Summary

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

Key word（**Isoprene**） （**Hormone**） （**Drought**）

Introduction and Purpose

Under a warming climate, heat waves and drought are observed to occur more frequently (Coumou and Rahmstorf, 2012). Forested ecosystems contribute most of the global emissions of biological volatile organic compounds (BVOCs) to the atmosphere (Guenther et al. 2012), and these emissions are expected to change with increasing frequency and intensity of climate extremes (Staudt and Peñuelas, 2010), which might persist following stress release. In the coming century under future climate change, the frequency and intensity of drought are projected to increase. Accurate simulation of future air quality and climate requires improving the understanding of isoprene emission response to drought conditions (Monson et al. 2007). Although the effects of temperature and CO_2 concentration on BVOC emissions, in particular, isoprene have been well studied, the effects of drought have not.

It is also essential to improve our understanding of the regulatory mechanism of isoprene emission, because present and predicted climate change causes a concurrent increase in the formation of these gases. Isolation of *IspS* upstream promoters from poplars, in silico analysis and reporter assays, gave signs on temperature and circadian regulation of *IspS* gene expression. It demonstrated that heat shock elements (HSEs), circadian motifs and different heat and light sensitive components prevailed in those promoters (Cinege et al. 2009; Schnitzler et al. 2010; Wiberley et al. 2009). Putative binding motifs of transcription factors of circadian rhythm and hormone signaling pathway was situated in the *IspS* upstream promoter sequences of *F. septica*. There is a probability that like the other terpene genes, *IspS* can be modulated by phytohormones by means of signal transduction, such as JA, CK, auxin, ethylene, and salicylic acid (SA) signaling that were shown to regulate genes of MEP, monoterpene, and sesquiterpene pathways (Ginis et al. 2012; Martin et al. 2002; Martin et al. 2003; Pateraki and Kanellis, 2010).

The main goal of the research was to advance our understanding of isoprene emission in less studied tropical environments concerning (i) molecular regulatory mechanisms of its biosynthesis and (ii) improvement of accuracy of predictive models currently used to predict future regional emissions from hot tropics.

Materials and Methods

F. septica cuttings were obtained from physiologically mature trees growing at the University of the Ryukyus, Okinawa, Japan (26°15′N, 127°46′E). Once rooted, the cuttings were transferred to 30-L plastic pots containing soil and organic matter (3:2, v/v) and cultivated outside under natural conditions. When

the saplings were 10 months old and approximately 1.5 m tall, they were randomly divided into control and treatment groups (n = 3 per group). During the experiment, both the control and treatment plants were moved to phytotron and grown under controlled environmental conditions at a day:night temperature of 30:25 °C with 50%–60% relative humidity, 300 μmol m^{-2} s^{-1} light intensity, and a 12-h photoperiod. In this experiment, I investigated the effect of isoprene emission under a short-term drought and the subsequent recovery period in a high-emitting broad-leaf tropical tree *F. septica* as a model. I represent the response of *F. septica*, against short-term water stress in terms of carbon-based secondary metabolites, physiological traits (photosynthesis, stomatal conductance, chlorophyll content, water potential, MDA and H_2O_2 content) and gene expression of MEP pathway and ROS signaling pathway.

In my second experiment, I gave my efforts to dissect the molecular regulation of isoprene emission applying five different exogenous hormones such as Auxin, ABA, CK, Gibberelic acid (GA) and JA in *F. septica*. I have compared the promoter sequence of the upstream region of *F. septica IspS* gene with poplar *IspS* gene. Besides, I have done a comprehensive analysis of the MEP pathway metabolites, IspS protein and transcript level dynamics of MEP pathway, circadian rhythm and different hormone signaling pathways during the spraying of the best effective hormone, Jasmonic acid to explore the molecular insights into isoprene emission.

In my last experiment, the most used isoprene emission predictive algorithm of the Guenther et al. 1993 (G-93) is optimized to improve model performance from tropical trees. The study used six tropical trees; *Bauhinia variegate* (camel's foot tree), *Calophyllum inophyllum* (ball tree), *Garcinia subelliptica* (fukugi tree), *Syzygium cumini* (black plum), *Syzygium samarangense* (wax jambu), and *Mangifera indica* (mango; red and yellow fruit). Diurnal observations of isoprene emissions under controlled conditions in the laboratory were done and an iterative optimization method "Ping-Pong" was used to parameterize the temperature and light response factors of the G-93 model without the need for fixing other variables and measuring one variable at a time.

Differences between the control and treatments were analyzed using Student's *t*-test with a significance level of $p < 0.05$. Correlations between different treatment parameters were evaluated by calculating the Pearson product-moment correlation coefficient. An ordination of the treatment parameters was performed using nonmetric multidimensional scaling (NMDS) based on the distance of $1 - |r|$.

Result, Conclusion and Consideration

The functional reasons for isoprene emission are still a matter of hot debate. A theory of the purpose of isoprene is that it is used as a mechanism by the plant to combat abiotic stresses, such as heat stress (Peñuelas et al. 2009). Siwko et al. (2007) provided evidence that isoprene stabilized lipid membranes and blocking "heat-induced phase transitions," helping the plant regulate temperature. Isoprene may additionally protect photosynthetic membranes by scavenging reactive oxygen species. An abundance of isoprene can cause indirect adverse effects on current and future air quality in an already polluted atmosphere.

I focus my discussion on the effects of concurrent stress factors such as drought on the biosynthesis and emission of isoprene, with the aim of further exploring isoprene functional roles in tropical plants challenged by environmental pressures associated to rapid climate change. This study aims at understanding the relationship between drought stress and isoprene emission. This understanding is vital

because drought is becoming increasingly widespread and is expected to rise in the future as a response to a warming world. By following physiological parameters, the proteins, the metabolites and the genes involved in isoprene biosynthesis and the anti-oxidation enzyme during and after the drought stress, I determined whether drought makes IspS more active and stimulate isoprene emission. The results also showed that short-term water stress promotes the production of isoprene emission based on their antioxidant properties and hence, to react with and quench reactive oxygen species (ROS) along with the anti-oxidation enzyme conferring tolerance to drought stress.

With the progression in plant science, it has been built up that phytohormones have the potential in reducing the detrimental impacts postured by abiotic stresses (Khan et al. 2013). Cross-talk between hormones is an incredibly dynamic sector of research that has profited from the current illustration of hormone signaling pathways. Besides, my insight into the molecular components and pathways that interacted hormone responses has enhanced tremendously in present times. JAs have been recommended specially to come up with plant stress responses due to its contribution as a signal of developmentally or naturally controlled articulation of different genes related to stress resilience (Kazan, 2015; Wasternack and Hause, 2013).

However, no information exists on the factors responsible for the changes in isoprene emission and whether JA is associated with that regulation for tropical trees. In my second experiment, the investigation was done to look at the impacts of JA on isoprene emission rates, metabolite pool sizes of MEP pathway, IspS proteins and transcripts level of *IspS*, hormone signaling, and circadian rhythm processes in *F. septica*. Results suggest that among the transcriptional factors, *MYC2* (JA hormone) and *LHY* (circadian clock) showed the negative correlation with isoprene emission and isoprene emission is regulated by coordinated transcriptional modulation of *IspS* gene by phytohormone and circadian rhythm signaling.

Modeling of isoprene emissions is most often estimated using Guenther et al. algorithms, considering the temperature and light dependence of emissions (Guenther et al. 1991; 1993). In these algorithms, a species-specific standard emission factor (Es, a constant that describes leaf emissions at standard conditions of typically 30 °C and photosynthetically active radiation of 1,000 μmol m^{-2} s^{-1}) is multiplied by temperature and light functions. Guenther et al. algorithms have been successfully used to model isoprene fluxes at spatial scales ranging from ecosystems to the globe (Guenther et al. 2006; 2012; Brilli et al. 2016). However, the temperature and light functions depend on empirically derived parameters, which may not be constant across different regions or climatic conditions (Arneth et al. 2008). Moreover, Es is known to vary, even within a given species, for example in response to weather extremes (Geron et al. 2016). Thus, the modeling algorithms often fail to reproduce isoprene emissions of ecosystems under stress, irrespective of whether stress is induced mechanically or by drought (Kaser et al. 2013).

Most of the algorithms developed based on observations from only temperate species that were generalized to all ecosystems (Guenther et al. 1991; Guenther et al. 1993; Monson et al. 2012; Niinemets et al. 1999). Driven by land cover distributions, vegetation emission factors (EFs) and environmental conditions, the Model of Emissions of Gases and Aerosols from Nature (MEGAN) can estimate emission fluxes of biogenic isoprene and other VOCs using simple mechanistic algorithms to account for the significant known processes controlling biogenic emissions (Guenther et al. 2006; 2012). The model has

estimated tropical trees to be responsible for 80% of global terpenoid emissions (Guenther et al. 2012), but emissions derived from satellite observations suggest those values are overestimated (Stavrakou et al. 2014). The model of MEGAN can simulate emission responses to some of the major driving variables, such as short-term variations in temperature and solar radiation, but the other factors are either missing or poorly represented.

Leaf-scale observations from tropical tree species are still very limited. I then conducted a more comprehensive study on improving the isoprene emission model in my last experiment. This study was carried out by the leaf-scale observations in the laboratory under controlled conditions and using a new modeling approach “Ping Pong” that improves the performance of G93 in predicting diurnal isoprene emissions from tropical trees.

Owing to the sparse amount of data, accounting for stress-induced BVOC emissions, hormone regulations, it is important to improve these weak points of modeling the global BVOC and calls for further research in this area.