University of São Paulo
“Luiz de Queiroz” College of Agriculture

Early responses of *Eucalyptus grandis* during rust infection

Alline Sekiya

Thesis presented to obtain the degree of Doctor in
Science. Area: Genetics and Plant Breeding

Piracicaba
2020

Alline Sekiya
Biotechnology Engineer

Early responses of *Eucalyptus grandis* during rust infection

Advisor:
Prof. Dr. **CARLOS ALBERTO LABATE**

Thesis presented to obtain the degree of Doctor in
Science. Area: Genetics and Plant Breeding

Piracicaba
2020

Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Sekiya, Alline

Early responses of *Eucalyptus grandis* during rust infection / Alline Sekiya - - Piracicaba, 2020.
99p.

Tese (Doutorado) - - USP / Escola Superior de Agricultura "Luiz de Queiroz".

1. *Austropuccinia psidii* 2. Metabolômica 3. Proteômica 4. Fisiologia vegetal I. Título

ACKNOWLEDGMENT

First of all, I thank God, for being the best author of my life.

My gratitude to my parents for believing on how my studies can impact on my personal development, even without any understanding of my scientific/academic life. I also thank my boyfriend, Gil, for staying with me at every single moment, always kindly and patient.

My special regards to my advisor, Professor Carlos Alberto Labate, for trusting in my work and supporting all my doctoral expectations.

I appreciate all the help that Mônica gave me during this period, by sharing her lab-work experiences and solving unexpected questions.

My sincere thanks to our lab expert, Thais, for being calm and patient to teach all the steps of proteomics and metabolomics so many times.

I wish to thank all the past, present and new members of Max Feffer Laboratory/ESALQ-USP whose assistance was a milestone in the completion of this thesis, specially to: Hana and Marisangela for being my first family in Piracicaba; to the post-docs Juliana and Fabrício for the scientific help and skills that contributed to data analyses; to my friends Vanessa, Ana Lúcia, Andressa Ducatti, Thalita and Gustavo for the emotional support and happy moments; to Felipe, Andressa Bini and Thiago Falda for starting and giving the basis for all the projects involving *Eucalyptus* rust.

My deepest gratitude to the professors of the Department of Genetics/ESALQ-USP for the competence to teach and qualify new professionals. I specially thank Professor Maria Carolina for the support during my teacher training, and Professor Claudia for the period I worked as a student representant and had the opportunity to follow her devotion to the students during the coordination of the Graduate Program of Genetics and Plant Breeding. I also thank other employees of ESALQ/USP: Segateli, Aparecido, João Pires and Sabino for contributing with the experiments; Léia, Silvia, Fernandinho, Valdir and Berdan for assisting me in the formalities of documents and presentations.

I also thank the GVENCK group for the experiences and extra-academic skills, and my friends Mariana Niederheitmann, Lillian Bibiano, Evelyn Couto, Letícia Lara, Jéssica Nogueira and many others with whom I had the pleasure to live and/or work together.

My expressive gratitude to Conselho Nacional de Desenvolvimento Científico and Tecnológico (CNPq) for the scholarship (Process 142379/2016-6) and to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support of my Doctoral Exchange Program in the United States (Process 88881.189205/2018-01).

And last, but not least, I'd like to thank Professor Steven Briggs for the great opportunity to learn more and improve so many skills, giving me the best experience in the University of California, San Diego. I'm also grateful to his wonderful team, Zhouxin Chen, Laura Gates, Ann Tong and Carla Omega, for the friendship and all the helpness they provided to make everything as easy as possible.

CONTENTS

RESUMO.....	5
ABSTRACT	6
1. INTRODUCTION	7
2. CONCLUSION	9
REFERENCES	11

RESUMO

Respostas iniciais de *Eucalyptus grandis* durante a infecção por ferrugem

Eucalyptus grandis (*E. grandis*) é a principal espécie florestal utilizada para produção de papel e celulose no Brasil, o segundo maior produtor do mundo. Entre as condições de estresse que podem afetar as plantações de eucalipto, a ferrugem causada pelo fungo biotrófico *Austropuccinia psidii* (*A. psidii*) pode interferir no desenvolvimento anual das árvores e conduzir a perdas de produtividade. Neste contexto, o objetivo deste trabalho foi estudar os eventos moleculares envolvidos nas respostas iniciais de *E. grandis* durante a infecção por ferrugem. Para isso, plantas de dois genótipos contrastantes, derivados de uma progênie de meios-irmãos de *E. grandis*, foram tratadas em condições de inoculação e controle para avaliar suas respostas fisiológicas, metabólicas e do proteoma durante 24 horas após a inoculação (hai). Os resultados mostraram que os genótipos iniciam uma divergência na resposta à infecção por *A. psidii* 12 hai, quando o genótipo resistente já detectou o fungo para induzir explosões oxidativas e produção de flavonoides como uma resposta efetiva contra o patógeno, ao mesmo tempo que o genótipo suscetível apresenta subversão do sistema imune na indução de detoxificação de EROS (espécies reativas de oxigênio) e supressão das respostas de hipersensibilidade. Portanto, este estudo possibilitou a compreensão de alguns dos mecanismos moleculares que ocorrem na interação entre *E. grandis* e *A. psidii* e a identificação de moléculas-chave que podem possivelmente auxiliar estudos futuros sobre a patologia da doença e, também, o melhoramento molecular.

Palavras-chave: *Austropuccinia psidii*, Metabolômica, Proteômica, Fisiologia vegetal

ABSTRACT

Early responses of *Eucalyptus grandis* during rust infection

Eucalyptus grandis (*E. grandis*) is the main tree species used to produce paper and pulp in Brazil, which is the second-largest global producer. Rust disease is among the most harmful conditions affecting Eucalyptus plantations, and it is caused by the biotrophic fungus *Austropuccinia psidii* (*A. psidii*), which can interfere with annual tree development and lead to productivity losses. In this context, we aimed to study the molecular events involved in the early response of *E. grandis* to rust infection. To accomplish these aims, contrasting plant genotypes derived from a half-sibling progeny of *E. grandis* were either inoculated with *A. psidii* or subjected to control conditions to evaluate their physiological, metabolomic and proteomic responses 24 hours after inoculation (hai). Our results showed that responses of the genotypes against *A. psidii* diverged at 12 hai, when the rust-resistant genotype detected the fungus and initiated both oxidative burst and flavonoid production as an effective defense against the pathogen. Simultaneously, the immune system of the rust-susceptible genotype was unable to induce the production of ROS (reactive oxygen species) and hypersensitive response was suppressed. Thus, our findings enabled us to understand some of the key molecular mechanisms that underly the interactions between *E. grandis* and *A. psidii*, identify key molecules for further study of the disease pathology, and important features for enhancing molecular breeding efforts.

Keywords: *Austropuccinia psidii*, Metabolomics, Proteomics, Plant physiology

1. INTRODUCTION

The genus *Eucalyptus* is comprised of several species that are distributed worldwide. Among these, *Eucalyptus grandis* (*E. grandis*) and its hybrids are the most important tree species used to produce pulp and paper in Brazil, which is the second largest producer globally (IBA, 2019). Rust, a harmful disease of *Eucalyptus* that is caused by the biotrophic fungus *Austropuccinia psidii* (*A. psidii*), can reduce the annual growth of trees, resulting in drastic yield reductions and economic losses (TAKAHASHI, 2002).

In *Eucalyptus*, rust is characterized by the growth of progressive yellow pustules on young plant tissues, specially leaves. Although some research has focused on the rust-resistance mediated by aging effects (SILVA et al., 2017, 2019), the high degree of susceptibility of plants in the first two years after planting has consistently motivated breeders to improve genetic resources to facilitate the production of resistant crops. Therefore, studies have been proposed the use of molecular markers based on DNA polymorphisms to select for resistant genotypes (LAIA et al., 2015; MAMANI et al., 2010). However, the mechanisms governing plant-pathogen interactions are usually complex and involve the regulation of both the transcription and translation of multiple genes into functional proteins. So, unfortunately, genes cannot always be used to predict phenotypes (JAFARI et al., 2017; XU et al., 2017).

In order to define the initial molecular events that take place as a result of plant-pathogen interaction, SHEN et al. (2017) stated that plant “early responses” against fungal disease encompass all biochemical processes that occur within the 24 hours period post-artificial inoculation. Within this time, the fungus will be able to completely invade host tissues and plant defenses will trigger resistance or susceptibility. To support investigations at the molecular level, multi-omics has been proved to be a promising tool to understand the mechanisms underlying plant defense responses and discover new molecules for breeding and gene-editing technologies.

In the next sections, we report our efforts to elucidate the mechanism used to mount an early response to *A. psidii* infection in two contrasting genotypes of *E. grandis*. First, we aimed to determine the time required for rust-resistant and rust-susceptible genotypes to begin divergent responses to pathogen invasion using the microscopic analysis of fungal development, creating a physiological time-course of the plant defense response, and by identifying protein abundance patterns associated with the initial plant response to the pathogen, using comparative proteomics. Second, we focused on secondary metabolic pathways and identified metabolites that were synthesized differently within the timeframe by each genotype assessed. The metabolites were

then associated with protein profiles by combining a time-course of metabolomic and proteomic datasets.

2. CONCLUSION

We conclude that the molecular interaction between *E. grandis* and *A. psidii* occurs within the first 24 hai, when pathogen induces haustorium formation in the rust-susceptible genotype and is unable to survive in the rust-resistant genotype. Physiological plant analysis also confirmed the existence of oxidative bursts that were produced in response to fungal invasion at 3 and 18 hai and played an important role in plant resistance. On the other hand, calloses deposition observed in susceptible plants provided evidence that the plants were weak and slow to perceive the fungus. At 12 hai, comparative proteomics revealed important mechanisms in which resistant plants defended themselves against *A. psidii*. These mechanisms induced proteins involved in reactive oxygen species (ROS) production and programmed cell death (PCD) mechanisms. Simultaneously, proteins related to pathogen perception and activating PTI (PAMP-triggered immunity) and ETI (effector-triggered immunity) responses were suppressed in the susceptible genotype.

Furthermore, combining metabolome and proteome analyses could help us to elucidate differences in the initial responses of two half-sibling genotypes of *E. grandis* to rust. The metabolome of R3 plants showed that flavonoids have a functional importance in rust resistance. Its association with proteins reveals crucial roles for pathogen recognition and signaling before 12 hai, changes in primary metabolic processes for energy delivery to produce targeted defenses after 12 hai, and modulation of oxidative species in the enhancement of immune activation against the biotrophic fungus, *A. psidii*. On the other hand, no specific response was determined for S4 plants infected with rust. Pathogen attack caused immune suppression of S4 plant metabolism until 12 hai, which lead to decreases in metabolite abundance at 24 hai. Although some important proteins for pathogen detection were identified in S4 plants, the initial accumulation of possible antioxidant metabolites and detoxifying proteins (until 6 hai) likely acted as ROS scavengers and induced rust-susceptibility by disabling defense responses such as hypersensitive reaction (HR) and PCD.

These findings suggest that rust-resistance is achieved through controlling ROS production and inducing PCD proteins, while rust-susceptibility is associated with the subversion on the molecular immune system affecting pathogen perception and signaling. The functions of proteins that were determined to be differentially modulated in response to pathogen require further study. Thus, comparative analyses used to study the early responses of *E. grandis* genotypes during rust infection enabled researcher to determine particular mechanisms that lead

to either resistance or susceptibility in plants, and was able to identify potential metabolites and proteins with key roles in the defense response.

REFERENCES

- IBA. **Dados Estatísticos: os números comprovam a força do setor de árvores plantadas.** Disponível em: <<https://www.iba.org/dados-estatisticos>>.
- JAFARI, M. et al. A logic-based dynamic modeling approach to explicate the evolution of the central dogma of molecular biology. **PLoS ONE**, v. 12, n. 12, p. 1–14, 2017.
- LAIA, M. L. et al. Identification of a sequence characterized amplified region (SCAR) marker linked to the *Puccinia psidii* resistance gene 1 (*Ppr1*) in *Eucalyptus grandis*. **African Journal of Agricultural Research**, v. 10, n. 18, p. 1957–1964, 2015.
- MAMANI, E. M. C. et al. Positioning of the major locus for *Puccinia psidii* rust resistance (*Ppr1*) on the Eucalyptus reference map and its validation across unrelated pedigrees. **Tree Genetics and Genomes**, v. 6, n. 6, p. 953–962, 2010.
- SHEN, Y. et al. The early response during the interaction of fungal phytopathogen and host plant. **Open Biology**, v. 7, n. 5, 2017.
- SILVA, R. R. et al. Pre-Infection Stages of *Austropuccinia psidii* in the Epidermis of Eucalyptus Hybrid Leaves with Different Resistance Levels. **Forests**, v. 8, p. 1–12, 2017.
- SILVA, R. R. et al. Limonene, a chemical compound related to the resistance of Eucalyptus to *Austropuccinia psidii*. **Plant Disease**, p. 1–38, 2019.
- TAKAHASHI, S. S. **Ferrugem do eucalipto: índice de infecção, análise temporal e estimativa de danos relacionadas a intensidade da doença no campo.** [s.l.] Universidade Estadual Paulista (UNESP), 2002.
- XU, G. et al. Global translational reprogramming is a fundamental layer of immune regulation in plants. **Nature**, v. 545, n. 7655, p. 487–490, 2017.