （**Tropical tree**） （**Molecular regulation**） （**G-93 Model**）

CHAPTER I – General Introduction

Several plants synthesize and emit the volatile hemiterpene isoprene (C_5H_8) from their leaves to the atmosphere, with huge implications on tropospheric oxidative capacity. Biogenic isoprene emitted by terrestrial vegetation, especially woody trees and shrubs accounts for most non-methane hydrocarbon flux into the atmosphere. Due to its amazingly huge flux (estimated at 500 – 750 Tg yr^{-1}) and high reactivity, isoprene has substantial effects on air quality through undergoing rapid photoxidation to generate toxic tropospheric ozone (especially in the presence of NOx), through its consumption of hydroxyl radicals and its potential to contribute to secondary organic aerosols (SOAs). Tropical and subtropical trees contribute more than half of this huge isoprene global annual flux, making tropical ecosystems the largest source of biogenic isoprene. Leaf isoprene emission rate is related to high temperatures and light intensity but mechanisms of how plants finely regulate isoprene biosynthesis in response to these environmental drivers are not yet fully elucidated. It is highly desired to uncover the underlying regulatory mechanisms of isoprene formation in response to environmental drivers to pave way for development of fully mechanistic, process-based models of isoprene emission. In the absence of mechanistic models, empirical and semi-mechanistic models are being used to predict regional isoprene emissions. In this regard, the Guenther (1993) model (G-93) is the most widely used empirical model and is the basis on which most new models were built upon. The G-93 model simulates leaf isoprene emission rate as a function of temperature and light intensity, and its parameters were determined by best-fit practices using observations from temperate plants. Several studies have reported on the model`s poor performance in predicting emissions from tropical tree species.

In this thesis, I used two tropical tree species *Ficus septica* and *Casuarina equisetifolia* to address the two questions above: (1) temperature regulation mechanisms of isoprene emission and (2) adequacy of G-93 model in predicting emissions from tropical plants.

CHAPTER II

Introduction

Isoprene is synthesized in plant chloroplasts by the action of isoprene synthase (IspS) on dimethylallyl diphosphate (DMADP), a product of the plastidic methylerythritol phosphate (MEP) pathway. The MEP pathway requires carbon substrates, energy and reducing equivalents from other plant pathways like photosynthetic and energy processes, linking isoprene biosynthesis to these other processes. Isoprene synthesis and its emission from leaves is very responsive to temperature and light intensity but many decades after these observations were made, the underlying regulatory mechanisms remain elusive, especially at medium-to-long term scales. The tropical broadleaf tree *F. septica* has been previously shown to cease isoprene emission when exposed to chilling temperatures of 12°C and to re-induce isoprene synthesis upon subsequent exposure to warmer temperatures of ≥ 30°C for 24 h. In this study, I sought to explore molecular regulation of isoprene emission in *F. septica* by using the low temperature suppression and recovery of isoprene biosynthesis in the tropical species.

Results and Conclusion

I conducted a gene expression and protein level analysis of *F. septica* plants under changing temperature using quantitative real-time PCR (qRT-PCR) and western blotting, respectively. In addition, DMADP pools in leaves were profiled by an LC-MS/MS method. Transcription levels were analyzed for 17 genes that are involved in metabolic pathways associated with isoprene biosynthesis, including isoprene synthase (IspS). Only changes in transcription and protein levels of IspS gene, but not the other assessed genes had identical temporal patterns to isoprene emission capacity, suggesting that isoprene emission in response to daily temperature was under IspS transcriptional regulation. A transcriptome study using RNA-seq under the temperature regime was also conducted. Changes in

DMADP pool did not explain observed changes in isoprene emission rate, suggesting that substrate-level regulation was not the main regulatory mechanism of isoprene biosynthesis in response to daily temperature change in the time frame used in this study.

CHAPTER III

Introduction

Results from Chapter II suggest that temperature regulation of basal isoprene emission in the leaves of the tropical tree *F. septica* likely proceeds by transcriptional regulation of IspS gene that ultimately adjusts available IspS protein in leaves. To gain deeper mechanistic insights in molecular regulatory mechanisms of isoprene biosynthesis, I cloned the upstream promoter region of IspS gene and conducted a more detailed analysis of MEP pathway metabolites and genes. In addition, I carried out a comprehensive analysis of RNA-seq data set generated in Chapter II by using correlation analysis, network constructions and co-expression analysis to gain global knowledge of potential plant processes that might be linked to isoprene biosynthesis.

Results and Conclusion

Bioinformatic analysis of SOLiD transcriptome data revealed a very close association of isoprene emission and IspS gene to genes in the plant hormone signal transduction pathway and circadian clock elements. Transcript levels of 29 genes in these pathways were validated by qRT-PCR. Interestingly, *cis*-acting regulatory elements identified on the cloned upstream promoter region of IspS were consistent with transcriptional regulation in response to light, heat, temperature, plant hormones and circadian rhythmicity. Gene expression and metabolites of the MEP pathway did not support the notion that the major regulatory mechanisms involved this substrate supply pathway. Altogether, results from this study were interpreted in proposing a molecular regulatory mechanism of isoprene biosynthesis that involves transcription factors in the circadian rhythm, ethylene, jasmonic acid and abscisic acid signal transduction pathways in conjunction with heat shock factors and their interactions.

CHAPTER IV

Introduction

In the last part of this thesis, I describe a study aimed at improving the performance of G-93 model in predicting isoprene emissions from tropical tree species. The model developed by Guenther and coworkers, the G-93 model, simulates leaf isoprene emission dependency on temperature and light intensity. The model parameters were determined by leaf-scale chamber observations of four temperate plant species and have been widely adopted in all regions and ecosystems with its default parameters. However, several studies in tropical tree species have reported on the inadequacies of the model to accurately predict emissions from those plants.

Results and Conclusion

To optimize and improve the G-93 model performance in predicting emissions from tropical trees, I conducted diurnal leaf-scale observations on tropical tree species *F. septica* and *C. equisetifolia* outdoors and used observations from temperate *Populus* trees for comparison. The G-93 model performed well in predicting isoprene emission from tropical trees during low temperature – low light intensity periods in the morning and late afternoon, but had poor performance at high temperature – high light intensities during mid-day. The model also performed fairly in predicting diurnal emissions from *Populus* throughout the day. To optimize the parameters of the G-93 model, a new iterative method that uses mutual and repetitive step-by-step optimization of the temperature (C_T) and light (C_L) dependency variables was developed, named "Ping-Pong" method. Results show that the temperature dependency of isoprene emission from tropical trees diverted from predictions of the G-93 by orders of magnitude, and that optimization by "Ping-Pong" improved model performance to predict 81-96% of diurnal emissions compared to 73-76% achieved by G-93. In addition, the proposed simple "Ping-Pong" optimization approach allows simultaneous optimization of temperature and light model parameters using real field data as input, unlike previously reported approaches that require use of controlled environments to observe one variable at a time.

CHAPTER V - General Conclusions

In this thesis, I present studies that aim at addressing two major areas of biogenic isoprene that can help close gaps in our understanding of its biosynthesis and emission: (i) exploration of molecular regulation mechanisms of basal isoprene emission rate and (ii) improvement of widely used G-93 model performance of isoprene emission in predicting emissions from plants in different ecosystems than temperate species used in its development. Results from Chapter II and III suggested that molecular regulatory mechanism of basal isoprene emission in response to temperature change likely proceeds by transcriptional regulation of IspS gene by upstream processes like circadian rhythm and plant hormone signal transduction pathways. In chapter IV, a simple approach for optimizing G-93 parameters, "Ping-Pong" optimization method is presented, and data from tropical tree species shows that their emission response to environmental drivers deviate from the assumptions of the G-93 model especially under high light – high temperature conditions. Optimization of the G-93 model parameters by using the "Ping-Pong" approach significantly improved its prediction of diurnal emissions from tropical species.