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**UNIVERSIDADE ESTADUAL PAULISTA - UNESP  
CÂMPUS DE JABOTICABAL**

**GENOMIC IDENTIFICATION OF MATE, ABC, AND MFS  
TRANSPORTERS IN *Citrus sinensis* AND EXPRESSION  
ANALYSIS OF CITRUS SPECIES INTERACTING WITH  
*Xanthomonas citri* subsp. *citri***

**Maria Heloisa Moreno Julião**  
Biotecnologista

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**Discente: Maria Heloisa Moreno Julião**

**Orientador: Prof. Dr. Alessandro de Mello Varani**

**Coorientador: Prof. Dr. Jesus Aparecido Ferro**

Dissertação apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Campus de Jaboticabal, como parte das exigências para a obtenção do título de Mestre em Agronomia (Genética e Melhoramento de Plantas).

J94g

Julião, Maria Heloisa Moreno

Genomic identification of MATE, ABC, and MFS Transporters in *Citrus sinensis* and expression analysis of *Citrus* species interacting with *Xanthomonas citri* subsp. *citri* / Maria Heloisa Moreno Julião. -- Jaboticabal, 2020

84 p. : il., tabs.

Dissertação (mestrado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal

Orientador: Alessandro de Mello Varani

Coorientador: Jesus Aparecido Ferro

1. Genômica Vegetal. 2. Proteínas Transportadoras. 3. Transcriptoma. I.

Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

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**CERTIFICADO DE APROVAÇÃO**

**TÍTULO DA DISSERTAÇÃO:** GENOMIC IDENTIFICATION OF MATE, ABC, AND MFS TRANSPORTERS IN *Citrus sinensis* AND EXPRESSION ANALYSIS OF CITRUS SPECIES INTERACTING WITH *Xanthomonas citri* subsp. *citri*

**AUTORA:** MARIA HELOISA MORENO JULIÃO

**ORIENTADOR:** ALESSANDRO DE MELLO VARANI

**COORIENTADOR:** JESUS APARECIDO FERRO

Aprovada como parte das exigências para obtenção do Título de Mestra em AGRONOMIA (GENÉTICA E MELHORAMENTO DE PLANTAS), pela Comissão Examinadora:

Prof. Dr. ALESSANDRO DE MELLO VARANI  
Departamento de Tecnologia / UNESP/Câmpus de Jaboticabal

Dra. SAURA RODRIGUES DA SILVA  
Pós Doutoranda do Departamento de Biologia Aplicada à Agropecuária / Faculdade de Ciências Agrárias e Veterinárias  
(VIDEOCONFERÊNCIA)

Profa. Dra. CLAUDIA BARROS MONTEIRO VITORELLO  
Departamento de Genética-ESALQ/USP / Piracicaba/SP  
(VIDEOCONFERÊNCIA)

Jaboticabal, 08 de maio de 2020

## CURRICULAR DATA OF THE AUTHOR

**Maria Heloisa Moreno Julião** – born on January 29, 1997, in Nova Andradina – MS, Brazil. She has a bachelor's degree in Biotechnology from the Federal University of Grande Dourados - UFGD (2014 - 2017). She was a member of the Research Group on Plant Breeding and Biotechnology, participating as a collaborator in research projects. Developed the course conclusion work entitled “Floral biology and in vitro germination of nasturtium pollen grains (*Tropaeolum majus* L.) in cultivation with chicken litter.” under the guidance of Dr<sup>a</sup>. Lívia Maria Chamma Davide. In the same period, she participated in the coordination of the extension project “Biotechnology for All” under the guidance of Dr<sup>a</sup>. Liliam Silvia Candido. She completed a mandatory internship in 2017 at Fundação MS - Pesquisa e Difusão de Tecnologias Agropecuárias, located in Maracajú - MS. In 2018, he joined the Graduate Program in Agronomy (Genetics and Plant Breeding), Faculty of Agricultural and Veterinary Sciences - Unesp/FCAV, Jaboticabal Campus, at the Laboratory of Biochemistry and Molecular Biology under the guidance of the professors Dr. Alessandro de Mello Varani and Dr. Jesus Aparecido Ferro. The dissertation project was developed as a fellow at the Coordination for the Improvement of Higher Education Personnel (CAPES).

“The task is not so much to see what no one has seen yet, but to think what nobody has thought yet, about what everybody sees.” (Arthur Schopenhauer)

## THANKS

To my family, for the love and support in all my choices. For teaching me to believe in my potential, to have faith and perseverance. For being my eternal safe harbor.

To my advisors, for the opportunity, confidence, patience and support in the years of work. Thank you for providing such an expressive personal and professional development, you are my examples of successful professionals.

To the professors of the Graduate Program in Agronomy (Genetics and Plant Breeding), other Unesp/FCAV programs, and my English teacher, for their willingness to help with whatever was possible. For all the knowledge, advice and criticism.

To all Unesp/FCAV employees, for allowing the university to function properly and to execute my project.

To the friends of the Laboratory of Biochemistry and Molecular Biology and the Department of Technology, for the joy in daily living, the spirit at coffee time and the outburst and consolation in difficult times. To all the colleagues at Unesp/FCAV that I met, for teaching me with their experiences, for making my days happier and for encouraging me to continue fighting for my dreams.

To the Unesp University Prayer Group, for filling me with the faith and hope I needed. To the Rotaract Club of Jaboticabal, for the opportunity to contribute to humanitarian projects and complete me personally. To the friends I met in the city of Jaboticabal and became true brothers. Thank you for so many good moments.

To all who contributed directly or indirectly to the execution of this work. My most sincere Thank you.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001



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**GENOMIC IDENTIFICATION OF MATE, ABC, AND MFS TRANSPORTERS IN  
*Citrus sinensis* AND EXPRESSION ANALYSIS OF CITRUS SPECIES  
INTERACTING WITH *Xanthomonas citri* subsp. *citri***

**ABSTRACT**

Plants as sessile organisms require the synthesis and accumulation of a large array of molecules involved in growth, development, and defense-related processes. The Multi-Antimicrobial Extrusion Protein (MATE), ATP-Binding Cassette (ABC) and Major Facilitator Superfamily (MFS) transporters are the largest families of membrane transporters in plants, playing a central role in the defense-related processes in plant-pathogen interactions. For instance, protecting Citrus species cells under the infection of *Xanthomonas citri* subsp. *citri* (Xac), the etiologic agent of the Citrus Canker type A, one of the most devastating Citrus diseases involved with serious economic and environmental impacts. Herein, we identified genes and transcripts from MATE, ABC, and MFS families using the available *Citrus sinensis* genome (v2.0 HZAU) and the re-annotated Citrus Reference Transcriptome (CRT) from CitrusKB Knowledge Base (<http://bioinfo.deinfo.uepg.br>). We identified 67 MATE, 91 MFS, and 143 ABC genes in the *C. sinensis* genome and 82 MATE, 139 MFS, and 226 ABC transcripts in the CRT. The transcripts were mapped in the *C. sinensis* genome revealing a high rate of paralogous genes and probably alternative splicing (AS) events, whose expression profiles and potential roles in the Citrus-Xac interaction were proposed. The tandem and dispersed copies along with genes that underwent AS events represents sources of transporters' genes diversity and complexity. Moreover, we also highlighted potential biotechnological targets, which seemed to contribute with the defense responses in the Citrus plants. The seventeen genes belong to MATE I, ABC C and G, and STP subfamilies, potentially involved in the transport of secondary metabolites and xenobiotics, may act as negative regulators of the disease and can limit the release of sugar from plant cells to apoplast. Overall, this work provides the first MATE, ABC, and MFS transporters' genomic data in *C. sinensis* and their transcriptome-wide characterization in Citrus species infected by Xac, providing fundamental information for studies concerning plant membrane transporters and their role in plant-pathogen interactions.

**KEYWORDS:** Comparative genomics, membrane transporters, differentially expressed genes, plant-pathogen interactions.

**IDENTIFICAÇÃO GENÔMICA DE TRANSPORTADORES MATE, ABC E MFS EM *Citrus sinensis* E ANÁLISE DE EXPRESSÃO EM ESPÉCIES DE CITROS EM INTERAÇÃO COM *Xanthomonas citri* subsp. *citri***

**RESUMO**

As plantas como organismos sésseis requerem a síntese e o acúmulo de uma ampla variedade de moléculas envolvidas no crescimento, desenvolvimento e processos relacionados à defesa. Os transportadores Proteínas de Extrusão Multi-Antimicrobianas (MATE), Cassete de Ligação de ATP (ABC) e Superfamília dos Facilitadores Maioritários (MFS) são as principais famílias de transportadores de membrana em plantas, desempenhando um papel central nos processos relacionados à defesa nas interações planta-patógenos. Por exemplo, protegem as células das espécies de Citros sob a infecção de *Xanthomonas citri* subsp. *citri* (Xac), o agente etiológico do Cancro Cítrico tipo A, uma das doenças de Citros mais devastadoras envolvidas em sérios impactos econômicos e ambientais. Aqui, identificamos genes e transcritos das famílias MATE, ABC e MFS usando o genoma disponível de *Citrus sinensis* (v2.0 HZAU) e o Transcriptoma Referência de Citros (CRT) re-anotado da base de dados CitrusKB (<http://bioinfo.deinfo.uepg.br>). Foram identificados 67 genes MATE, 91 MFS e 143 ABC no genoma de *C. sinensis* e 82 transcritos MATE, 139 MFS e 226 ABC no CRT. Os transcritos foram mapeados no genoma de *C. sinensis*, revelando uma alta taxa de genes parálogos e putativos eventos de splicing alternativo (AS), cujos perfis de expressão gênica e potenciais papéis na interação Citros-Xac foram propostos. As cópias de genes em tandem e cópias dispersas juntamente com genes que possivelmente sofreram eventos de AS representam fontes de diversidade e complexidade dos genes transportadores. Além disso, nós destacamos potenciais alvos biotecnológicos que parecem contribuir com as respostas de defesa das plantas cítricas. Os dezessete genes pertencem às subfamílias MATE I, ABC C e G e STP, potencialmente envolvidas no transporte de metabólitos secundários e xenobióticos, podem atuar como reguladores negativos da doença e limitar liberação de açúcar das células das plantas para o apoplasto. No geral, este trabalho fornece os primeiros dados genômicos de transportadores

MATE, ABC e MFS em *C. sinensis* e sua caracterização no transcriptoma de espécies de Citros infectadas por Xac, fornecendo informações fundamentais para estudos sobre transportadores de membrana em plantas e seu papel nas interações planta-patógeno.

**PALAVRAS-CHAVE:** Genômica comparativa, Transportadores de Membrana, Genes Diferencialmente Expressos, Interações Planta-Patógeno.

## LIST OF ABBREVIATIONS

|          |   |
|----------|---|
| ABC      | ATP-Binding Cassette  |
| AIC      | Akaike Information Criteria   |
| AS       | Alternative Splicing  |
| CRT      | Citrus Reference Transcriptome  |
| CC       | Citrus Canker type A  |
| CitrusKB | A Knowledge Base for Transcriptome of Citrus species and <i>Xanthomonas citri</i> subsp. <i>citri</i> Interactome |
| Chr      | Chromosome  |
| CsMATE   | MATE genomic sequences from the <i>Citrus sinensis</i> genome   |
| CsABC    | ABC genomic sequences from the <i>Citrus sinensis</i> genome  |
| CsMFS    | MFS genomic sequences from the <i>Citrus sinensis</i> genome  |
| CstMATE  | potential MATE sequences expressed by Citrus species  |
| CstABC   | potential ABC sequences expressed by Citrus species   |
| CstMFS   | potential MFS sequences expressed by Citrus species   |
| DE       | Differentially Expressed  |
| DTX      | Detoxification Proteins   |
| FBT      | Folate-Biopterin Transporter  |
| MATE     | Multi-Antimicrobial Extrusion Protein   |
| MFS      | Major Facilitator Superfamily   |
| MW       | Molecular Weight  |
| pI       | Isoelectric point   |
| PHT      | Phosphate Transporter   |
| STP      | Sugar Transporter Protein   |
| WGD      | Whole-genome/segmental duplication  |
| Xac      | <i>Xanthomonas citri</i> subsp. <i>citri</i>  |

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## 1. INTRODUCTION

Plant-pathogen interactions result in macro and microscopic changes in the host plant, involving a wide range of morphological, biochemical, genetic, and molecular processes (Schenk et al., 2000; Melotto et al., 2006; Uchida and Tasaka, 2010; Cheval and Faulkner, 2018). The defense-related processes triggered by plants during the interaction are fast and targeted to counter-attack the pathogen to maintain the cellular homeostasis (Jones and Dangl, 2006). One of the most studied and devastating plant diseases affecting all commercial citrus varieties in production areas around the world is the Citrus Canker type A (CC), caused by the Gram-negative bacterium *Xanthomonas citri* subsp. *citri* (Xac) (Behlau et al., 2010).

The Xac infects the citrus tissues through the penetration in stomatal pores or wounds made by thorns and insects. The symptoms of infection start like high injuries soaked with water and evolve until it forms the cankers, reaching plant defoliation and premature fall of fruits (Gottwald et al., 2002). Moreover, distinct susceptibility levels to Citrus Canker across Citrus species (de Carvalho et al., 2015) were observed, reflecting in a complex array of plant-pathogen interaction and defense-response mechanisms.

Among the mechanisms conducted during the defense responses, plants developed and expanded transport mechanisms to excrete or sequester a broad range of synthesized molecules and xenobiotics to provide an efficient immune response (Tegos et al., 2002; Wang et al., 2016b). A large number of primary and secondary active transporter genes found in plants improve the plant competition and adaptation to stress conditions (Hwang et al., 2016), such as under the Citrus Canker disease. The major transporters families Multi-Antimicrobial Extrusion Protein (MATE), ATP-Binding Cassette transporters (ABC), and Major Facilitator Superfamily (MFS) play an essential role in the plants' membrane trafficking network (Shoji, 2014), are ubiquitously present in prokaryotic and eukaryotes organisms, and present distinct protein structures and source of energy used to execute the transport of molecules (Fath and Kolter, 1993; Takanashi et al., 2014a).

Identifying and understanding the features and roles of membrane transporter proteins in plant species, as the MATE, ABC, and MFS transporters in Citrus species has only been possible due to the growing number of genomic and transcriptomic data available in the public databases. Despite that, information concerning membrane transporters in Citrus species is still patchy. Thus, the importance of citriculture along with

the constant threatening to citrus cultivation and the posing substantial economic impacts of the citrus canker, and also the possibility of adding scientific advance to the knowledge on membrane transport proteins prompted us to characterize and investigate the role and impact of these important gene families into the Citrus-Xac interaction.

As part of our efforts to understand the Citrus-Xac interaction, we recently build a knowledge base for transcriptome of Citrus and Xac interactome (CitrusKB, <http://bioinfo.deinfo.uepg.br/citrus>), providing data from Citrus species of divergent Citrus Canker susceptibility levels (e.g. from the less susceptible cultivars: 'Kumquat' *Fortunella* spp., Tangerine mandarin 'Satsuma' *C. unshiu*, and Tangerine mandarin 'Ponkan' *C. reticulata*, to the intermediate susceptible as Sweet oranges 'Pera Rio' and 'Valencia' *C. sinensis*, and highly susceptible as Sweet oranges 'Hamlin' and 'Bahia' *C. sinensis*, and Mexican lime 'Galego' *C. aurantifolia*) (Ferrasa et al., 2020). Using the CitrusKB database along with publicly available *Citrus sinensis* genome (v2.0 HZAU), we identified, classified, and compared the MATE, ABC, and MFS families in the *C. sinensis* genome and in the Citrus Reference Transcriptome (CRT).

We identified the genomic context in which the membrane transporters genes exist to understand how duplicate genes have contributed to their expansion and function and highlighted promising candidates for further exploitation from subfamilies MATE I, ABC C, ABC G, and STP based on their potential role in the Citrus-Xac interaction. Taken together, our results are not only providing novel insights about the main membrane transporters families in Citrus defense-related processes but also revealing a complex layer of the history of these gene families expansions and the transcriptional regulation under a plant-pathogen interaction.

## 6. CONCLUSIONS

MATE, ABC, and MFS transporters are involved beyond development and survival processes in plants, are essential proteins under plant-pathogen interactions due to their role in the excretion or uptake of macronutrients, defense-related compounds, xenobiotics, and metabolic products. Five MATE, eight ABC, and fourteen MFS subfamilies were identified in *Citrus sinensis*, of which their putative roles and impact during the Citrus-Xac interaction revealed potential targets for functional studies and a common transport of secondary metabolites and xenobiotics. Moreover, the subfamilies MATE I and ABC G seem to be key players in the management of the compounds produced by plants during the defense responses. Altogether, the transcriptional evidence of both membrane transporter family members based on CRT data supports the proposal for a highly complex pattern of gene expression regulation during the Citrus-Xac interaction, which reflects the

intricate defense-related processes in CC susceptibility levels between Citrus cultivars. Also, alternative splicing and gene duplication events may represent evolutionary strategies to increase the membrane transporters' numbers and functional diversification in Citrus species for proper stress responses to Citrus Canker disease. Altogether the findings from this work offer useful information, highlighting membrane transporters for potential biotechnological applications, and representing a basis for the functional characterization of MATE, ABC, and MFS transporters in Citrus species, helping to address several biological questions concerning the plant membrane transporters families.

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