

**Investigating the Molecular Basis of Volatile-
mediated Plant Indirect Defense against
Herbivorous Insects Using Functional and
Comparative Genomics**

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Shuhua Yuan

December 2007

Brief Abstract

Volatile organic compounds (VOCs) play important roles in plant indirect defense against herbivorous insects by attracting the natural enemies. I first used a tritrophic model system involving rice, rice fall armyworm (*Spodoptera frugiperda*), and the parasitoid *Cotesia marginiventris* to discover and characterize the volatile terpenoids and *TPS* genes involved in the indirect defense of rice against the insect herbivory with integrated functional genomics analyses. Seven rice *TPS* genes were found to be significantly up-regulated by both microarray and real-time PCR analyses, with one characterized as a linalool synthase and two as sesquiterpene synthases. The products of all three characterized genes covered most of the volatile terpenoids emitted by the fall armyworm-damaged rice. The products of all three characterized genes covered most of the volatile terpenoids emitted by the fall armyworm-damaged rice. In addition to the insect treatment, responses induced by defense hormone jasmonic acid were also examined with volatile analysis, gene expression profiling and enzyme assays. Only one *TPS* gene in the microarray analysis was up-regulated during the early response to jasmonic acid, and the gene was characterized as *OsLMS* (rice limonene synthase). Moreover, a homolog gene with similar sequence was characterized with limonene synthase activity and named *OsLMS2*. Both genes exhibited a time-dependent expression up-regulation upon jasmonic acid treatment. The regulation of terpenoid volatile emission was also examined from the perspective of diurnal cycle. Our results showed that the emission of volatile terpenoids was linked to the diurnal cycle;

however, the base level emissions were different among the products of three genes. The volatile terpenoid biosynthesis and emission were regulated at both *TPS* gene expression level and the substrate level. To further understand the molecular evolution of plant indirect defense against herbivorous insects, I also investigated the evolution of the TPS gene family using comparative genome analyses. These analyses revealed both a rapid evolution of the *TPS* gene family and a conserved group of monoterpene synthase with a deep evolutionary origin. Biochemical analysis of one of the poplar *TPS* genes in the conserved group showed linalool synthase activity.

To the Graduate Council:

I am submitting herewith a dissertation written by Shuhua Yuan entitled “Investigating the Molecular Basis of Volatile-mediated Plant Indirect Defense against Herbivorous Insects Using Functional and Comparative Genomics.” I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plants, Soils and Insects.

Feng Chen, Ph.D.
Major Advisor

C. Neal Stewart Jr., Ph.D.
Co-advisor

We have read this dissertation and recommend its acceptance:

Robert M. Augé, Ph.D.

Arnold M. Saxton, Ph.D.

Juan Jurat-Fuentes, Ph.D.

Accepted for the Council:

Carolyn R. Hodges
Vice Provost and
Dean of the Graduate School

(Original signatures are on file with official student records.)